OCCURRENCE OF MYOCARDITIS IN RODENTS INFECTED WITH *SCHISTOSOMA MANSONI*

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Abstract. Myocarditis is a complication of *Schistosoma mansoni* infection, although the literature does not provide much information regarding the frequency of myocarditis. In order to analyze the relationship between myocarditis and *S. mansoni* infection, different laboratory animals were infected with different dose of cercariae. At different weeks of post infection the hearts of infected animals were collected and processed for histopathological examination. Myocarditis was characterized by interstitial inflammatory cell infiltration with or without granuloma. ddY and ICR infected mice showed eosinophilic egg-granuloma in the heart where as neither eosinophil nor egg-granuloma were observed in the heart of infected gerbils. Higher number of eosinophils and greater size of the granuloma were found in the ddY mice than ICR mice. The number of eosinophils was significantly higher in severe myocarditis. Incidence of myocarditis was higher in ddY mice (69% with 100) than ICR mice (35%) and gerbils (23%). The results indicate that ddY mice were more susceptible to *S. mansoni* infection in the development of myocarditis and myocardial severity was associated with greater eosinophil infiltration. These findings suggest that eosinophils might be involved in the development of myocarditis, although the involvement of immunological reaction can not be ruled out.

INTRODUCTION

Myocarditis is defined as a pathological process characterized by an inflammatory infiltrate of the myocardium with necrosis and/or degeneration of adjacent myocytes (Aretz *et al*, 1986). Schistosomiasis characterized clinically by an increase in eosinophil counts in the peripheral blood and pathologically by cell accumulation around the invading parasites or the ova retained in the host tissue (Mahmoud, 1982). The literature does not contain much information to the frequency with which schistosome ova are found in the heart and the effects of such deposition on the myocardium. There are few human cases recorded of such lesions in the myocardium. The first reported case was as "an occasional ovum surrounded by a few inflammatory cells" in the myocardium of both ventricles (Clark and Grafe, 1935) and adult worm in a branch of the coronary artery (Gelfand and Alves, 1959). Kamo *et al* (1970) mentioned that the schistosome infections have influenced on the heart but frequently overlooked. Acute infection by *S. japonicum* egg causes myocardial necrotic abscess, mostly in the left ventricle. On histology revealed myocarditis composed of mostly eosinophils and mononuclear cells and perivascular in distribution (Gelfand and Alves, 1959).

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Genetic background, age, gender and immune status may have influences in the development of myocarditis (Cheever, 1965; Gauntt *et al*, 1988). The purpose of present experiment was to clarify the kinetics of the development of myocarditis in rodents infected with *S. mansoni*.

MATERIALS AND METHODS

Parasites

A Puerto Rican strain of *Schistosoma mansoni* maintained in *Biomphalaria glabrata* snails and Mongolian gerbils, *Meriones unguiculatus*, was used throughout the experiments. Cercariae were used within one hour of being shed.

Animals

In the present study, we used 5-7 weeks old male ddY mice and ICR mice; gerbils of either sex weighing 60-80 g were used at the time of infection. These animals were bred in the Experimental Animal Facility of Hirosaki University School of Medicine. Animals were fed pellets and water *ad libitum*. All animal experiments were performed, according to the Guidelines on Animal Experimentation as set out by Hirosaki University.

Infection

The animals were anesthetized by intraperitoneal injection of 30 mg/kg Nembutal® (pentobarbital sodium; Abbott / Laboratories, North Chicago,

USA). The infections with cercariae were carried out by the ring method of Smithers and Terry (1965). The mean number of cercariae used in each animal was calculated from 6 random aliquots of cercarial suspension. For cercarial penetration, one-hour time was allowed after which the water in the ring was examined for non-penetrating cercariae.

