SCHISTOSOMA MANSONI: RELOCATION OF PARASITES TO LUNGS FROM HEPATIC PORTAL SYSTEM IN RODENTS

Toshihiro Ozaki, Takashi Inaba, Hiroshi Sato, Mahfuza Nargis, Moinuddin Chisty and Haruo Kamiya

Department of Parasitology, Hirosaki University School of Medicine, Hirosaki 036, Aomori, Japan

Abstract. The prevalence and development of adult worms in the lungs of mice and gerbils infected with *Schistosoma mansoni* was investigated. All infected BALB/c mice harbored the schistosomes in their lungs at 10-12 weeks post-infection, showing the distinct relocation of adult worms to the lungs. from the hepatic portal system. The male and female flukes from lungs of BALB/c mice were significantly smaller than those from livers. The percentage of gravid females in lungs was considerably lower than that in the livers. The number of eggs recovered from lungs of BALB/c mice and gerbils having lung female worms, however, was higher than that from animals without lung females, indicating egg deposition of lung females. The number of eggs detected in the brains correlated well with the number of eggs from the lungs in BALB/c and ICR mice. Out of 119 infected gerbils at 8 weeks post-infection, only two animals had egg-emboli in the brain vessels, although many eggs embolized in the lungs of those animals. These data suggest that transfer of worms to the lungs from livers involves reduction of worm recovery from the portal circulation, and also pulmonary pathology of the disease.

INTRODUCTION

In mammalian hosts, the migration of *Schistosoma mansoni* or *S. japonicum* schistosomula in the circulating system usually terminates at the hepatic portal system. However, many reports on the occurrence of adult lung worms of these helminth parasites in laboratory animals or natural hosts have been published (Moore *et al*, 1949; Hewitt and Gill, 1960; Lancastre *et al*, 1976; Oshima *et al*, 1978; Kamiya *et al*, 1980; Elsaghier *et al*, 1989; Elsaghier and McLaren, 1989; Imbert-Establet and Combes, 1992; Theron *et al*, 1992; Cheever *et al*, 1994). In spite of these papers, the detailed kinetics of adult *S. mansoni* lung worms in mice and Mongolian gerbils, *Meriones unguiculatus*, is not obvious. Thus, investigation of the prevalence and development of *S. mansoni* lung worms in these rodent hosts may provide important clues to the mechanisms involved in the migration of parasites and the nature of pulmonary schistosomiasis. Additionally, the present study could also provide some complementary information concerning the problems between the pulmonary involvement in schistosomiasis mansoni and resistance referred to "concomitant immunity".

Correspondence: Dr Haruo Kamiya, Fax: 81-172-39-5045.

MATERIALS AND METHODS

Parasites

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

Animals

In the present study, 8-10 week-old BALB/c mice and ICR mice, gerbils weighing 60-80 g were used at the time of infection. These animals were bred in the Institute for Animal Experimentation, Hirosaki University School of Medicine. Animals were fed food pellets (CE-2, CLEA, Japan) and water *ad libitum*. All animal experiments in this paper followed the Guidelines for Animal Experimentation of Hirosaki University.

Infection of animals

All animals were anesthetized by intraperitoneal injection of 30 mg/kg Nembutal® (pentobarbital sodium; Abbott Laboratories, North Chicago, USA). And the infection with cercariae were carried out by

the "ring method" of Smithers and Terry (1965). The mean number of infecting cercariae was calculated from 6 random aliquots of larval suspensions. One hour was allowed for cercarial penetration, after which the water in the ring was examined for nonpenetrating cercariae.

Recovery of parasites

Animals were killed by an anesthetic overdose, at various times post-infection. The vena cava and periesophageal veins were ligated just above the diaphragm, to avoid the possibility that some worms might be forced into the pulmonary circulation. After removing the livers, mesenteries and lungs of all animals, these organs were compressed between glass plates and examined for the parasites under a dissecting microscope. The worms found in those organs were collected with tweezers carefully. Soon after the recovery, the worms were fixed in the boiled Lillie's buffered 10% formalin solution.

Measurements of worm length

Worm length was recorded according to Ozaki and Kamiya (1993). The body profile of fixed schistosomes was traced using a Universal Projector, UP-350 (Olympus, Tokyo), and their length were calculated with Comcurve-5 (Digital curvemeter, Koizumi, Tokyo).

Egg counts in organs

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). Detection of eggs in brain tissues of those animals was done by the method (Kamiya *et al*, 1994). 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 cerebellar portions. They were then compressed between two slide glasses and then the number of eggs detected in the brain tissues was counted under a microscope.

