

Defect of Resistance of Jird (*Meriones unguiculatus*) to Reinfection of *Schistosoma mansoni* and its Development after Treatment

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Abstract: Resistance to reinfection after primary infection with *Schistosoma mansoni* was examined by the recovery technique of schistosomula from lungs in jirds (*Meriones unguiculatus*). After 8, 12 and 16 weeks of primary infection, no resistance was observed when the recovery rate of schistosomula from reinfected group was compared with that of previously uninfected group. Effective treatment of jirds with eight-week old patent infections with praziquantel resulted in slight development of resistance to the challenge when their recovery rate of schistosomula was compared with that of the primarily infected jirds. There was no difference in the recovery rate between infected jirds with incomplete treatment and previously uninfected jirds. Jird is a convenient animal model which does not develop resistance to reinfection with *S. mansoni*.

Key words: *Schistosoma mansoni*, *Meriones unguiculatus*, Resistance to reinfection, Treatment, Praziquantel.

INTRODUCTION

A great difficulty lies in the research on a resistance of patients to reinfection with schistosomes. Many studies of immunity to schistosomes have been performed so far in experimental animals. In recent years, the baboon, rhesus monkey, mouse and rat have been used extensively for studying immunity to schistosomes mainly because they develop immunity to reinfection partially or totally. However, when green monkey (*Cercopithecus sabaeus*) and chimpanzee were used as a host, little or no resistance to reinfection was observed (Ritchie *et al.*, 1967; Sadun *et al.*, 1970). Thus, a development of immunity differs considerably from one animal to the other. Therefore, a study of immunity in a new combination of host and schistosome has been expected to be done.

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Jird (*Meriones unguiculatus*) has been known to be a good experimental animal for several parasitic diseases. Some parasitologists recommended jird as an ideal experimental animal of schistosomiasis because the pathological change seen was similar to that of man (Yingrui *et al.*, 1983) or because schistosome could be easily maintained in jird (Ehrlich, 1976). However, jird has never been used for the study of immunity in schistosome infection. In the present study, we measured resistance to reinfection with *Schistosoma mansoni* in jird and examined the influence of chemotherapy on the development of resistance.

MATERIALS AND METHODS

Parasite and animal host: A Puerto Rican strain of *Schistosoma mansoni* was used in this study. It was originally from Juntendo University, Tokyo and has been maintained in our laboratory through passage in *Biomphalaria glabrata* and golden hamster. The snails are usually exposed individually to 5-7 miracidia overnight at 25-27°C. Jirds (20 to 40 weeks old, weighing from 53 to 80 g) used in this study were the colony which has been maintained for many years in our laboratory. They were randomized before use.

Infection: Jirds were given an anesthetic, pentobarbital (Nembutal[®], ABBOTT), shaved on the abdomen and exposed to cercariae for 30 min following the method of Erickson (1974). A batch of 20 to 40 snails infected about 5 weeks ago was used as the source of cercariae in each experiment. After animals were detached from the water infested with a given number of cercariae, the water was examined for the remaining cercariae. Then, the number of cercariae which successfully invaded the hosts was estimated. The animals in any experiment were exposed randomly and not in the order of experiment group.

Recovery of schistosomula and adult worms: To recover schistosomula from the lungs of jirds, a modification of the technique outlined by Sher *et al.* (1974) was used. Jirds were anesthetized with Nembutal and exsanguinated by cutting femoral arteries prior to perfusion. The lungs were perfused by heparinized Hanks' solution, chopped into small pieces and suspended in 20 ml of RPMI 1640 medium (pH 7.4) supplemented by 2 ml of fetal calf serum. The experiment was undertaken under sterilized conditions. The specimens were shaken at 37°C for 3 hrs and incubated at 37°C for 45 hrs in a CO₂ chamber. After the incubation, the specimens were filtered to remove the pieces of lungs and the filtrate was washed several times by centrifugation. Residual red blood cells were lysed by a rapid washing with chilled hemolytic buffer followed by the replacement with Hanks' solution. The pellet in the final 1 ml of fluid was suspended and the suspension was dropped on a glass plate (8 × 12cm) with a pipette. The schistosomula in each drop were counted under a dissecting microscope under 40 x magnification. In the experiment where adult worms had to be recovered in addition to the lung recovery, animals were not exsanguinated.

