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Brain Involvement in Murine Schistosomiasis Mansoni

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Abstract

Brain involvement in murine schistosomiasis mansoni was investigated. One hundred and fifty-seven (52%) of 300 male ddY mice infected with *Schistosoma mansoni* showed schistosome egg-embolization in their brain from 6 to 14 weeks post-infection. Distribution of those eggs in the brain was not significantly different among 3 divided brain portions; the right, the left half-cerebrum and the cerebellum. However, the number of eggs detected in the brains of infected mice was correlated well with parasite burdens and also with the number of eggs recovered from the lungs. Furthermore, the different infection routes such as percutaneous infection, subcutaneous or intraperitoneal injection of cercariae did not significantly influence the frequency on cerebral egg-embolization. No fluke was detected in the brains of infected animals. Infected ICR male mice also showed frequent occurrence of cerebral egg-embolization at 9 weeks post-infection, but no egg was found in the brains of jirds, *Meriones unguiculatus*, from 8 to 12 weeks post-infection. The aetiology of cerebral schistosomiasis mansoni was discussed with special reference to hepatosplenic form of the disease.

Key words: *Schistosoma mansoni*, brain involvement, mouse, *Meriones unguiculatus*

Intracerebral granuloma caused by *Schistosoma japonicum* ova in a patient with Jacksonian epilepsy was first described by Yamagiwa (1890), who is also known for the first production of artificial carcinoma by rubbing coal-tar on rabbit ears. Since then, many papers on the various aspects of cerebral schistosomiasis have been published (Kane and Most, 1948; Chang *et al.*, 1957; Hayashi *et al.*, 1977, 1987; Bird, 1978; Pittella and Lana-Peixoto, 1981; Zhang and Jiang, 1985; Scrimgeour and Gajdusek, 1985; Chen and Mott, 1988a, b; Ariizumi, 1991), but the frequency and aetiology of the disease remain obscure. Thus, investigation of the brain involvement in animals experimentally infected with *Schistosoma* spp., may provide some clues to understand the features of cerebral schistosomiasis. The present paper provides the information on these issues, which are responsible for the morbidity of schistosomiasis patients, with special reference to their relationship to the schistosome egg-embolization in the brain.

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Materials and Methods

Parasite

A Puerto Rican strain of *Schistosoma mansoni* maintained in *Biomphalaria glabrata* and ICR mice was used throughout the experiments.

Animals and infection

Five-week-old male ddY and male ICR mice were purchased from a local animal breeder (Funabashi Experimental Animal Farm, Chiba, Japan). Male jirds, *Meriones unguiculatus*, 50 to 60 g of body weight at the time of infection, bred in the Institute for Animal Experiment, Hirosaki University School of Medicine, were used. Percutaneous infection was done by the ring method (Smithers and Terry, 1965). Intraperitoneal and subcutaneous injection of cercariae were also carried out to assess the possibility of whether the different routes of infection might cause a difference in aetiology or frequency of cerebral schistosomiasis. Animals were

fed food pellets and water *ad libitum*.

Detection of eggs in brain tissues

Infected animals were sacrificed under ether anaesthesia. The brain was carefully removed from the cranium and then divided into three portions; the right, the left half-cerebrum with respective rhinencephalon and the cerebellal portions. They were then compressed between two slide glasses and then the number of eggs detected in the brain tissues was counted under a light microscope.

Pulmonary egg count

Lungs of infected animals were excised and digested with 4% KOH in PBS (pH 7.2) and the number of eggs in lungs was counted (Cheever *et al.*, 1984).

Parasite recovery

The adult schistosome worms were recovered by means of retrograde portal perfusion (Smithers and Terry, 1965). To check for any remaining worms, the liver and mesentery of each perfused animals were compressed under a dissecting microscope.

Pathological findings

Brain tissue sections were processed by routine tissue sectioning procedure and stained with haematoxylin-eosin.

Statistical analysis

Statistical significance of the results was calculated using Student's *t*-test, with $P<0.05$ as the minimal level of significance acceptable.

Results

Frequency of egg-emboli in brains of ddY mice infected with S. mansoni

(1) Gross observation: Throughout the experiments, infected mice occasionally exhibited the psychomotor disorders, such as neck-stiffness or circling movement. Fresh haemorrhagic or granulomatous foci due to the egg-emboli were seen in the brains under leptomeningial membrane of infected mice 6 to 14 weeks p.i. (Fig. 1A).

