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ANALYSIS OF THE INTERACTING COMPONENTS BETWEEN LARVAL *SCHISTOSOMA MANSONI* AND SCHISTOSOME-SUSCEPTIBLE AND RESISTANT *BIOMPHALARIA GLABRATA*

WILLARD O. GRANATH, JR. & FRANCES J. SPRAY

Department of Microbiology University of Montana, Missoula, Montana 59812, USA

Cell-free hemolymph (serum) and hemocytes from Schistosoma mansoni-*susceptible (PR albino M-line) and resistant (10-R2) strains of* Biomphalaria glabrata *were compared by SDS-PAGE, immunoblotting and radioiodination. Whole serum of both snail strains is dominated by hemoglobin (Hb) (MW = 160 Kd). SDS-PAGE. of Hb-depleted serum indicated that the 10-R2 strain has dominant polypeptides in the 50 to 30 Kd range whereas PR albino snails have few low MW proteins.*

Antibodies raised to whole PR albino and 10-R2 serum, and the 160 Kd (Hb) band reacted similarly in immunoblot assays. Analysis of hemocytes revealed that 10-R2 snails have a surface-exposed protein at about 80 Kd which is not present on PR albino hemocytes. An examination of primary cultured sporocysts indicated the presence of four major surface proteins (40, 50, 55, 70 Kd) and two minor surface-exposed polypeptides (92, 170 Kd). Antibodies raised against live, intact sporocysts reacted almost exclusively with sporocyst-surface proteins when tested by immunoblotting.

The internal defensive mechanisms of *Biomphalaria glabrata* have been the subject of much research since it is an important vector of *Schistosoma mansoni* in nature. Such studies have clearly shown that there are three basic components associated with the internal defense response of *B. glabrata* to the larval schistosome, namely circulating blood cells, called hemocytes, soluble hemolymph components, and the sporocyst surface (e. g. C. J. Bayne, 1983, *Biology of Mollusca*, Academic Press, New York, p. 407-486). Currently we are beginning a systematic molecular analysis of these three components using a host-parasite model composed of PR albino M-line *B. glabrata* (PR albino), which is 90-100% susceptible to infection by *S. mansoni* (NIH-Sm-PR-1 strain) and 10-R2 *B. glabrata* which are virtually 100% resistant to the same strain of parasite (C. S. Richards 1975, *Ann. N. Y. Acad. Sci.*, *266:* 394-410; C. S. Richards, 1975, *Parasitology, 70:* 231-241). The following are preliminary results of experiments which have utilized the techniques of sodium dodecyl sulfate-poluacrylamide gel electrophoresis (SDS-PAGE), immunoblotting (W. O. Granath, Jr. et al., 1987, *J. Invert. Pathol.*, 198-208) and radioiodination (R. C. Judd, 1982, *Infect. Immun.*, *37:* 632-641).

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Studies on *B. glabrata* serum, which we define as cell-free hemolymph, were conducted on whole serum and a hemoglobin-depleted fraction. To create this fraction, serum was centrifuged at 50,000 X g at 4 °C for 3 hours. The resulting supernatant was thus depleted of hemoglobin (Hb). Samples to be used for SDS-PAGE were then solubilized with a solution with or without 2-mercaptoethanol (2-ME).

Whole serum and the Hb-depleted supernatant from resistant 10-R2 and susceptible PR albino *B. glabrata* were separated in a 10% acrylamide gel. Whole serum of both *B. glabrata* strains are dominated by Hb, which migrates at about 160 Kd when solubilized with 2-ME, and over 200 Kd without 2-ME. Although the same amount of sample was applied to the gel (5 μl) the PR albino strain appears to have more total protein than the 10-R2 strain. Otherwise, the polypeptide profiles are strikingly similar between the *B. glabrata* strains. In comparison, several snail strain differences were observed in the Hb-depleted supernatants separated by SDS-PAGE. The 10-R2 strain has many polypeptides in the 45 to 30 Kd range, which were not observed in the PR albino strain. There are

also several differences in the migration of bands between strains in the 200 to 50 Kd range. Further, this experiment indicated significant differences in the migration patterns between samples (whole serum and Hb-depleted fractions) solubilized with and without 2-ME revealing that many serum proteins are modified upon reduction and thus most possess disulfide bonds.

