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Comparison of Host-Parasite Relationships of *Schistosoma margrebowiei* and *Schistosoma mansoni* in Mice: Pathology

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ABSTRACT

The pathology of mice experimentally infected with *Schistosoma margreboweie* and *S. mansoni* was compared. Pathological changes associated with the eggs were described in the liver, intestine and spleen. The distribution of eggs of both the species within the intestine of the host was found to be very similar, with the majority being located in the intestine and a small number in colon. The rate of hepatic egg accumulation in *S. margrebowiei* was found to differ significantly from the *S. mansoni*. Multiple-egg granuloma size was greater in *S. margrebowiei* than in *S. mansoni* infected livers and egg numbers in multiple-egg granulomas was also greater in *S. margrebowiei*, but single egg granulomas in *S. mansoni* were larger than in *S. margrebowiei*. In acute infected mice, a greater degree of spleno-megaly was induced by *S. margrebowiei*, 25% of infected mice showed multiple-egg granulomas in the spleen; whereas, no eggs granulomas were present in spleens from *S. mansoni* infected animals.

Key Words: *Schistosoma margrebowiei*; *Schistosoma mansoni*; Mice; Pathology

INTRODUCTION

The pathology related to infection with the helminth *S. mansoni* is a product of the host's cellular immune response to parasite eggs and results in characteristic granuloma formation in the liver and intestines (Smithers *et al.,* 1982). Schistosomal egg granulomas consist of well circumscribed aggregates of macrophages, eosinophils, and T and B cells. Egg granuloma formation in the liver results in scar formation, that may lead to portal hypertension, haemorrhage and death. It has been shown that schistosomal granuloma formation represents a delayed-type hypersensitivity (DTH) reaction (Warren *et al.,* 1967) mediated by CD4+ helper T cells sensitized to egg antigens (Phillips *et al.,* 1977).

Hepatic lesions in experimental schistosome infection consist primarily of granulomas around schistosome eggs, portal and diffuse inflammation, focal parenchymal necrosis and fibrosis; the last is principally associated with the granulomas in most host species. The severity of hepatic lesions clearly increases as the number of worm pair increases. The size of granulomas of hepatic fibrosis is readily quantified. This paper reports comparative pathology of host-parasite relationships of *S. margrebowiei* and *S. mansoni* in mice.

MATERIALS AND METHODS

Parasite. A Puerto Rican strain of *S. mansoni* was maintained in albino *Biomphalaria glabrata* snails and random-bred BKTO strain mice (Taylor *et al*., 1969). *S. margrebowiei* (originally obtained from Lochinvar National Park, Zambia) was maintained in the laboratory using *Bulinus natalensis* as intermediate host that had been maintained for many years at University of Wales UK.

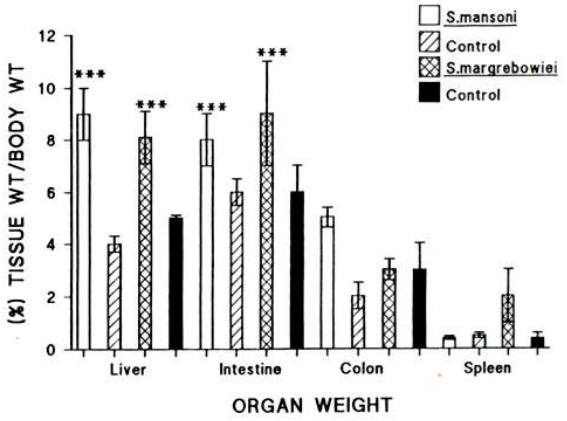
Methods of infecting and perfusing mice. Age-matched male and female mice were anaesthetized and infected with 140 *S. mansoni* or *S. margrebowiei* cercariae percutaneously. The technique employed (Doenhoff *et al.,* 1978a) was adopted from Smithers and Terry (1965). The experiment was terminated when mice were found ill (*S. mansoni* at 49 days or at 59 days *S. margrebowiei* after infection).

Histology. When the experimental mice were killed, the representative samples of liver, spleen, and intestine were taken from all the infected animals for histopathological studies. Organs and tissues were fixed in Heidenhain's Susa, embedded in Historesin, sectioned at 4 µ and stained with polychrome or Haematoxylin and Eosin stain and examined.

RESULTS AND DISCUSSION

Histologically, liver granulomas were found in all autopsied mice of both groups. The newly formed granulomas mostly consisted of one egg (single egg granuloma) or many eggs (multiple egg granulomas) in the center surrounded by variable numbers of eosinophils, some neutrophils, epithelioid cells, plasma cells and lymphocytes. In later stage, a fibrotic tissue reaction occurred and the surrounding liver sinusoids were dilated. Most of the granulomas occupied portal veins of various sizes and provoked an intense perivascular infiltration of eosinophils, plasma cells and lymphocytes. The eggs and resultant lesions were found to cause severe obstruction of portal vessels. In some mice, the main perivascular reaction consisted of a dense lymphocytic infiltration surrounding portal branches from the smallest to the largest. The major cell types present in the granulomas arising from both schistosome infections did not differ from each other, but significant differences were found in the degree of

Fig. 1. Effect of *Schistosoma mansoni* and *S. margrebowiei* infections on the relative weight of tissues in the mouse. ***=p<0.001



organomegaly compared to uninfected controls (Fig.1). This was pronounced in both *S. margrebowiei* and *S. mansoni* infected animals (p<0.001) for both liver and intestine responses, in *S. mansoni* infected animals for the colon and in *S. margrebowiei* for the spleen weight changes in the tissues following infection with either species of schistosome did not differ significantly between the sexes of experimental mice.