(2) Prevalence: Out of 300 infected ddY mice in 3 experiments, 157 (52%) mice had egg-emboli in the cerebral blood vessels. The range of the frequency was from 42–80% (Table 1). As shown in Table 1, the mice infected percutaneously with 500 cercariae exhibited the highest occurrence of egg-emboli, but infectous routes did not distinctly affect the occurrence of egg-emboli in the brains of infected mice. Even though 69% of the mice infected with 250 or 500 cercariae were positive for eggs in the brain at more early stage of infection of 47 to 55 days (6–7 weeks) p.i. (Table 1; E-2), no adult worm was seen in the brain tissues of the infected mice. By histopathological examination of egg-associated cerebral lesions (EACLs) in mice infected with 50 or 100 cercariae from 8 to 14 weeks p.i., mature and immature eggs were seen in the blood vessels of cortex, medulla or leptomeninges. These egg-emboli were frequently recognized at the junction of brain vessels, resulting in large granulomatous lesions (Fig. 1B). Haemorrhagic (Fig. 1C) and extensive eosinophil infiltration in egg granuloma were the characteristic feature (Fig. 1D). As shown in Table 2, at 8 weeks p.i., only one mouse infected with 100 carcariae was positive for EACLs. However, the prevalence of EACLs distinctly increased at 10 weeks p.i. and the mean number of eggs in the brain was significantly larger in the mice infected with 100 cercariae than those in mice infected with 50 cercariae.

(3) Distribution of eggs in brain tissues: There was no distinct difference in the distribution of eggs among the three divided portions; namely the right and left halves of the cerebrum, and the cerebellum of mice with EACLs. However, more frequent EACLs in the cerebellum were observed as compared to the cerebrum (data not shown).

(4) Egg-emboli in lungs of infected mice: Mean number of eggs in the lungs of mice with EACLs, infected with either 50 or 250–500 cercariae, was significantly higher than those without EACLs (Table 3). These findings suggest that the number of eggs transmitted from hepatic portal vein to lung blood vessels may have significant correlationship with the occurrence of EACLs in infected mice.

(5) Worm burdens and EACLs: It was shown that the occurrence of EACLs depended on the number of adult pairs present (Table 4). As the worm burdens increased, the frequency of egg-emboli also increased. Mean number (37 ± 2) of adult pairs/mouse