To further analyse *B. glabrata* serum, antibodies to this material were raised in rabbits. Moreover, since it appeared that Hb was the dominant protein in the sera of both snail strains, we attempted to produce rabbit antisera to *B. glabrata* Hb. To do this, the 160 Kd polypeptide was purified 2-times by SDS-PAGE, eluted from the gel, and then used to immunize rabbits. These reagents were then used in immunoblot assays. Whole snail sera and the Hb-depleted supernatants of both *B. glabrata* strains were solubilized with a solution containing 2-ME. Five μ l of a 1:10 dilution of whole snail sera and 35 μ l of undiluted supernatants were separated in a 10% SDS-PAGE gel, electroblotted, and then probed with anti-10-R2 serum or with anti-PR albino serum. Both antibody preparations reacted similarly. These antibodies bound to many polypeptides in whole snail sera and supernatants, although some differences in staining intensity between snail strains were observed. Next, similar immunoblots were conducted but, in this case, samples were solubilized with a solution without 2-ME. As in the previous immunoblots, both antibody preparations reacted similarly, although there were some differences in staining intensity. Whole *B. glabrata* sera and Hb-depleted supernatants (solubilized with or without 2-ME) were subjected to further immunoblot analysis by probing with antibody derived against snail Hb. Many polypeptides in the sera and supernatant of both *B. glabrata* strains reacted with the anti-Hb antibody indicating that they may be Hb-related proteins. It should be noted that immunoblots incubated with antibody raised against whole snail serum, have more polypeptides in the 150 to 60 Kd range. Further, all of the immunoblot experiments again indicated that *B. glabrata* serum proteins are modified by reduction.

To examine the second component involved in schistosome-snail interactions, hemocytes from 10-R2 and PR albino snails were studied by SDS-PAGE and surface radioiodination. The polypeptide profiles appear to be similar between snail strains except for a band at approximately 80 Kd that is present in 10-R2 hemocytes which does not appear in hemocytes of the PR albino strain. Autoradiography of ^{125}I surface-labeled hemocytes from both snail strains revealed the presence of surface-exposed proteins in the 200 to 45 Kd range. However, the 80 Kd polypeptide present in 10-R2 hemocytes, but lacking in PR albino cells, is exposed on the 10-R2 hemocyte surface. Perhaps this polypeptide, apparently unique to the surface of resistant 10-R2 hemocytes, will be shown to be important in larval schistosome recognition. To work towards this goal, we are analyzing intramolluscan stages of *S. mansoni*. We are examining *in vitro* transformed primary sporocysts by SDS-PAGE, autoradiography and immunoblotting. SDS-PAGE revealed many bands in the 200 to 14 Kd range. However, autoradiography of surface-iodinated sporocysts indicated the presence of four major surface proteins at approximately 40, 50, 55 and 70 Kd, and two minor surface-exposed polypeptides at about 92 and 120 Kd. Sporocysts separated by SDS-PAGE were also probed with antiserum raised against live, intact sporocysts by immunoblotting. This antiserum reacted with all of the surface proteins and a band at about 60 Kd. It is anticipated that such information will be valuable in evaluating the role of sporocyst surface proteins in parasite recognition or evasion by *B. glabrata*.

In conclusion, these data show that there are some specific differences between serum polypeptides and hemocyte surface-exposed proteins of the 10-R2 and PR albino strains of *B. glabrata*. These differences may be important in mediating resistance and/or susceptibility to larval *S. mansoni*. Moreover, it is possible that these differences may be quantitative rather than qualitative. Although the functional significance of the observed sporocyst antigens are not yet understood, it is anticipated that ongoing research will reveal their importance, as well as hemocyte and serum proteins, in schistosome-snail interactions.