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Mouse Strain Type is Not Selective for a Laboratory-adapted Strain of *Schistosoma mansoni*

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Abstract

We genotyped pooled adult worms of *Schistosoma mansoni* from infected CF1, C57BL/6, BALB/c, and BALB/c interferon gamma knockout mice in order to investigate if mouse strain differences selected for parasite genotypes. We also compared differentiation in eggs collected from liver and intestines to determine if there was differential distribution of parasite strains in the vertebrate host that might account for any genotype selection. We found that mouse strains with differing immune responses did not differ in resistance to infection and did not select for parasite genotypes. *S. mansoni* genotypes were also equally distributed in tissues and there was no difference between adult and egg allele frequencies. Differing immune responses in these mouse strains does not significantly affect the degree or the nature of host resistance to schistosomes.

Different hosts show differing degrees of susceptibility to *Schistosoma mansoni* infection. Some mammals, such as foxes and rabbits, are absolutely resistant (Von Lichtenberg et al., 1962; Peck et al., 1983), while others demonstrate selection of certain *S. mansoni* genotypes (LoVerde et al., 1985; Bremond et al., 1993). Although rats of the species *Rattus rattus* can maintain the life cycle, *R. norvegicus* cannot (Alarcón de Noya et al., 1997). Primates also are variably permissive with rhesus macaques (*Macaca mulatta*) showing a high degree of resistance, whereas baboons are susceptible (Sadun et al., 1966). The intermediate host range is even more finely restricted. Schistosomes in some cases are better habituated (i.e. a larger proportion of the population can be infected) to snails for the same geographic region than snails from outside of the region (Files and Cram, 1949; Michelson and DuBois, 1978; Sulaiman and Ibrahim, 1985; Manning et al., 1995; Incani et al., 2001).

Selection can be studied in terms of population differentiation based on neutral markers (Freeland et al., 2010). In many instances, beneficial mutations occur in a single or limited group of individuals, and following a purifying selection, survivors represent a defined subpopulation of the original. The result is genome-wide differentiation. While genetic drift and bottlenecks will also reduce diversity, over a single generation in a large population selection is the likely explanation for a subpopulation failing to represent a random draw from the parent population (Merilä and Crnokrak, 2001). We used the population differentiation index Jost's D to investigate two questions. First, whether mouse strain (and thereby the type of host immune response) strongly selects for specific genotypes of *S. mansoni*. Second, whether the allele frequencies of *S. mansoni* eggs found in the liver and the intestine of infected mice differed from those observed in adult worms from the same mice.

In order to determine whether variations in immunological response or other differences in mouse strain biology were selective in the short term, we infected 4 different mouse strains with *S. mansoni* and genotyped the resultant adults. The outbred strain CF1 and the inbred strains C57BL/6 and BALB/c were obtained from Charles River Laboratories (Wilmington, Massachusetts). C.129S7(B6)-*Ifng*^{tm1Ts}/J mice, a congenic BALB/c line carrying targeted knockout of the interferon gamma gene (IFNg-KO) were obtained from Jackson Laboratories (Bar Harbor, Maine). CF1 mice present a combined humoral and cellular response to *S. mansoni* infection, while C57BL/6 mice have predominantly cellular responses and humoral responses predominate in BALB/c mice, respectively (James and DeBlois, 1986). The IFNg-KO mutation in C.129S7(B6)-*Ifng*^{tm1Ts}/J mice further suppresses the reduced cellular responses found in the BALB/c strain leaving them even more dependent on humoral responses. All mice were 5–6 week old females and were housed in groups of 5 in filter top cages. All procedures were approved by the Case Western Reserve University Institutional Animal Care and Use Committee.

Biomphalaria glabrata snails infected with *S. mansoni* strain PR-1 were obtained from the Biomedical Research Institute (Rockville, Maryland). For collection of cercariae, patent snails were transferred to a container of water and exposed to bright light for one hour. Cercariae were then concentrated on ice, counted, and used for infections; those not used were sedimented and stored at –20 °C for later DNA extraction.

