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***Schistosoma mansoni*: sex-specific modulation of parasite growth by host immune signals**

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Abstract

Development of female schistosomes from infectious cercariae to mature egg-producing adults requires both male schistosomes and an intact adaptive immune system. By examining single sex infections in immunodeficient mice, we provide evidence that female schistosome development is not directly influenced by the adaptive immune system, whereas male development is. Our data are consistent with a sequential model of schistosome development, where the adaptive immune system signals development of mature males, which subsequently stimulate development of mature females. The male schistosome therefore appears to play a central role both in transducing signals from the adaptive immune system and in facilitating female development.

Keywords

Schistosoma mansoni; Trematode; Developmental biology; Dioecy; Recombination activating gene; Adaptive immunity

Many helminth species are capable of modulating their development in response to environmental and host factors. Frequently, developmental responses appear to facilitate parasite survival and transmission under adverse conditions (reviewed in Davies and McKerrow, 2003). Developmental responses are best represented amongst the Nematoda (Gibbs, 1986; Michel, 1974), but there are examples of similar responses in some platyhelminths (Shoop and Corkum, 1984, 1987). Several lines of evidence indicate that schistosome development within the definitive host is somewhat plastic and amenable to modulation in response to host factors. Using a murine model of schistosome infection, we have shown that *Schistosoma mansoni* is capable of developmental responses to factors that emanate from the host's adaptive immune system and that CD4⁺ αβ T lymphocytes are integral in supplying the immune signals to which the parasite responds (Davies et al., 2001). Specifically, schistosomes developed more slowly, produced fewer eggs, and attained a smaller adult size in immunodeficient mice when compared to parasites from wild type controls. The biological relevance of developmental responses in schistosomes is not yet clear but the observation that potential causes of immune dysfunction, such as malnutrition (Desai et al., 1980) and co-infection with other pathogens (Ashford et al., 1992; Chung et al., 1995; Keiser et al., 2002; Keusch and Migasena, 1982; Thiong'o et al., 2001), are commonplace in schistosomiasis-endemic areas suggests that developmental plasticity in blood flukes is an important parasite adaptation and may be of significance in understanding the pathogenesis and epidemiology of schistosomiasis.

The Schistosomatidae are unusual platyhelminths in that they are dioecious rather than hermaphrodite. An interesting consequence of dioecy in this family is that, in most species, schistosome development to mature adult parasites is also contingent on the transmission of signals between males and females, as female worms fail to develop fully in the absence of males (Basch, 1991). In *S. mansoni*, Vogel (1941) considered female development to proceed in two consecutive phases within the definitive host. The first phase, which spans the first month of infection, is independent of males. The second phase of female development, from the beginning of the second month of infection until adulthood, is dependent on the presence of mature male schistosomes. A consequence of the male-independent and male-dependent phases of female growth is that females recovered from mice infected with only female cercariae for 4 weeks or longer are all uniformly small, having attained a length approximately half that of mature females (Vogel, 1941). Thus, in the absence of males, female development proceeds normally for the first month of infection, but is then arrested unless males are present to signal development to mature adulthood (Vogel, 1941). The manner by which the male schistosome induces female development has been the subject of speculation and supply of nutritive material or hormonal signals by males to females have both been postulated (Basch, 1991). Evidence for a male-derived lipid-based factor that stimulates female development has been presented (Shaw et al., 1977; Popiel and Erasmus, 1981).

In contrast, to female development, male development appears to be largely unaffected by the presence of the opposite sex. Vogel (1941) and Armstrong (1965) both concluded that male growth and development were independent of the presence of females, while another study reported small reductions in parasite length and number of testes in males from unisexual infections (Zanotti et al., 1982).

The observation that host–parasite and male–female interactions can modulate female schistosome development prompted us to examine the interactions between these factors. Two models of how the adaptive immune system might influence female development can be postulated. First, the adaptive immune system may influence female development directly, and might thus affect female development during the early, male-independent phase of development. Second, female development may be independent of direct stimulation by the adaptive immune system. In this latter case, the adaptive immune system would only affect females during the later, male-dependent phase of development when female development is dependent on mature males (Vogel, 1941). To discriminate between these possibilities, we examined the development of females in the absence of both the adaptive immune system and male parasites by infecting immunodeficient mice with single sex female infections. We postulated that if female development is directly affected by the adaptive immune system, phenotypic differences would be evident when females isolated from wild type and immunodeficient hosts were compared. In contrast, if the adaptive immune system acts only indirectly on the female, via the male, we postulated that female development in the absence of males would be identical in both wild type and immunodeficient mice. Finally, we compared the development of male parasites in wild type and immunodeficient mice to assess whether compromised male development due to absence of adaptive immune signals could contribute indirectly to compromised female development.