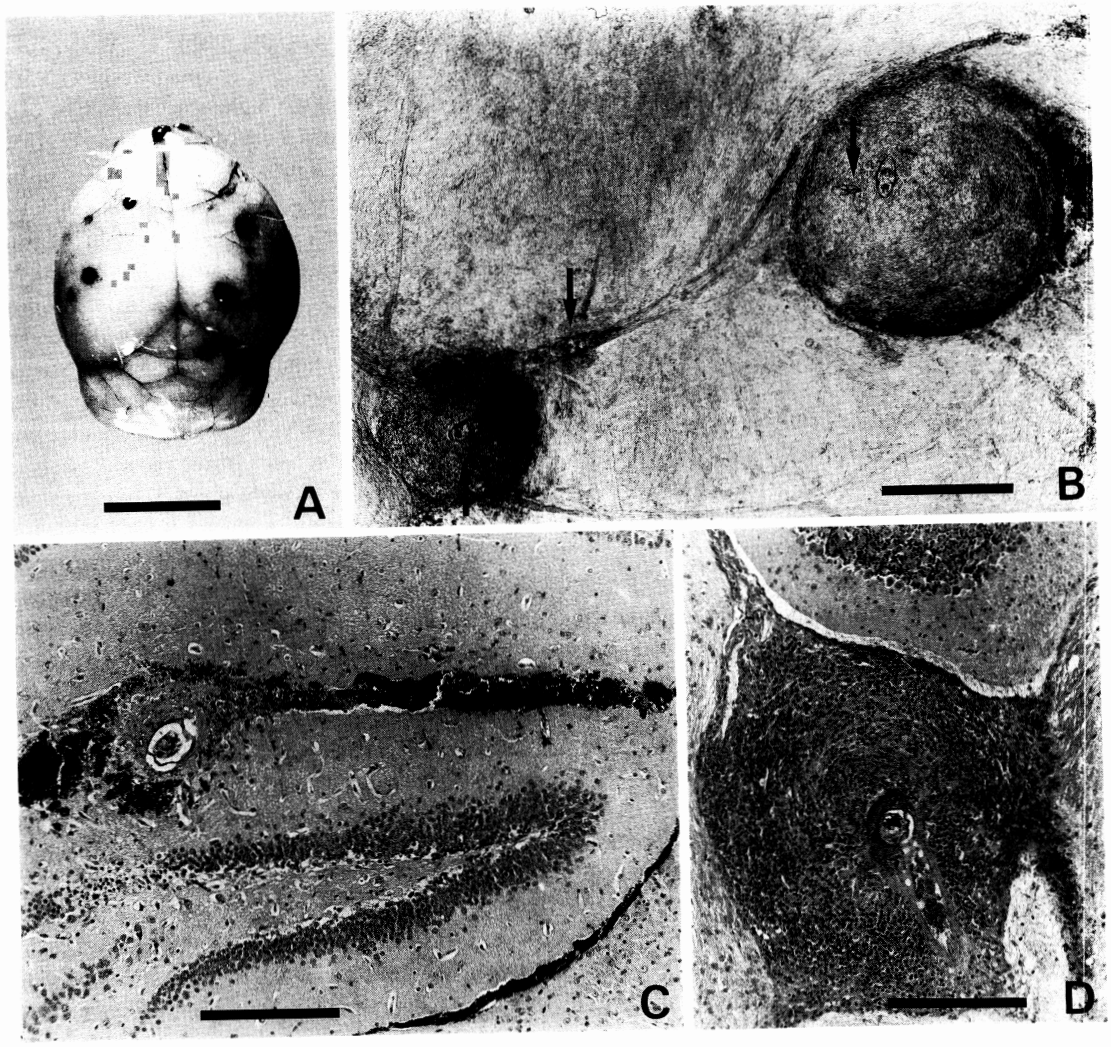

Fig. 1 shows the egg-associated cerebral lesions of ddY mice infected with *Schistosoma mansoni*. (A) Gross observation of a brain with many schistosome egg-associated lesions, 10 weeks p.i. Scale bar=5 mm. (B) Granuloma formation around mature eggs and egg-shell (↑) embolized at the junction of cerebral vessels, 13 weeks p.i. Bar=0.5 mm. (C) Newly formed egg-associated lesions with haemorrhagie in hippocampus, 7 weeks p.i. Bar=0.2 mm. (D) An egg embolized in vessel of celleberum, with extensive eosinophil accumulation, 10 weeks p.i. Bar=0.2 mm.

with EACLs was also significantly higher than those (26±2) without EACLs ($P<0.001$).

Egg-emboli in brains and lungs of ICR mice and jirds

Since the infected ddY mice showed high prevalence of EACLs, we investigated whether this phenomenon was in ICR mice. All of the ICR mice, however, showed EACLs at 9 weeks p.i. and a large number of eggs in lungs was also recorded. In contrast to the ICR mice, all of the 39 infected jirds, 8 to 12 weeks p.i., did not have any EACLs, though many eggs were seen in the lungs of these animals (Table 5).

Table 1 Frequency of egg deposition in brains of male ddY mice infected with *Schistosoma mansoni*

Experiment number	Infection routes	Weeks post-infection	Number of cercariae infected	Frequency of egg deposition
E-1	Percutaneous (PC)	8~14	50	12/25 (48)*
	PC	8~14	100	19/24 (79)
E-2	PC	6~7	250	17/26 (65)
	PC	6~7	500	12/15 (80)
	Intraperitoneal (IP)	6~7	500	12/17 (71)
	Subcutaneous (SC)	6~7	500	10/16 (63)
E-3	IP, SC	8~13	250~500	75/177 (42)
Total		–	–	157/300 (52)

*: Number of mice with deposited eggs in brain/no. examined mice (%).