Histopathological study

Animals were killed by anesthetic over dose, at various times post-infection (*pi*) Heart were removed and immediately fixed in 10% neutral buffered formalin. All collected samples were routinely processed, embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin (HE) and/or Mason's trichrome staining. Average 4 sections were made of each heart and were examined microscopically. The number of lesions and their contained cells were counted. In each heart 10 representative lesions, were selected from 4 sections (HE). The number of infiltrated inflammatory cells at each lesion was counted in 10 high power field and the number of eosinophil infiltrated in 0.0156 mm^2 square area in each lesion, delineated with a grid-pattern eye piece, was counted at a magnification x400 and summed up. The sizes of the egg granulomas were measured with the help of an ocular micrometer. Mean sizes of the granuloma were determined from 10 randomly selected granuloma of the representative lesions. Myocarditis was categorized as severe (+++), moderate (++) and mild (+) depending upon the number of inflammatory cells collection in the heart. More than 500 cells, 500 to 200 cells and less than 200 cells were categorized as severe, moderate and mild, respectively.

Statistical analysis

Statistical significance of the results were determined using Student's *t*- test and chi-squared χ^2 test, with $p < 0.05$ as the minimum level of significance acceptable.

RESULTS

ddY mice

ddY mice showed higher incidence (Table 1) of myocarditis, 55% when infected with 50 cercariae and 69% with 100 cercariae. The majority of cases of myocarditis developed between the 10th and 14th weeks. The myocardial injury was typically multifocal, characterized by inflammatory cell infiltration and associated with egg-granuloma (Fig 2A, B). The cells in the egg-granuloma were mostly mononuclear cells and eosinophils (Fig 2C). The numbers of eosinophils were significantly higher in the ddY mice than ICR mice and gerbils (Table 2). Eosinophil infiltration in the myocardial lesions was peaked at 10th week *pi* and there after gradually decreased (Fig 1). Mean size ofthe granuloms was 657±48 μm (mean±SE) in diameter. Some of the egg-granulomas exhibited destroyed eggshells, surrounded by eosinophils and macrophages (Fig 2B). ddY mice infected with 100 cercariae showed a greater number of cases of severe myocarditis than those infected with 50 cercariae (Table 3) and myocardial severity was directly proportional to the extent of eosinophil infiltration (Fig 3).

ICR mice

Incidence of myocarditis was 35% in ICR mice. At 10th week *pi* 6 of 10 ICR mice were positive for myocarditis and the occurrence gradually reduced in successive weeks (Table 1). In contrast to ddY mice, ICR mice showed significantly lower numbers of eosinophils (Table 2) and smaller sized egg granulomas. The size of the granulomas was 471±23 μm (mean±SE) in diameter. Severe myocarditis was not found in the ICR mice (Table 3).

Gerbils

Gerbils infected with 100-cercariae showed 23% with myocarditis (Table 1). In contrast to ddY and ICR mice, gerbils did not show any egg-granulomas in the heart. Severe myocarditis was also not found (Table 3). Most myocarditis cases were mild in form, and were characterized by inflammatory cell infiltration without eosinophils and egg-granulomas. Inflammatory cells were mostly mononuclear cells (Fig 2D) and a few neutrophils.

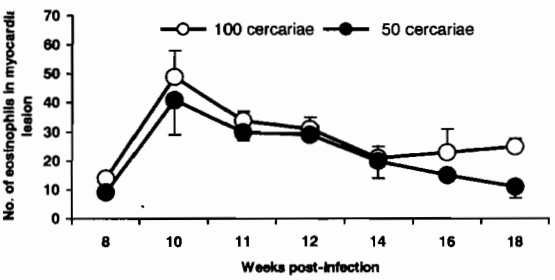

Fig 1–Kinetics of eosinophil infiltration in myocardial lesion of ddY mice infected with *Schistosonna mansoni*. Animals were 5 to 6 at each week as indicated. Each point represents no. of eosinophils (mean±SD) in 0.156 mm^2 area.