Statistical analysis

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

RESULTS

Prevalence of adult schistosomes in lungs of mice and gerbils infected with *S. mansoni*

BALB/c mice: At 6 weeks post-infection, few adult worms in the lungs of infected mice were detected, but approximately half number of mice had lung worms at 8 weeks. Thereafter all mice harbored schistosomes in the lungs (Table 1). Additionally, at 8 weeks post-infection, the mean number of adult worms in lungs ± SE was 2 ± 1, but thereafter significantly increased (Table 2). As shown in Fig 1, the percentage of mean worm recovery from lungs and livers was examined at 6-12 weeks post-infection. It should be noted that the sum of the mean percentages from these two organs were almost identical (approximately 30% of challenge parasites), throughout the present study.

Table 1

Occurrence of adult lung worms in mice and gerbils infected with *Schistosoma mansoni*.

Animal	No. of cercariae infected	Weeks post-infection				
		6	8	10	12	Total
BALB/c mice	210	1/31*	14/31	21/21	16/16	52/99
ICR mice	200	2/16	1/16	2/6	4/10	9/48
Gerbils	100	nd	8/34	11/34	2/9	21/77
Gerbils	190	2/5	36/61	3/4	nd	41/70

* : No. of animals with adult worms in lung/examined.
nd : not done.

Table 2

Mean number of adult worms in lungs of mice and gerbils infected with *Schistosoma mansoni.*

Animal	No. of cercariae infected	Weeks post-infection			
		6	8	10	12
BALB/c mice	210	0	2 ± 1	11 ± 2	19 ± 3
ICR mice	200	0	0	2 ± 1**	2 ± 1**
Gerbils	100	nd	1 ± 1	1 ± 0**	0**
Gerbils	190	1 ± 1	4 ± 1	3 ± 2*	nd

Mean number of animals examined was 21 at each week indicated.
Each point represents the mean ± SE of values.
* : $p < 0.02$; ** : $p < 0.001$, comparing worm numbers from BALB/c mice and the others.
nd : not done.

ICR mice: At 6 weeks post-infection, 2 of 16 ICR mice were positive for lung worms, but the occurrence of lung worms was considerably lower than that of BALB/c mice or gerbils infected with 190 cercariae (Table 1). In contrast to BALB/c mice, ICR mice did not show the distinct relocation of adult worms to the lungs from the hepatic portal system (Table 2).

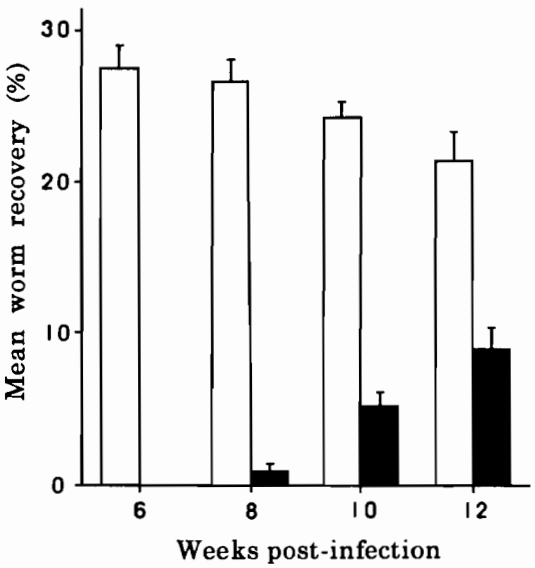

Fig 1–Percentage of mean worm recovery from the lungs and livers of BALB/c mice infected with *Schistosoma mansoni.*
Mice were exposed to 210 cercariae. Animals used were 16~31 in number, at each week indicated. Each point represents the mean ± SE of values. (■), Worms recovered from lungs; (□), worms recovered from livers.

Gerbils: Gerbils infected with 190 cercariae exhibited a higher occurrence and a greater number of lung worms than that of the infected with 100 cercariae (Tables 1, 2). It was indicated that the prevalence of lung worms depended on the number of cercariae infected.

Development of worms

Worm length: The body length of flukes recovered from lungs and livers of BALB/c mice from 8 to 12 weeks post-infection is shown in Table 3. The male and female flukes from lungs were significantly smaller than those from livers. Furthermore, out of adult lung worms, only a few paired worms could be detected, though many couples were seen in the livers.

Fecundity of parasites: Fecundity of female worms from lungs and livers of BALB/c mice were examined at 10, 12 weeks post-infection as shown in Table 4. The percentage of gravid *S. mansoni* in lungs was considerably lower than that of females in the livers.

Eggs recovered from the lungs

The mean number of eggs in the lungs of BALB/c mice and gerbils with lung female worms was significantly higher than that without lung

Table 3

Length of worms recovered from livers and lungs of BALB/c mice infected with *Schistosoma mansoni*.