The adult worms were recovered by a modified technique described by Smithers and Terry (1965). The animals were anesthetized with Nembutal and perfused by heparinized Hanks' solution. The mesenteric vein was carefully examined and the liver was crushed to check the remaining worms.

Stool examination: Jirds were kept individually in separate cages to collect their stool specimen. The cage was floored with a mesh which facilitate masses of faeces to pass through. A tray was placed under the cage and was covered with wet paper to prevent the faeces from drying out by the time of examination. Five Kato-Katz thick smears were prepared from each sample. The method is identical to that described by Katz *et al.* (1972) except that cardboard templates are replaced by plastic ones with a smaller hole which delivers a mean weight of 9.395 mg of faeces and the original screens are replaced by the larger mesh-size screens (50 per inch).

Treatment of animals: Praziquantel (Biltricide^R, Bayer A. G.) and metrifonate (Bilarcil^R, Bayer A. G.) were used to treat animals. The drugs were administered by a stomach tube after suspension in water (praziquantel: 60 mg/ml, metrifonate: 25mg/ml).

RESULTS

Recovery of schistosomula from lungs of primarily infected jirds: To ascertain the peak recovery of schistosomula, the pattern of the recovery of schistosomula from lungs of primarily infected jirds were determined. The experiment was repeated twice.

In the first experiment, 20 female jirds were exposed to 100 cercariae each and divided into 5 groups each of 4 animals. The lung recovery was performed on each group for 5 successive days from 3 to 7 days after infection. The number of recovered schistosomula is shown in Table 1a. The recovery of schistosomula was the highest on day 6 followed by day 4. In the second experiment, 6 male and 4 female jirds were exposed to 200 cercariae each and were examined from 4 to 6 days after infection. Over 20% of the number of cercariae which had been supposed to penetrate the host successfully was recovered on day 4, 5 and 6 days after infection (Table 1b). Although the difference of the mean recovery rates among the days examined was not statistically significant, the recovery rate of each animal on day 5 was rather constant.

Based on the results of the 2nd experiment, the lung recovery of schistosomula in the following experiments was performed on the 5th day after infection.

Experimental treatment of jirds: To determine the dosages of the drug which cause complete or incomplete cure, a preliminary experiment was designed. Twenty-one male and 3 female jirds were exposed to 100 cercariae each and were divided into six groups. Two of the animals died immediately after the infection and 4 animals died during the course of treatment owing to an over-dose of the anesthetic. The first egg appeared in the stool on the 42nd day and all the animals turned positive for the eggs by the 53rd

Table 1a. Recovery of schistosomula from lungs of primarily infected jirds (1st experiment)*

	Days after infection				
	3	4	5	6	7
No. of animals	4	4	4	4	4
No. of schistosomula recovered (Mean±S.E.)	5.75±1.4	27.5±0.29	11.75±0.95	41.75±7.81	7.75±0.63

* Jirds were exposed to 100 cercariae each; remaining cercariae in the infested water was not counted.

Table 1b. Recovery of schistosomula from lungs of primarily infected jirds (2nd experiment)*

	Days after infection		
	4	5	6
No. of animals	3 (2M 1F)**	4 (2M 2F)	3 (1M 2F)
% recovery of schistosomula (Mean±S.E.)	28.6±7.9	31.2±2.7	22.4±3.7

* Jirds were exposed to 200 cercariae each; The number of cercariae remained in the infested water after the infection ranged from 1 to 10.

** M: Males, F: Females

day after infection. The geometric mean of number of eggs in stool was 603 per gram on day 54. On the 56th day, the treatment was started according to the protocol shown in Table 2. Two weeks after the beginning of treatment, animals were sacrificed and adult worms were recovered.

The results of adult worm recovery are shown in Table 2. In every animal, male flukes were found more than females. In the control group, the average recovery rate was 42.7%. Metrifonate was not effective at all at the given dosage. At the dosage of 500 mg/kg of body weight for 5 days, praziquantel provided a complete treatment and its efficacy was dose dependent. Lower dose of praziquantel caused insufficient cure. The inactive adult worms, the colour of which turned dark, were sometimes recovered from the treated jirds.