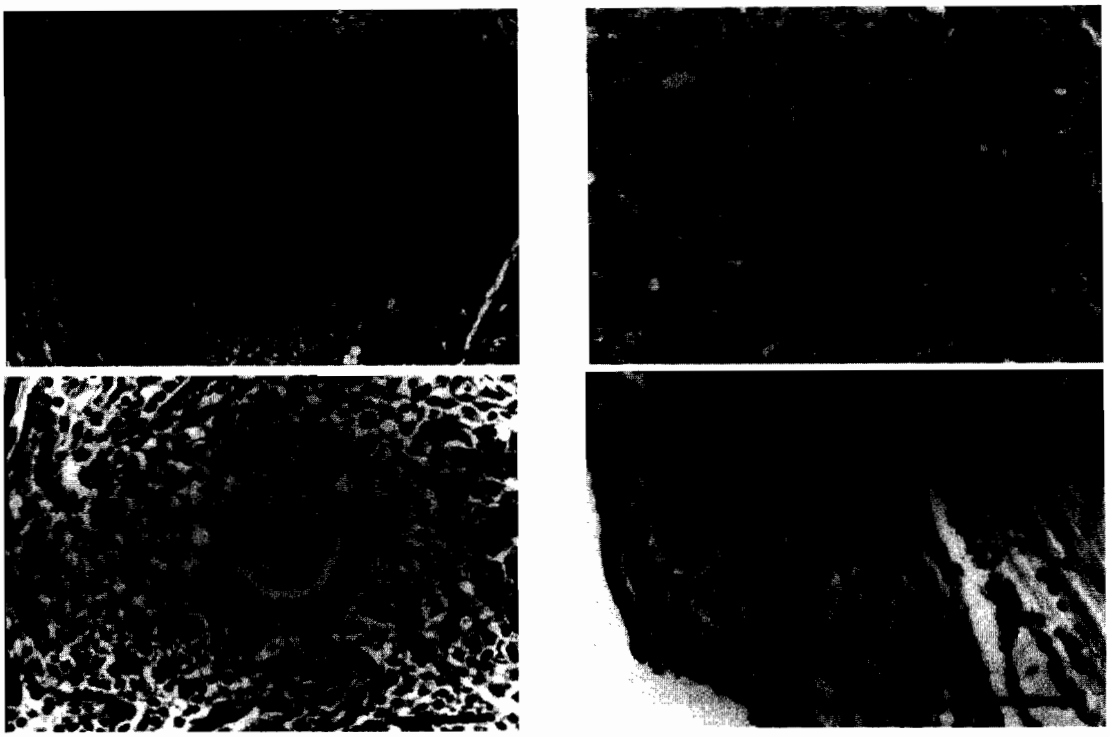

Fig 2–Hematoxylin and eosin staining of myocardial lesions of ddY mice (A,B,C) and gerbils (D) infected with *Schistosoma mansonion* 10th *pi* week. A) Egg-granuloma with cellular infiltration; B) Egg-granuloma showing macrophages and eosinophils are attached to the eggshell of *S. mansoni* (arrow); C) Area adjacent to the egg-granuloma showing eosinophils accumulation; D) Myocarditis without eosinophilic egg-granuloma, endomyocardium showing infiltration of mononuclear cells; bar =50 μm (B,C,D) and 200 μm (A).

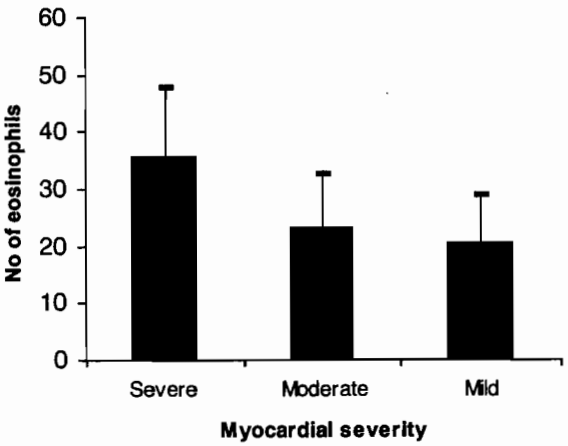

Fig 3–Relationship between myocardial severity and eosinophil infiltration in the myocardial lesion of ddY mice infected with *Schistosoma mansoni*. Total of 47 out of 77 mice showed myocarditis, severe myocarditis, n=17; moderate, n=20 and mild, n=10. Each point represents number of eosinophils (mean±SD) infiltrated in 0.156 mm^2 area. Eosinophil infiltration was evaluated by Student's *t*-test and found to be highly significant ($p < 0.001$) comparing with severe myocarditis.

