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Time series analysis of the transcriptional responses of *Biomphalaria glabrata* throughout the course of intramolluscan development of *Schistosoma mansoni* and *Echinostoma paraensei*

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ABSTRACT

Successful colonization of a compatible snail host by a digenetic trematode miracidium initiates a complex, proliferative development program requiring weeks to reach culmination in the form of production of cercariae which, once started, may persist for the remainder of the life span of the infected snail. How are such proliferative and invasive parasites able to circumvent host defenses and establish chronic infections? Using a microarray designed to monitor the internal defense and stress-related responses of the freshwater snail *Biomphalaria glabrata*, we have undertaken a time course study to monitor snail responses following exposure to two different trematode species to which the snail is susceptible: the medically important *Schistosoma mansoni*, exemplifying sporocyst production in its larval development, or *Echinostoma paraensei*, representing an emphasis on rediae production in its larval development. We sampled eight time points (0.5, 1, 2, 4, 8, 16 and 32 days p.i.) that cover the period required for cercariae to be produced. Following exposure to *S. mansoni*, there was a preponderance of up-regulated over down-regulated array features through 2 days p.i. but by 4 days p.i. and thereafter, this pattern was strongly reversed. For *E. paraensei*, there was a preponderance of down-regulated array features over up-regulated features at even 0.5 days p.i., a pattern that persists throughout the course of infection except for 1 day p.i., when up-regulated array features slightly outnumbered down-regulated features. Examination of particular array features revealed several that were up-regulated by both parasites early in the course of infection and one, fibrinogen related protein 4 (FREP 4), that remained significantly elevated throughout the course of infection with either parasite, effectively serving as a marker of infection. Many defense-related transcripts were persistently down-regulated, including several fibrinogen-containing lectins and homologs of molecules best known from vertebrate phagocytic cells. Our results are consistent with earlier studies suggesting that both parasites are able to interfere with host defense responses, including a tendency for *E. paraensei* to do so more rapidly and strongly than *S. mansoni*. They further suggest mechanisms for how trematodes are able to establish the chronic infections necessary for their continued success.

1. Introduction

The associations between digenetic trematodes and their molluscan intermediate hosts are specific, complex and long-lasting. The digenean developmental program in molluscs often takes weeks to unfold and the production of cercaria that follows can be sustained for months or even years (Curtis, 2003). The molluscan host is partially or wholly castrated after infection (Sorensen and Minchella, 2001) and not uncommonly dies with an unresolved infection. Such associations pose several questions of fundamental interest for those interested in unraveling the mechanistic bases of host–parasite interactions. Furthermore, many digeneans are of medical or veterinary significance and by knowing more about their associations with molluscs, we may reveal mechanisms whereby their adverse affects can be controlled.

The digeneans *Schistosoma mansoni* and *Echinostoma paraensei* both successfully infect the Neotropical planorbid snail *Biomphalaria glabrata*. Experimental infections of laboratory-reared M-line *B. glabrata*, with either parasite, are successful at least 90% of the time. In the case of *S. mansoni* which exemplifies a "sporocyst only" mode of larval development, a miracidium initiates infection by penetrating the snail, often the head–foot. Within hours the miracidium casts off its miracidial plates and transforms into a mother sporocyst that grows, and by a clonal process of asexual reproduction, begins to produce elongate daughter sporocysts. Depending on the temperature but usually at about 18 days p.i., daughter sporocysts exit the expanded mother sporocyst and migrate to the

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host's digestive gland/ovotestis complex where they settle, become amorphous in shape, and begin to produce either additional sporocysts or cercariae, again by a clonal, asexual reproductive process (Pan, 1965). Fully developed cercariae are usually released from daughter sporocysts at about 1 month post-exposure and are then shed from the infected snail's body. Cercarial production can continue for several months, typically until the infected snail dies.

Echinostoma paraensei exemplifies the "sporocyst-rediae" mode of larval development. A miracidium of this species loses its ciliated plates as it penetrates the snail tegument (Ataev et al., 1997). Once in the snail the parasite, now referred to as a sporocyst, migrates via the snail's circulatory system to the heart and by 2–3 days p.i. has attached to the ventricular wall. Here the sporocyst rapidly grows and produces mother rediae, one of which, at least in the isolate maintained in our laboratory, is produced before the others, at day 6 p.i. Mother rediae then colonize other parts of the snail and produce daughter rediae which eventually colonize the host's digestive gland/ovotestis where they commence production of cercariae. As with *S. mansoni*, cercariae are first released at about 1 month p.i. Although the overall length of the pre-patent period is comparable for both parasites, and development culminates with the production of cercariae which can then continue to be produced over many months, their developmental programs are quite distinct.