Weeks post-infection	Livers		Lungs	
	Male worms	Female worms	Male worms	Female worms
8	6.9 ± 0.2 mm	7.2 ± 0.3	6.2 ± 0.2*	-
10	7.0 ± 0.1	7.4 ± 0.2	5.5 ± 0.1**	5.0 ± 0.2**
12	7.3 ± 0.2	7.8 ± 0.3	4.7 ± 0.2**	5.0 ± 0.3**

The number of measured flukes from livers and lungs was 20, respectively at each week indicated.
Each point represents the mean ± SE of values.
* : $p < 0.02$; ** : $p < 0.001$, comparing worm length from livers and lungs.

Table 4

Fecundity of *Schistosoma mansoni* female worms, recovered from livers and lungs of BALB/c mice.

Weeks post-infection	Percentage of female worms with uterine eggs from	
	livers	lungs
10	75 (40)	8 (26)
12	83 (40)	25 (24)

Parentheses show the number of female worms examined.

females (Table 5). These findings suggest that female worms could lay in the lungs, and also the eggs were flowed from hepatic portal system to lung blood vessels.

Eggs in brain tissues

BALB/c and ICR mice infected with this parasite showed high prevalence of egg-associated cerebral lesions, and also mature and immature eggs were detected in the brain vessels. As the number of eggs in lungs increased, the number of eggs in brains also significantly increased (Table 6). These findings suggest that the number of eggs in lungs may have a significant correlation with the number of eggs in brains of these mice. In contrast to the mice, 2 of 119 infected gerbils at 8 weeks post-infection had egg-emboli in the cerebral blood vessels (Table 6), though many eggs were recovered from the lungs of gerbils. No adult worm was detected in the brain tissues of any infected animals.

Table 5

Mean number of eggs in lungs of BALB/c mice and gerbils infected with *Schistosoma mansoni*.

Weeks post-infection	Animal	No. of cercariae infected	Mean no. of eggs ± SE recovered from lungs	
			with female worms	without female worms
8	Gerbils	190	7,300 ± 2,000	330 ± 110*
10	BALB/c	210	13,500 ± 2,700	4,000 ± 1,800*
12	BALB/c	210	20,000 ± 4,300	6,400 ± 3,600

Mean number of animals examined was 16 at each week indicated.
* : $p < 0.01$, comparing egg numbers from lungs with and without the female worms.

Table 6

Mean number of eggs in brains and lungs of mice and gerbils infected with *Schistosoma mansoni*.

Animal	No. of cercariae infected	Weeks post-infection	No. of animals with eggs in brain /no. examined	No. of eggs in brains /animal	No. of eggs in lungs /animal
BALB/c mice	210	6	0/22	0	5 ± 4†
		8	9/24	5 ± 3	610 ± 280
		10	15/23	6 ± 3	10,000 ± 2,100*
		12	12/17	13 ± 8*	18,000 ± 3,900*
ICR mice	210	6	0/17	0	2 ± 1
		8	7/17	1 ± 1	39 ± 13
		10	12/17	12 ± 5	1,000 ± 700
		12	4/7	33 ± 19	1,600 ± 1,000
Gerbils	190	8	2/119	0	3,700 ± 1,100

† : Mean number of eggs recovered ± SE

* : $p < 0.001$, comparing egg numbers from BALB/c and ICR mice.

DISCUSSION

A transfer of schistosomes from the porto-mesenteric system to the lungs starts between the 6th and the 8th weeks post-infection in BALB/c mice infected with *Schistosoma mansoni*, followed by significant increase of worm numbers detected in lungs (Tables 1, 2). It should be noted that the worms started laying eggs at 6 weeks post-infection. And the magnitude of relocation of worms to the lungs was greater in BALB/c mice than ICR mice and gerbils in the present study. The question why or how the some adult worms can move to the lungs is not well understood. However, it appears difficult for developed schistosomes to pass the pulmonary blood vessels because of the disproportion between vasculature and worm sizes. It might be possible that smaller and less developed worms are shunted preferentially to the lungs. Indeed, Theron *et al* (1992) observed that sexually immature worms were located in the lungs of *Rattus rattus* infected with *S. mansoni* from Guadeloupe (West Indies). Additionally, Oshima *et al* (1978) suggested that a large amount of glucose necessary for *S. japonicum* development is available in the liver, but there is not enough in the lungs. These observations might explain the significantly lower development of worms in the lungs than in the livers (Tables 3, 4).

Gerbils infected with 190 cercariae exhibited higher occurrence and greater number of lung worms than those infected with 100 cercariae (Tables 1, 2), indicating the possibility that the prevalence of lung flukes depended on the number of cercariae infected. This result conforms well with the idea that the relocation of schistosomes in the lungs of *Rattus rattus* infected with *S. mansoni* is correlated with the total worm burden (Imbert-Establet and Combes, 1992).