DISCUSSION

Cardiopulmonary schistosomiasis is considered a complication of hepatoportal disease with collateral circulation (Cheever and Andrade,1967; Andrade and Andrade, 1970; Manson-Bahr, 1982; Watt *et al*, 1985). Eggs bypass the hepatic filter and are carried to lungs and then to the brain (Kamiya *et al*, 1994), where they become embolized. Transfer of schistosomes from the porto-mesenteric system to the lungs starts between 6th and 8th week *pi* followed by a significant increase in the number on subsequent weeks (Ozaki *et al*, 1997), eventually results in pulmonary hypertension and then corpulmonale (Andrade and Andrade, 1970). Thus myocarditis would be expected to occur late in the course of the disease.

In our present study, ddY mice were found to be the most vulnerable for myocarditis of schistosomiasis mansoni. Higher incidence of myocarditis was observed in ddY mice than ICR mice and gerbils. ddY mice infected with 100 cercariae ex-

Table 1
Occurrence of myocarditis in ddY mice, ICR mice and gerbils infected with *Schistosoma mansoni*.

Animal	No. of cercariae infected	Weeks post infection							Total
		8	10	11	12	14	16	18	
ddY mice	50	1/6*	4/6	4/6	4/6	4/6	3/6	3/6	23/42 (55)
ddY mice	100	2/5	4/5	4/5	4/5	4/5	3/5	3/5	24/35 (69)
ICR mice	300	4/10	6/10	N*	3/10	ND	1/10	ND	14/40 (35)
Gerbils	100	1/10	2/10	ND	4/10	3/10	2/10	2/10	14/60 (23)

Occurrence of myocarditis was evaluated by chi-square test (χ^2) and found highly significant ($p < 0.001$) and parenthesis shows the percentage of myocarditis.
*: No. of animal with myocarditis/animal examined; ND = not done

Table 2
Number of eosinophils in the myocardial lesion of ddY, ICR mice and gerbils infected with *Schistosoma mansoni*.

Animal	No. of cercariae infected	Weeks post infection						
		8	10	11	12	14	16	18
ddY mice	50	9	41±12	30±3	29±2	20±6	15±1	11±4
		(1)	(4)	(4)	(4)	(4)	(3)	(3)
ddY mice	100	14±1	49±9	34±3	31±4	21±4	23±8	25±3
		(2)	(4)	(4)	(4)	(4)	(3)	(3)
ICR mice	300	15±1	21±2*	ND	7±2*	ND	7*	ND
		(4)	(6)		(3)		(1)	
Gerbils	100	0*	0*	ND	0*	0*	0*	0*
		(1)	(2)		(4)	(3)	(2)	(2)

Each point represents the numer of eosinophils (mean±SD) infiltrated in 0.0156 mm^2 area in each lesion; a total of 10 representative lesions were counted and summed up; parenthesis shows the number of positive cases of myocarditis examined for eosinophil; ND = not done.
*: Infiltration of eosinophils was evaluated by Student's *t*-test and found highly significant, where $p<0.01$, comparing with both 50 and 100 cercariae infected ddY mice.