Resistance to reinfection: The resistance to reinfection was examined at a given interval after a primary infection. Jirds were infected with 30 cercariae each and challenged with 200 cercariae at 8, 12 and 16 weeks after the primary infection. The degree of resistance was measured by comparing the number of schistosomula from the lungs of jirds infected previously and that of control jirds which received challenge infection alone.

The results of the experiments are shown in Table 3. At any particular week examined in this study, there was no significant difference in the percent recovery of schistosomula between the previously infected and primarily infected (control) groups. It is

Table 2. Effect of treatment on adult worm recovery

Treatment group*	No. of animals	% recovery of adult worms**; Mean±S.E.
1	2	48.7± 8.9 (100.0)***
2	3	0.4± 0.4 (0.0)
3	2	10.7± 2.4 (59.1)
4	3	15.5± 4.6 (0.0)
5	4	33.6± 8.7 (96.6)
6	3	42.1±13.0 (100.0)

* The protocol of the treatment;

Group 1 Metrifonate 200 mg/kg/day for 5 days

Group 2 Praziquantel 500 mg/kg/day for 5 days

Group 3 Praziquantel 250 mg/kg/day for 5 days

Group 4 Praziquantel 250 mg/kg/day for 3 days

Group 5 Praziquantel 100 mg/kg/day for 1 day

Group 6 No treatment (Control)

** Jirds were exposed to 100 cercariae each. The number of cercariae remained in the water after the infection ranged from 1 to 20.

*** The numbers in parentheses show the percentage of active worms in the whole worms recovered in each group.

Table 3. Lung recovery of schistosomula from lungs of reinfected jirds

Animals	Time of challenge infection* (Weeks after primary infection)		
	8	12	16
reinfected group	26.6±1.6** (n=4)	34.8±2.7 (n=10)	35.9±4.6 (n=8)
previously uninfected (control)	24.4±6.5 (n=2)	35.5±2.7 (n=12)	31.8±4.1 (n=6)

* Jirds were exposed to 200 cercariae each for the challenge infection. The number of cercariae remained in the water after the infection ranged from 0 to 14.

** % recovery of schistosomula; Mean±S.E.

likely that jirds do not induce resistance to reinfection of *S. mansoni* by week 16.

Effect of treatment on resistance to reinfection: Two groups of animals were treated with praziquantel 8 weeks after a primary infection; a group, completely treated, which were given praziquantel at the dosage of 500 mg per kg for 5 days and another group, incompletely treated, given 250 mg per kg for one day. The animals were then challenged with 200 cercariae 4 weeks after the beginning of treatment. The number of schistosomula recovered from the challenge infection in treated jirds were compared to those recovered in the controls (infected and untreated jirds, and primarily infected jirds). Before and after the treatment, stool examinations were performed to confirm the efficacy of treatment. The geometric mean of the egg count from all infected jirds was 267 per gram before treatment. The efficacy of the treatment was also determined by the number of

adult worm recovered and the existence of live eggs in liver when animals were sacrificed for the lung recovery of schistosomula. No live egg was in the livers of jirds which had been completely treated. However, 9 out of 11 animals incompletely treated had live eggs yet in the livers. In our experiment, the complete treatment refers to an absence of live adult worms in the host and the incomplete treatment refers to an existence of live worms.

The results of the lung recovery of schistosomula and the effect of treatment, egg counts per gram of stool (EPG) and adult worms recovery, are shown in Table 4. The recovery rate of schistosomula from lungs of jirds which had been completely treated with praziquantel was less than those from the other groups. The difference was statistically significant ($t=2.2963$, d.f.=20, $0.025 > P > 0.01$). These results may indicate that the treatment with praziquantel induced a certain degree of resistance to reinfection of *S. mansoni* in jirds. However, there was no difference in the percent recovery of schistosomula between the incompletely treated group and the controls.