No significant differences were found in the proportion of the liver taken up by granulomatous tissue (Table I). The multiple-egg granuloma size was greater in *S. margrebowiei* than in *S. mansoni* livers and egg numbers in multiple-egg granulomas was also greater in *S. margrebowiei* infections (Figs. 2 & 3), but single egg granulomas in *S. mansoni* were larger than those of *S. margrebowiei* (Figs.4 & 5).

Fig. 2. Mouse liver 59 days post-infection with *Schistosoma margrebowiei.* Note the well formed multiple-egg (E) granuloma (G); C= cellular reaction; H= hepatocyte . Stain: Haematoxylin and eosin

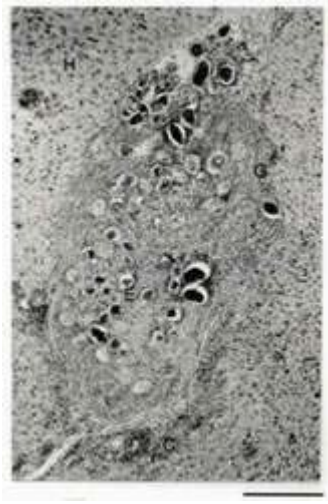
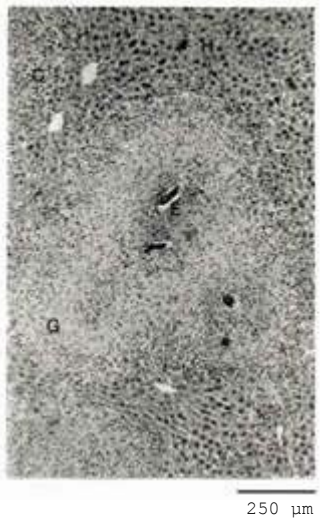


Fig. 3. Mouse liver 49 days post-infection with *Schistosoma mansoni.* Note the well form multiple-egg (E) granuloma (G); C= cellular reaction; H= hepatocyte; Stain: Haematoxylin and eosin



Of particular interest was the greater degree of splenomegaly induced by *S. margrebowiei* infection (Table II). In 25% of mice infected with *S. margrebowiei,* multiple-egg granulomas were found present in spleen (Fig. 6); whereas, no eggs or granulomas were present in the spleens from *S. mansoni*-infected animals (Fig. 7). Although granulomas were absent in 56% of mice infected with *S. mansoni,* yet they developed a marked splenomegaly (Table II). The spleens from *S. mansoni*-infected mice did appear to differ histologically from the normal controls. The spleenic changes consisted of follicular hyperplasia and increased cellularity of the red pulp including lymphocytes,

Table I. Granuloma formation in liver of mice infected with schistosomes

Parasite group	Sex	% area of granulomas ± S.D.
	Female	27.8±15.3
Schistosoma margrebowiei	Male	27.4±14.6
	Average	27.4±14.9
	Female	22.1±13.3
Schistosoma mansoni	Male	25.3±16.2
	Average	23.7±14.8

Table II. Spleen weight change in mice infected with schistosomes

Parasite	No. infected	Splenic enlargement (% of mice affected)		Granulomas present (% of mice)
		Marked	Moderate	
S. margrebowiei	16	43	43	25
S. mansoni	16	56	0	0

Fig. 4. Mouse liver 59 days post-infection with *Schistosoma margrebowiei.* Note the single egg (E) granuloma(G) H=hepatocyte Stain: Haematoxylin and eosin

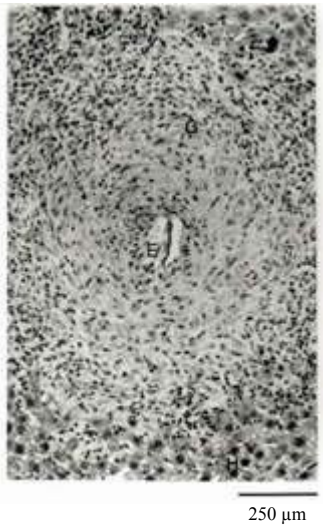


Fig. 6. Mouse spleen 59 days post-infection with *Schistosoma margrebowiei.* Note the well formed multiple-egg (E) granuloma(G). Wp=white pulp; RP=red pulp; T=Trabeculae. Stain: Haematoxylin and eosin

polymorphs. In intestine, the granulomas were most predominant in the outer serosa causing extensive thickening and infiltration of the latter layer. The number and size of granulomas were greater in *S. margrebowiei* (Fig. 8) than in *S. mansoni* infections (Fig. 9). Apart from granulomas, there was usually severe inflammation and desquamation of superficial glands with congestion and eosinophilic infiltration of the mucosa.