hibited higher occurrence of myocarditis than those infected with 50 carcariae (Table 1). The myocarditic lesions of the ddY and ICR mice were associated with eosinophil infiltration and egg-granulomas but gerbils did not show any egg-granulomas and/or eosinophils. It is to be noted that occurrence of myocarditis in ddY mice peaked between the 10th and 14th week *pi* (Table 1). Myocarditis in mice was due to entrapment of schistosome ova in the myocardium while passing through the coronary vessels. There are few cases recorded of such lesions in the myocardium. Kamo *et al* (1970) mentioned that *Schistosoma* have an influence on the heart but this is frequently overlooked. Bertand *et al* (1978) showed myocarditis among 3 of 37 patients (8.1%) without pulmonary hypertension. Higher prevalence of egg associated lesions in the brain was observed in BALB/c mice, ddY mice and ICR mice. In contrast to the mice, almost no eggs were detected in the brain vessels of infected gerbils, though many eggs were embolized in the lungs (Kamiya *et al*, 1994; Ozaki *et al*, 1997). In gerbils, the reason why schistosome ova do not pass to the heart and brain is not clear. Different vascular systems or alterations in the portal vasculature might facilitate egg transportation from the liver or the lungs to the brain vessels (Mitchell, 1989). Portal-systemic collateral veins were larger in BALB/c mice than B6 mice (Cheever *et al*, 1994). Genetic background and immune status of the mouse and other hosts may have influence on the development of myocarditis (Cheever, 1965; Gauntt *et al*, 1988).

In our results, ddY mice exhibited larger sized egg-granulomas in comparison to ICR mice, whereas gerbils showed no such egg-granulomas. Hsu *et al*

Table 3
Myocardial severity in ddY, ICR mice and gerbils infected with *Schistosoma mansoni*.

Animal	No. of cercariae infected	Severe	Moderate	Mild
ddY mice	50	4/23 * (17)	10/23 (44)	9/23 (39)
ddY mice	100	13/24 (54)	10/24 (42)	1/24 (4)
ICR mice	300	Nil	6/14 (43)	8/14 (57)
Gerbils	100	Nil	5/14 (36)	9/14 (64)

*: No of occurrence/examined positive myocarditis; parenthesis shows the percentage of occurrence. Myocarditis was categorized as severe, moderate and mild depending upon the number of inflammatory cell infiltration in the heart. More than 500 cells, 500 to 200 cells and less than 200 cells were categorized as severe, moderate and mild, respectively.

(1973) reported that granulomata in the permissive hosts were larger than that in non-permissive hosts but contrasting findings have also been reported (Akpom *et al*, 1970). Yingrui *et al* (1983) mentioned smaller size liver granulomas in gerbils but the granuloma cells showed more well-developed mitochondria, golgi apparatus, endoplasmic reticulum and lysosomes than the cells of mice granulomas. Myocarditis associated with immunological reaction also has been reported (Bertrand *et al*, 1978). A characteristic feature associated with the formation of egg-granuloma was the type of cells recruited to the site of egg. Our results demonstrate that substantial proportions of eosinophils were present in the myocarditic lesion (Table 2) and their concentrations were significantly ($p < 0.01$) higher in severe myocarditis (Fig 3). Eosinophils and their toxic granules may play a role in either causation or aggravation of the heart lesion. It has been shown that eosinophil granules are toxic to mammalian cells, including heart cells (Gleich and Adolphson, 1986; Young *et al*, 1986; Molina *et al*, 1988) and eosinophils can also destroy intact eggs of *S. mansoni in vitro* (James and Colley, 1976). Deposition of a major basic protein has been correlated to tissue damage in the heart (Tai *et al*, 1987) and an immuno-histochemical study of the eosinophilic cationic proteins (ECP) showed a strong correlation between the ECP and tissue necrosis in all organs (Fredens *et al*, 1988). Thus a role of eosinophils in host defense is plausible. Eosinophill recruited for the purpose of killing parasites and/or removing debris could cause additional myocardial damage.

It is interesting to note that neither eggs nor eosinophils were found in the myocardial lesions of any infected gerbils in the present study, although myocarditis was also observed. Most myocarditis was mild in form and composed mostly mononuclear cells and a few neutrophils. Our findings support the speculation that the development of myocarditis in gerbils might be immunologically mediated, since pulmonary arteritis is a tissue immunological reaction and myocarditis is also a manifestation of circulating antibody (Bertrand *et al*, 1978). These findings suggest a role of eosinophils in pathological development of myocarditis, although the involvement of immunological reactions against circulating antigens cannot be ruled out. The present result indicates that both murine and gerbil models of schistosomiasis will be useful for investigating further the etiology, pathogenesis and treatment of myocarditis.

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