Fifteen mice of each strain (three replicate groups each consisting of five mice per strain) were infected with 50–70 cercariae to prime the immune response to the parasite. The replicate groups were infected at intervals of 5 days with cercariae collected from the same cohort of infected snails (cercarial Lot A). Snail counts declined over the shedding period with 201, 149, and 82 snails shed for the first, second and third replicate groups, respectively. After 42 days, the mice were treated with praziquantel (Sigma, St. Louis, Missouri) at a dosage of 400 mg/kg administered by oral gavage in a suspension of 100 mg/mL in 7% Tween 80 and 3% alcohol, followed by a second dose 7 days later. One week after the second praziquantel treatment, the mice were reinfected by subcutaneous injection with 250 cercariae from a second lot of PR1 infected snails (cercarial Lot B). For reinfection, 164, 141, and 98 snails were shed for the three replicate groups. The third replicate group of IFNg-KO mice was lost prior to completion of the experiment when the cage was accidentally flooded, leaving only two replicate groups for this strain.

Seven weeks after the second infection, blood samples were collected from each mouse via tail bleeds for serological testing and the following day adult worms were collected via portal vein perfusion and mesenteric dissection. Worms were sexed and counted, and statistical analysis was done using R version 2.7.2 (R Development Core Team, 2008). Average worm counts per mouse (Table 1) did not significantly differ between strains (Welch two-sample t-test $p = 0.5$ for all comparisons). As has been reported previously (Mitchell et al., 1990; Morand et al., 1993), the observed sex ratio of worms was biased toward males. The lower male worm sex ratio observed in the CF1 strain was not significant (Pearson's Chi-squared test $p > 0.1$ for CF1 vs. all other strains). Sera from the five mice in each strain replicate group were pooled, and IgG reactivity against 0.1 µg and 1 µg of soluble worm antigen extract was evaluated by ELISA (King et al., 1996). For all strains, an endpoint titer of 1:125,000 was observed, indicating little difference in humoral immune response between strains.

DNA was extracted from samples using the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA). Prior to extraction, worms from all 5 mice in each replicate group were pooled. PCR and genotyping at 14 microsatellite loci and data analysis to calculate the genetic differentiation index *D* was performed as described previously (Blank et al., 2010). We

compared the degree of differentiation among replicate groups within the same mouse strain as well as between different strains and parasite lots. Allele frequencies were obtained by genotyping the pool of all worms obtained by perfusion. Significant genetic differentiation was not observed between worms collected from replicates within a given mouse strain nor when comparing all worms from a given mouse strain against those from other strains or against cercariae from Lot B, the source for the final parasite infection (Table 2). A small yet significant degree of differentiation was observed between cercarial Lot A and the worms from all strains as well as cercarial Lot B (D range 0.043–0.052). Allelic diversity was also slightly lower in Lot A compared to the other samples (data not shown), suggesting that some degree of bottlenecking may have occurred within the first group of snails. Genotype frequencies for the CWRU strain of *S. mansoni* were included in the analysis and demonstrated a high degree of differentiation consistent with that seen earlier between the CWRU and PR-1 strains (Blank et al., 2010).

No selection was observed between mouse strains, but it is not known whether the schistosome eggs which remain trapped in the liver differ genetically from those which pass through to the intestine and are eventually excreted. A differential distribution of eggs between the liver and the intestine that would effectively lead to selection and misrepresentation of the adult population if only stool eggs were collected. Clearly parasites whose eggs are preferentially retained in the liver are likely to be less competitive and over time would be eliminated from the population. We therefore examined whether egg genotypes were preferentially deposited in liver or intestines of infected animals.

Five CF1 mice were infected with 100 cercariae and 5 more with 200 cercariae, in case worm density influenced the distribution of eggs. Adult worms were perfused from the portal vein 49–54 days post infection, and eggs were isolated from livers and small intestines by selective filtration after homogenization of the tissues (Dresden and Payne, 1981). Eggs from five mice were pooled and counted in a hemocytometer (Table 3). More eggs were observed in the intestine than in the liver, consistent with earlier reports (Incani et al., 2001), although counts were obtained from too few mice for statistical testing. Allele frequencies of worms and eggs from each mouse and of the infecting cercariae were determined as above. Genetic differentiation between cercariae, worms, and eggs was practically nil in all comparisons (Table 4), indicating no differential genotypic localization. It is likely that the genotypes found in the intestine would also be found in the same proportion in the feces and therefore transmitted to subsequent generations, but fecal eggs were not tested. Additionally, we found that egg genotypes in aggregate are representative of adult genotypes. Within this laboratory host no selective pressure was identified at any level.