The *S. mansoni* life cycle was maintained in the laboratory using *Biomphalaria glabrata* snails and golden hamsters (*Mesocricetus auratus*) as intermediate and definitive hosts, respectively (Smithers and Terry, 1965). All vertebrate animals were housed at the Veterans Affairs Medical Center San Francisco, in accordance with protocols approved by the Institutional Animal Care and Use Committee. Livers from infected hamsters were homogenized in PBS/0.7% trypsin and incubated at 37 °C for 1 h to recover parasite eggs. To generate snails infected with a single miracidium, *S. mansoni* eggs were hatched in fresh water and individual miracidia were separated into wells of 96-well plates. Single *B. glabrata* were then added to each well and

infection was allowed to proceed overnight. At nine weeks post infection snails were shed individually under light and cercariae from each snail (~100 cercariae) were collected in microfuge tubes. Genomic DNA was isolated using a DNeasy Tissue Kit (Qiagen) and cercarial sex was determined by PCR amplification of the female-specific W1 repeat (Gasser et al., 1991).

Male and female cercariae were collected and used to infect groups of recombination activating gene- (RAG-) 1-deficient (RAG-1^{-/-};B6;129S7-*Rag1*^{tm1Mom} (Mombaerts et al., 1992)) and wild type control C57BL/6 mice percutaneously via the tail skin. Groups of five animals were used in every experiment and all animals were age- and sex-matched. Mice were euthanized at 42 days post infection and schistosomes were recovered from the hepatic portal system by perfusion. Parasites were immediately fixed in 4% neutral-buffered formaldehyde and digital images were obtained using a Nikon Coolpix 4500 digital camera. Parasite length was measured from the digital images using “Image J” software (<http://rsb.info.nih.gov/ij/>). Each parasite was measured 3 times and a mean value was determined. Fifteen randomly selected worms from each sample population were measured. Differences in parasite length between groups were analyzed by *t* test. All experiments were performed at least twice.

Interestingly, *S. mansoni* females recovered from single sex infections of C57BL/6 (Fig. 1A) and RAG-1^{-/-} mice (Fig. 1B) were phenotypically identical. Determination of parasite length from digital images confirmed these observations. There was no significant difference in the lengths of females from wild type C57BL/6 and RAG-1^{-/-} mice ($P = 0.8127$ returned by unpaired *t* test), which had mean lengths of 3.013 and 3.085 mm, respectively (Fig. 1C). Thus, in the absence of males, female parasites developed comparably in both immunodeficient, and wild type hosts. These observations indicate that female development during the early male-independent phase of development is independent of adaptive immune signals. As female development does not proceed beyond this early phase in the absence of males (Vogel, 1941), we cannot determine what the respective contributions of adaptive immune system, and male parasites are in the later, male-dependent phase of development. However, these findings suggest that subsequent immune effects on female development later in infection might also be indirect, mediated via the male schistosome.

To specifically examine the effects of the adaptive immune system on male schistosome development, we compared the phenotypes of males from wild type and immunodeficient hosts. Significantly, males isolated from single sex-infected RAG-1^{-/-} mice (Fig. 2A) were visibly smaller than those isolated from wild type controls (Fig. 2B). Of particular note were the numerous very small males isolated from RAG-1^{-/-} mice and a relative paucity of oxidized heme in the guts of these parasites (Fig. 2B). Determination of parasite length from digital images confirmed these observations—*S. mansoni* males isolated from single sex infections of wild type C57BL/6 mice had a mean length of 4.044 mm, while those from RAG-1^{-/-} animals had a mean length of 3.160 mm (Fig. 2C). The difference in mean parasite length between the two groups was significant, with an unpaired *t* test returning a *P* value of 0.0088. This result confirms that male development is significantly influenced by adaptive immune signals. Importantly, these observations provide a mechanism by which the adaptive immune system might influence female development during the later, male-dependent phase of female development, as female development during this phase requires mature males (Vogel, 1941).

Development of female schistosomes from cercariae to mature, egg-producing adults requires a complex interplay between signals from male schistosomes (Vogel, 1941), and the adaptive immune system (Davies et al., 2001). However, the data we present here support the conclusion that female schistosome development is not directly influenced by the adaptive immune system (Fig. 1), whereas male development is (Fig. 2). We can therefore propose a sequential model of schistosome development, where the adaptive immune system signals development of

mature males, which subsequently stimulate development of mature females. In such a model, the male plays a central role both in transducing signals from the adaptive immune system and in facilitating female development. This model has important implications for future studies aimed at elucidating how schistosomes respond to adaptive immune signals, as the male parasite can now be considered as the primary recipient of these signals.

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Fig. 1. Female *S. mansoni* development in single sex-infected wild type and RAG-1^{-/-} mice. (A, B) Female *S. mansoni* were isolated from single-sex infected wild type (A) and RAG-1^{-/-} (B) mice at 42 days post-infection. Scale bars represent 1 mm. (C) Length of female parasites isolated from single sex-infected wild type (■) and RAG-1^{-/-} (▲) mice, as determined from digital images using ImageJ software.

Fig. 2.

Male *S. mansoni* development in single sex-infected wild type and RAG-1^{-/-} mice. (A, B) Male *S. mansoni* were isolated from single-sex infected wild type (A) and RAG-1^{-/-} (B) mice at 42 days post-infection. Note the numerous very small forms and diminished black heme gut pigment in the worms isolated from RAG-1^{-/-} mice (B). Scale bars represent 1 mm. (C) Length of male parasites isolated from single sex-infected wild type (■) and RAG-1^{-/-} (▲) mice, as determined from digital images using ImageJ software.