Table 4. Lung recovery of schistosomula from challenged jirds which had been infected and treated previously

	No. of Animals	E.P.G.* (G.M.**)	No. of worms recovered***			% recovery of schistosomula#
			M	F	I	
Infected & untreated	10	462	2.80	2.83	0.53	34.8±2.7
Infected & incompletely treated	11	122	2.23	2.41	0.45	33.9±2.2
Infected & completely treated	10	16	0.0	0.07**	0.71***	27.7±1.9
Previously uninfected (control)	12	—	—	—	—	35.5±2.7

* No. of eggs per gram of stool. The stool examination was performed about 3 weeks after treatment. The viability of eggs in stool was not examined.

** G.M.: Geometric mean was obtained by adding 1 to each value before calculation and 1 was reduced from the mean after the calculation.

*** Results of primary infection: M; Males, F; Females, I; Immature worms: Jirds were exposed to 30 cercariae each. The number of cercariae remained in the water ranged from 0 to 3.

Results of challenge infection: Jirds were exposed to 200 cercariae each. The number of cercariae remained in the water ranged from 3 to 14. Lung recovery was performed 3 to 4 weeks after completion of treatment.

** One dead.

*** Nine inactive worms recovered from 6 jirds.

DISCUSSION

The development of resistance to reinfection with schistosome differs much from one experimental animal to another. The baboon, rhesus monkey, mouse and rat were reported to develop immunity to reinfection partially or totally (Reviewed by Smithers and Terry, 1976; Phillips and Colley, 1978). However, green monkey and chimpanzee did not show

any resistance to reinfection (Ritchie *et al.* 1967; Sadun *et al.* 1970). Recently, James and Sher (1983) reported a strain of mouse which did not develop sufficient resistance to reinfection after primary infection with irradiated cercariae.

In the present study, we examined resistance of jird to reinfection with *S. mansoni* by using a lung recovery procedure at scheduled intervals after primary infection. The percent recovery of schistosomula by challenge infection from the lungs of previously infected jirds was not reduced in comparison with those from previously uninfected jirds. Jird seems to be an animal which shows no resistance to reinfection with *S. mansoni*.

A considerable controversy has arisen as to whether or not acquired immunity plays a significant role in limiting infection in man. While a concept of concomitant immunity (Smithers and Terry, 1969) has been widely accepted, the degree of exposure to infested water was shown to be a major factor controlling the infection (Dalton and Pole, 1978). On this context, protective immunity may not occur in man. Therefore, further studies on protective immunity against *Schistosoma* should be made with any host animals that acquire strong or less resistance to reinfection. Jird is well worthy of a convenient animal model which does not develop resistance to reinfection.

The effect of treatment on resistance to reinfection has been exclusively studied by using mice, so far. The studies with *S. mansoni* indicate that resistance tends to decline shortly after treatment (Cheever *et al.* 1965; Doenhoff *et al.*, 1980). In contrast to these findings, Warren *et al.* (1977) and Andrade and Azevedo de Brito (1982) reported a long persistent resistance after treatment. Our study suggested possible development of resistance to reinfection with *S. mansoni* after complete treatment in jirds. This may indicate that the destruction of worms induced resistance in jirds. Further investigation is necessary to determine the mechanism of possible resistance which develops after the treatment with drug in jirds and how long jirds maintain resistance to reinfection after treatment.

Some parasitologists claim that reduced recovery of schistosomula from lungs may not accurately reflect resistance to reinfection in mice (Doenhoff *et al.*, 1978; Dean *et al.*, 1978). In the present study we measured the resistance to reinfection by lung recovery technique alone and concluded that jird showed no resistance to reinfection. The result might be different when the elimination of worms could be examined beyond the lung stage. A further experiment would be desirable to determine the resistance by the perfusion procedure aiming at the matured worm.

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スナネズミ (*Meriones unguiculatus*) を用いたマンソン住血吸虫症の研究 —再感染に対する抵抗性—

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スナネズミのマンソン住血吸虫に対する再感染抵抗性を肺から schistosomula を回収する方法で観察した。初感染から 8, 12, 16 週後に challenge infection を行った結果, いずれの時期でも既感染群と初感染群 (対照群) の間で肺からの schistosomula の回収率に差はなかった。しかし感染後 8 週目に praziquantel で治療すると, 不完全に治療した群では対照群との差はないものの, 完全治療群では再感染に対する抵抗性の発現が見られた。スナネズミはマンソン住血吸虫に対して通常は再感染抵抗性を示さない動物モデルとして有用と考えられる。

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