Table 2 Number of eggs deposited in brains of male ddY mice infected with *Schistosoma mansoni**

Weeks post-infection	Mean number of eggs ± S.D. detected in brains of mice infected with	
	50 cercariae	100 cercariae
8	0 (0/5)	5 (1/5)†
10	10±4 (4/5)	58±32 (5/5)‡
11	16±15 (2/5)	67±37 (5/5)‡
13	19±10 (5/5)	58±22 (4/4)‡
14	16 (1/5)	31±16 (4/5)‡
Mean±S.D.	13±4 (12/25)	48±13 (19/24)‡

*: Data were deduced from Experiment 1 of Table 1.
†: Number of mice with eggs in brain/no. examined mice in parentheses.
‡: $P<0.05$

Discussion

The aetiology of cerebral lesions in schistosomiasis has not been well elucidated and still remains controversial. The prevalence of cerebral schistosomiasis due to egg deposition has been reported to be low ranging between 2–4% in the patients with schistosomiasis japonica (Kane and Most, 1948; Hayashi *et al.*, 1987; Ariizumi, 1991) and also in schistosomiasis mansoni (Chen and Mott, 1988b), despite many case reports on the involvement of central nervous system (Chen and Mott, 1988a, b; Ariizumi, 1991). Although establishment of experimental animal model of cerebral schistosomiasis has been reported to be difficult (Hayashi, 1985; Jane *et al.*, 1970), it is surprising that the occurrence of the egg-associated cerebral

Table 3 Deposition of eggs in lungs of *Schistosoma mansoni* infected male ddY mice with and without the eggs in the brain*

Experiment number	Number of cercariae infected	Weeks post-infection	Number of eggs in lungs of mice±S.D.	
			with eggs in brains(n)	without eggs in brains(n)
E-1	50	8~14	610±170 (12)†	170±70 (13)
E-2	250~500	6~7	1280±390 (51)†	460±100 (23)

*: Data were deduced from Experiment 1 and 2, in Table 1. Parentheses show the number of mice examined.
†: $P<0.05$

Table 4 Relationship between the number of paired flukes and occurrence of egg deposition in brains of ddY mice infected with *Schistosoma mansoni**

Number of adult pairs recovered	Number of mice examined	Number of mice with eggs in brains	Percentage
1~10	30	5	17
11~20	31	12	39
21~30	34	13	38
31~40	29	14	48
41~50	24	14	58
51~	29	17	59
Total	177	75	42

*: Data were deduced from Experiment 3 of Table 1. Mice were infected subcutaneously or intraperitoneally with 250~500 cercariae 8 to 13 weeks previously.

Table 5. Number of eggs in brains and lungs of male jirds and ICR mice infected percutaneously with *Schistosoma mansoni*

Animal groups	Number of cercariae infected	Weeks post-infection	Number of animals with eggs in brain /no. examined	Number of parasites recovered	Number of eggs in brain /animal	Number of eggs in lungs /animal
Jirds	200	8	0/15	n.d.	0	2150±720*
		9	0/ 6	n.d.	0	2290±1050
	80	9~12	0/18	n.d.	0	4120±2570
ICR mice	300	9	6/ 6	78±7	29±16	1370±740

*: Data were expressed as mean±S.D.
n.d.: not done.

lesions (EACLs) in infected male ddY mice was as high as 52% in the present experiments. The development of animal models will therefore contribute to study of the pathogenesis, aetiology and treatment of cerebral schistosomiasis.

The aetiology of cerebral schistosomiasis due to the 3 major human-infecting species of *Schistosoma* is not the same; central nervous system involvement in schistosomiasis haematobia and mansoni has been suggested to depend on egg-emboli through the vertebral plexus vein of Batson (1940), because of high prevalence of egg-emboli in the spinal cord rather than in the brain tissues (Chen and Mott, 1988b). However, relatively high prevalence of cerebral schistosomiasis mansoni was recently reported in patients with hepatosplenic schistosomiasis (Pittella and Lana-Peixoto, 1981). Also the prevalence of EACLs corresponded well with the occurrence of cor pulmonalae in those patients (Scrimgeour and Gajdusek, 1985). Our results showed that the mean number of eggs in the lungs was significantly higher in mice with EACLs than those without (Table 3), and this supports the observation reported for human.

The number of pulmonary eggs corresponded proportionately with the worm burdens in the infected mice (data not shown). More flukes in infected mice produced significantly more egg-em-

boli in brains (Table 4). Therefore, we suggested that there was a correlation among worm burdens, number of eggs in lungs and the occurrence of EACLs (Table 2, 3, 4). Since *S. mansoni* worms start to deposit eggs in the portal and mesenteric vein at 5–6 weeks p.i., it may be supposed that in mice with heavy worm burdens, high prevalence of EACLs occur even before 8 weeks p.i. This supposition was also supported by the fact that 51 of 74 (69%) heavily infected mice showed the cerebral egg-emboli at 6–7 weeks p.i. (Table 1). Thus hepatosplenomegaly may not be correlated with egg deposition in the brains.