The results of the present study demonstrate minor differences in the effects of *S. mansoni* infection on mice as compared with *S. margrebowiei*. The presence of eggs in the tissues, in particular, plays a central role in the evolution of the host's immuno-pathological responses (Boros, 1989; Doenhoff *et al*., 1985) and strongly influences both the development of concomitant immunity (Dean, 1983) and the efficacy of Praziquantel (Doenhoff *et al.,* 1987; Fallon & Doenhoff, 1994).

Histologically, in the liver newly formed granulomas mostly consisted of one or many eggs surrounded by a variable amount of infiltration with eosinophils, some

Fig. 5. Mouse liver 49 days post-infection with *Schistosoma mansoni.* Note the single egg (E) granuloma (G); H= hepatocyte; Stain: Haematoxylin and eosin

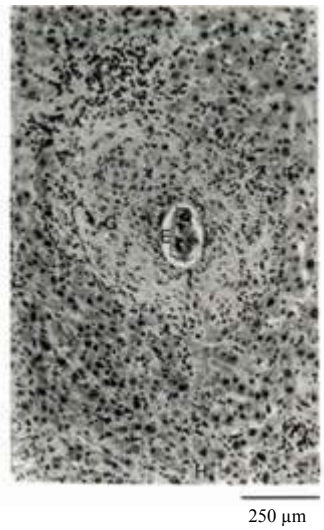


Fig. 7. Mouse spleen 49 days post-infection with *Schistosoma mansoni.* Note the absence of eggs and granulomas; Rp=red pilp; wp= white pulp; MC= megakaryocyte; Stain: Haematoxylin and eosin

Fig. 8. Mouse intestine 59 days post-infection with *Schistosoma margrebwiei.* Note the extensive thickening of the muscle layers due to severe inflammation; G= granuloma; M= muscle layers; V= villi; K= crypt of Lieberkuhn; Stain: Haematoxylin and eosin

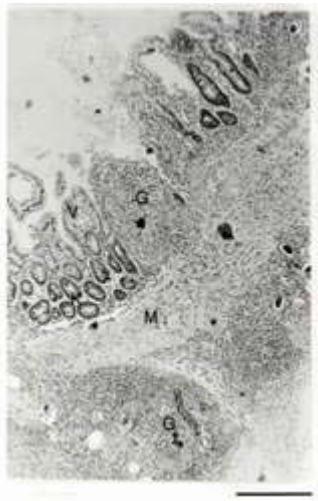


Fig. 9. Mouse intestine 49 days post=infection with *Schistosoma mansoni;* G= granuloma; K= crypt of Lieberkuhn; M= muscle layer; V=villi; Stain: Haematoxylin and eosin

neutrophils, epithelioid cells, plasma cell, and lymphocytes and in later stages a fibrotic reaction and some giant cells were also found, similar results were shown by Fransen *et al*. (1992) in *S. margrebowiei* infected hamsters and mice. Splenomegaly is prominent in both species, but 25% of *S. margrebowiei*-infected mice showed a variable number of eggs, surrounded by a granulomatous reaction (Fransen *et al*., 1992), deposited in the spleen. However, neither eggs nor granulomas were found in the spleen of any *S. mansoni*-infected mice. Fig. 8 clearly shows that eggs are deposited in the spleen of *S. margrebowiei*-infected mice leading to granuloma formation. The presence of giant cells and a large number of eosinophils indicate an immunological response by the infected host. Butterworth *et al*. (1975) have shown that eosinophils are capable of killing schistosomes *in vitro*. The spleen is a large lymphatic organ, which contains a large number of lymphocytes, specialized vascular spaces, a meshwork of reticular cells and reticular fibers and a rich supply of macrophages. The functions credited to the spleen include, lymphocyte production, antibody production, destruction of red blood cells and storage of blood. However, despite the importance of these functions, the spleen is not essential to life and is surgically removed in some conditions.

In the liver, major differences were found in granuloma size. Multiple-egg granuloma size was greater in *S. margrebowiei* than *S. mansoni.* Whereas, single-egg granulomas were larger in *S. mansoni*. Granuloma formation around *S. mansoni*, *S. haematobium* and *S. japonicum* were compared by Warren and Domingo (1970), mean granuloma diameters around *S. haematobium* eggs were smaller than those around *S. mansoni*. The number of eggs in multiple-egg granulomas in the present study was very much higher in *S. margrebowiei* than in *S. mansoni*.

In intestine, the granulomas were most prominent in the outer serosa causing extensive thickening and infiltration of this layer (Fig. 8) but the reaction was greater in *S. margrebowiei* than in *S. mansoni.* This significant difference might be due to the variation in distribution and number of eggs laid by the parasite. In studies of such phenomena, the comparison of schistosome species or strains can be considered valid only when infection characteristics of the parasites (i.e. worm recovery, prepatent period and fecundity) have been taken into account so as to ensure similar duration of tissue egg deposition and similar tissue egg densities.

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