Around 70% of 129/Ola mice infected with *S. mansoni* were characterized by the absence of schistosome worms or eggs in the hepatic portal system and with the occurrence of adult schistosomes in the pulmonary vasculature (Elsaghier *et al*, 1989). By means of the vasculature casting technic, Elsaghier and McLaren (1989) suggested that the alterations in the portal and pulmonary vasculature of these mice might facilitate worm relocation from the liver to the lungs. Recently, Cheever *et al* (1994) reported that dislocation of *S. mansoni* flukes to the lungs appeared to be related to the degree of portal-systemic shunting. They also suggested that portal-systemic collateral veins were larger in BALB/c mice than in B6 mice. A shift of *S. mansoni* to the lungs in mice following drug therapy has been reported (Hewitt and Gill, 1960). Thus, the

relocation of adult worms to the lungs from livers seems to be complex and multifaceted, although different schistosome strains, different parasite and host species should also be taken into consideration, in comparing with the results previously reported by other authors (Cheever *et al*, 1994).

The number of eggs recovered from the lungs of BALB/c mice and gerbils having lung female worms was greater than that from animals not harboring lung females in the present study (Table 5). Some of female flukes in the lungs of BALB/c mice were gravid (Table 4), although the worms from lungs were significantly smaller than those from livers (Table 3). These findings suggested that female flukes could lay eggs in the lungs. Indeed, Elsaghier *et al* (1989) showed that some lung-located schistosomes could feed, pair and lay eggs in 129/Ola mice infected with *S. mansoni*. In contrast to this, Cheever *et al* (1994) reported that worms could not lay eggs in the intrapulmonary arterial branches of infected BALB/c mice, and the number of eggs in the lungs also reflected greater shunting.

Almost no eggs were detected in the brain vessels of infected gerbils, although many eggs embolized in the lungs of those animals (Table 6), this being consistent with our previous report (Kamiya *et al*, 1994). And also, Kamiya *et al* (1994) proposed the significant correlationship between the occurrence of egg deposition in brains and the number of eggs in lungs of *S. mansoni* infected ddY mice. In the present study, a lot of eggs were detected in the lungs of infected BALB/c and ICR mice, and also the number of those eggs gradually increased with time (Table 6). The number of eggs detected in the brains was correlated well with the number of eggs from the lungs in infected BALB/c and ICR mice (Table 6). However, the reason why more many eggs were detected in the brains of ICR mice than those of BALB/c mice (Table 6), even though the number of eggs recovered from the lungs of BALB/c mice was about 10 times greater than that from ICR mice at 10 and 12 weeks post-infection, is not clear. Different vasculature systems or alterations in the portal and pulmonary vasculature might facilitate the egg transportation from the livers or the lungs to the brain vessels (Mitchell, 1989; Elsaghier and McLaren, 1989).

It should be noted that pulmonary schistosomiasis caused by eggs and migrating adult schistosomes might contribute to the concomitant immunity to *S. mansoni* infection. For instance, Dean *et al* (1978) reported that challenge schistosomula encountered the focal inflammatory responses stimulated by eggs in the lungs of mice infected with *S. mansoni*, as a consequence of which they might be killed. The level of concomitant immunity to *S. mansoni* that develops gradually after the start of oviposition in mice correlates with the degree of portal hypertension and the number of egg granulomas in the lungs (Dean *et al*, 1981). Furthermore, Imbert-Establet and Combes (1992) showed a relationship between resistance to reinfection and the presence of schistosomes in the lungs of *R. rattus* infected with *S. mansoni*. Therefore, concomitant immunity to schistosomiasis mansoni might be related, at least in part, to pulmonary pathology caused by eggs, and also perhaps migrating adult schistosomes.

It is also noteworthy that shunting of adult worms to the lungs contributes to a reduction in the average number of worms recovered from the mesenteric circulation. It should be noted that approximately half number of the lung schistosomes were dead in BALB/c mice infected with *S. mansoni* (Cheever *et al*, 1994), and lung worms evoked an eosinophil-enriched inflammatory reaction which ultimately encapsulated the worms, when parasites expired in infected 129/Ola mice (Elsaghier *et al*, 1989). *S. mansoni* eggs might be destroyed more rapidly in the lungs than in the liver and gut (Almedia and Andrade, 1983; Cheever *et al*, 1994). It is stressed, therefore, that factors leading to abnormal adult worm and/or eggs tranfer from livers to the lungs, and retarded development of schistosomes in the lungs might contribute to the resistance to *S. mansoni* infection.

ACKNOWLEDGEMENT

This study was supported in part by Grant-in-Aid for scientific research (055 8113) from the Ministry of Education, Science, Sport and Culture, Japan.

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