To establish such long-term infections, digeneans must persistently thwart the host's immune system. Two commonly invoked ideas are that the parasite is either undetected by the host immune response (Yoshino and Bayne, 1983; Hanelt et al., 2008), or the parasite is able to interfere with or suppress the host response in order to establish and maintain infection (Boswell and Bayne, 1986; Loker et al., 1986, 1992). Based on a comprehensive series of in vivo experiments, it was shown that a strain of *B. glabrata* resistant to *S. mansoni* infection, if first infected with *E. paraensei* sporocysts, would lose their resistance to *S. mansoni*, an outcome attributable to the strong interfering capacity of *E. paraensei* larvae (Lie et al., 1977; Lie and Heyneman, 1979). It was concluded that all trematodes studied by Lie and co-workers that developed in *B. glabrata* were able to interfere with this snail's defense responses, and interference was suggested as a mechanism of general significance for understanding the success of trematodes in infecting molluscs (Lie et al., 1982).

Interference by *E. paraensei* has been shown to target hemocytes which are a critical component of the snail defense response against trematode infection (Loker et al., 1989, 1992). *Echinostoma paraensei* sporocysts (Loker et al., 1989) as well as sporocyst excretory/secretory products interfere with phagocytosis of particles (Noda and Loker, 1989) and with hemocyte binding to, and encapsulation of, *E. paraensei* and *S. mansoni* sporocysts (Loker et al., 1992). Hemocytes in the presence of either sporocysts or daughter rediae of *E. paraensei* exhibited abnormal behavior (Adema et al., 1994).

Studies of other snail-trematode systems support the interference hypothesis. Humbert and Coustau (2001) noted that hemocytes from a strain of *B. glabrata* susceptible to *Echinostoma caproni* exhibited diminished adhesiveness and phagocytosis when exposed to parasite secretory/excretory products. Iakovleva et al. (2006) observed reduced rates of phagocytosis of hemocytes of *Littorina littorea* following exposure to the secretory products of echinostome and heterophyid rediae. A number of studies suggest that prior infection of a snail by one trematode can facilitate the snail's colonization by other trematodes (Appleton, 1983; Southgate et al., 1989; Abrous et al., 1998). At the transcriptional level, that *S. mansoni* can also have suppressive effects on host responses has also been shown by Knight et al. (2009) who noted that successful infection caused a down-regulation of defense-related transcripts.

Results from other parasite-invertebrate host model systems exhibiting prolonged parasite development times also suggest that parasite-mediated interference with host defenses occurs. This has been reported with *Plasmodium*–mosquito interactions (Boete et al., 2004), as well as with parasitoid development in arthropods. Parasitoids often possess symbiotic viruses that are responsible for the observed immunosuppression (Davies and Vinson, 1988; Labropoulou et al., 2008; Suderman et al., 2008). Parasite-induced immunosuppression has also been reported in human–trematode interactions (Duvaux-Miret et al., 1992; Cervi et al., 1996), and a number of other parasite infections of vertebrates and invertebrates (Sanad et al., 1991; Guimaraes et al., 1997; Zuniga et al., 2000).

Although effects consistent with trematode-mediated interference have been documented early in the course of infection, additional study is needed to determine how long a state of interference might be maintained by digeneans, given the length and complexity of their developmental patterns in snails. Further documentation of the actual molecular effects of interference on the snail is also needed. Also, although resistance to trematode infection has been associated with various mechanisms such as refractoriness to trematode interference (Humbert and Coustau, 2001), or expression of high levels of certain immune mediators, (Mitta et al., 2005; Vergote et al., 2005; Bayne, 2009), the immunological mechanisms underlying resistance and the targets of parasite interference remain poorly known.

To help rectify these shortcomings, we report our most recent results using a *B. glabrata* oligo-based microarray focused on transcripts known to be involved in immune and stress responses. We have previously used this array to show that *B. glabrata* is capable of mounting diverse immune responses to different immune challenges, as assessed, 0.5 days p.i. (Adema et al., in press). A number of immune features were both up- and down-regulated following exposure to either *S. mansoni* or *E. paraensei*, suggesting that neither parasite is able to initiate infection without alerting defense responses from the host. At this single, relatively early time point, a predominance of down-regulated features was noted in snails exposed to *E. paraensei*, whereas snails exposed to *S. mansoni* exhibited a strong predominance of up-regulated features (Adema et al., in press).

In the present study we monitored the transcriptional profile of susceptible M-line *B. glabrata* snails at several time points (0.5, 1, 2, 4, 8, 16 and 32 days) following exposure to *S. mansoni* or *E. paraensei*. We demonstrate that *S. mansoni* infection is associated with an early preponderance of up-regulated transcripts followed by a sharp trend towards a persistent predominance of down-regulated features throughout the course of development. Exposure to *E. paraensei* provoked a predominance of down-regulated features throughout the entire period of development. We also highlight particular immune features that merit additional study based on the transcriptional profiles observed.

2. Materials and methods

2.1. Live material and experimental treatments

M-line *B. glabrata* used in these studies can serve as an intermediate host for both *E. paraensei* and *S. mansoni* (PR-1 strain). Both snails and trematodes were maintained at the University of New Mexico as previously described, and for both trematodes, snails (4–8 mm diameter) were exposed individually to 15–20 miracidia per snail in the wells of a 24-well plate, in artificial spring water (Loker and Hertel, 1987). Size-matched snails were sham exposed to serve as controls. Snails from all groups were kept for 12 h in 24-well plates to remove conditions of the