On the other hand, many eggs deposited in the hepatic portal vein in due course of infection resulting in egg granulomas which causes hepatic fibrosis and then splenomegaly followed by the development of the hepatosplenic schistosomiasis. This pathology leads to the retention of the portal blood flow, and hypertension of the hepatic blood pressure (Bird, 1978; Chen and Mott, 1988a, b), which in turn may result in the frequent establishment of collateral blood circulation and artero-venous shunts in the liver as well. These physiological and pathological changes induces the passive transfer of eggs into lungs and then into the systemic circulation which favour egg-transportation into the brain, thus resulting in the cerebral schistosomiasis. This speculation may be supported by the issues which wild rats of Leyte, Philippines, infected with *S. japonicum* also showed the frequent egg-emboli in the systemic, non-splanchnic organs, such as spleens and kidneys (Kamiya *et al.*, 1980).

Another possible route through which the eggs can have access to the brain is the peculiarity of the blood circulation of the lungs. In the lungs, pecurial vesicular circulation between veins and arteries has been described (Kasai, 1989). This indicates that eggs deposited in hepatic portal veins may successfully reach the lung-vascularity via newly established blood circulation, such as the hepatic portal-venous shunts caused by the egg-induced granulomas. Eggs might then be easily transferred into the pulmonary vein from the artery through bronchial veins and will get into the brain tissues through the systemic blood circulation.

It is also noteworthy that no fluke was detected in the brains of all the infected animals, irrespective of cercarial dose, infection routes and infection period. Although flukes may sometimes arrive successfully at the brain, produce eggs *in situ* and then causes cerebral schistosomiasis (Chen and Mott, 1988a, b), the present findings indicate that this aetiology may be rare in murine schistosomiasis mansoni.

The prevalence of cerebral egg-emboli in autopsy finding varied from 4% (Alves, 1958) to 26% (Pittella and Lana-Peixoto, 1981) in schistosomiasis mansoni patients. However, some of these patients were asymptomatic in neurological dysfunction. This finding conforms well with our results in that most of the infected mice did not show symptoms of psychomortor disorders, except in some cases which showed circling movement or neck-stiffness, despite that more than half of the infected mice had egg-emboli in the brains (Table 1). On the other hand, a variety of the neurological symptoms including headache, confusion and epilepsy has been attributed to cerebral ova deposition in the schistosomiasis japonica (Kane and Most, 1948; Chang *et al.*, 1957; Hayashi *et al.*, 1977, 1987; Ariizumi, 1991), and schistosomiasis mansoni patients (Bird, 1978; Pittella and Lana-Peixoto, 1981; Scrimgeour and Gajdusek, 1985).

Our results showed that frequency of egg distribution in the brain did not significantly differ in the right, the left half-cerebrum and the cerebellum portions, but in human cases egg-emboli was reported to be more frequent in the left half of the cerebrum (Chang *et al.*, 1957). Recently, Zhang *et al.* (1985) reported 11 cases of successful surgical operation involving cerebellum schistosomiasis japonica.

It is interesting that no egg was detected in the brains of all infected jirds in the present study (Table 5), though many eggs embolized in the lung-vasculature of those animals. This may indicate the possibility that the different host species, host strains or host different immunogenetic factors, such as HLA-linked genes might possess different vasculature system or aetiology which facilitate the egg transportation into the systemic circulation from portal or pulmonary vascular system (Mitchell, 1989; Ohta *et al.*, 1987). Unlike jirds, ICR mice showed high prevalence of cerebral egg-emboli as the ddY mice (Table 5). Both of ddY and ICR mice, but not the jirds, exhibited the distinct hepatosplenic form of

schistosomiasis mansoni. However, severe combined immunodeficiency mice (CB-17/SCID), infected with 200 cercariae of *S. mansoni* 6 to 8 weeks p.i., did not show any egg-emboli in the brain tissues (data not shown). Those did not show any hepatosplenic form of the disease. Moreover, it has been reported that egg granuloma formation is suppressed in SCID mice infected with *S. mansoni* (Amiri *et al.*, 1992). Our findings support the speculation that the development of hepatosplenic schistosomiasis might partially favour the frequent occurrence of cerebral egg-emboli in chronically infected animals.

The present results indicate that the murine model of schistosomiasis will be useful for investigating the aetiology, pathogenesis and treatment of cerebral schistosomiasis mansoni.

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