Although susceptible mammalian host species and intermediate host geographic strains demonstrate differential susceptibility to *S. mansoni* genotypes, we observed no strong selection of parasite genotypes based on mouse strain and mouse immunologic profile. Mouse strain differences have been demonstrated with regard to *S. mansoni* pathology and resistance to reinfection, although our results do not reflect the lower resistance to reinfection shown earlier in C57BL/6 mice (Colley and Freeman, 1980; Dean et al., 1981). A proposed mechanism for resistance, the shunting of schistosomula from the liver back into the venous system, appears to be a result of egg-induced pathology or mouse strain specific structure of hepatic-portal vasculature (reviewed in Wilson, 2009) rather than a specific immunological response to infection.

Since the initial infection of mice with cercarial lot A and treatment with PZQ was intended to induce an immunologic response to maximize differences in survival of parasite genotypes, it is possible that naïve animals would show differences in parasite genotypes based on non-immunologic host factors, such as differing biochemistry or anatomy. The study was

designed to detect strong selection which would be apparent within a single generation, so it is possible that over multiple generations a more subtle degree of selection would become apparent.

Previous studies have reported selection for particular *S. mansoni* alloenzyme alleles when *S. mansoni* populations from baboons (LoVerde et al., 1985) and rats (Bremond et al., 1993) were maintained in laboratory mice. Since at least one of the allozymes selected against may be antigenic in mice and provoke an immune response (Bremond et al., 1993), these studies are not directly relevant to our work with neutral markers. Laboratory mice and rabbits were reported to select for different microsatellite alleles when a field population of *S. japonicum* was transferred into the two species (Shrivastava et al., 2005) based on the number of private alleles found in the mouse and rabbit derived subpopulations, although repeated selection for those same alleles was not demonstrated. Because the PR-1 strain of *S. mansoni* has been maintained as a laboratory population since the 1950s (Lewis et al., 1986), it is possible that the phenotypic diversity upon which immunological selective sweeps could act has been lost due to bottlenecking, random genetic drift, or selection early in the establishment of the line.

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Table 1

Worm counts and sex ratios by mouse strain

Strain	Total Worms ^a	Mice	Mean worms/mouse	M/F ratio ^b
CF1	255	15	17.0	1.87
C57BL/6	199	15	13.3	2.62
BALB/c	230	15	15.3	2.43
IFN γ KO	162	10	16.2	2.60

^aT-test worm counts all comparisons 0.5^bT-test sex ratios CF1 for all comparisons <0.05

Table 2

Pairwise differentiation and 95% confidence intervals of worm and cercariae populations. Inter-strain differentiation values are reported above the diagonal; average within-strain differentiation among replicate groups is reported in boldface.

	CF1	C57/BL6	BALB/c	IFNg-KO	Cerc Lot A	Cerc Lot B	CWRU
CF1	0.005 (0, 0.018)	0 (0, 0.004)	0 (0, 0.005)	0 (0, 0.007)	0.051 (0.043, 0.061)	0.001 (0, 0.003)	0.478 (0.461, 0.494)
C57	0 (0, 0.014)	0 (0, 0.001)	0 (0, 0.006)	0 (0, 0.008)	0.043 (0.033, 0.052)	0.002 (0, 0.006)	0.483 (0.466, 0.499)
BALB		0 (0, 0.014)	0 (0, 0.001)	0 (0, 0.008)	0.051 (0.041, 0.061)	0.001 (0, 0.004)	0.483 (0.467, 0.499)
IFNg-KO			0 (0, 0)	0 (0, 0)	0.043 (0.030, 0.057)	0.001 (0, 0.006)	0.478 (0.459, 0.496)
Cerc Lot A					0.008 (0.005, 0.011)	0.062 (0.060, 0.066)	0.526 (0.511, 0.540)
Cerc Lot B					0.003 (0.002, 0.004)	0.003 (0.002, 0.004)	0.475 (0.465, 0.488)

Table 3

Counts of worm pairs and eggs isolated from the liver and small intestine of CF1 mice.

Cercariae infection dose	Average counts (number of mice counted)		
	Worm pairs	Liver eggs	Intestine eggs
200	56 (n=5)	4000 (n=3)	5500 (n=3)
100	24.8 (n=5)	1000 (n=2)	6000 (n=2)

Table 4

S. mansoni genetic differentiation within CF1 mice.

	<i>D</i> (95% C.I.)		
	Liver eggs	Worms	Cercariae
Intestine eggs vs.	0.000 (0.000-0.000)	0.001 (0.00–0.002)	0.005 (0.004–0.007)
Liver eggs vs.		0.000 (0.000-0.000)	0.005 (0.004–0.007)
Worms vs.			0.005 (0.004–0.006)