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Host-Parasite Relationships in Schistosomiasis Mansoni and Japonica in Rhesus Monkeys: Interhost Worm Transfers

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Interest in the host-parasite relationship in schistosomiasis has been greatly stimulated by the experiments of Smithers and colleagues in which surgical interhost transfers of adult or juvenile schistosomes have been performed. They have shown that *Schistosoma mansoni* adults can be surgically transferred from rodents or rhesus monkeys into normal or immunized rhesus where the worms resume egg laying or are destroyed by host reactions evoked by host-specific antigens (Smithers *et al.*, 1969). Monkeys immunized with mouse spleen and liver cells or with erythrocytes with Freund's complete adjuvant destroy worms transferred from mice, while worms from other rodents or monkeys survive and resume egg production in similarly treated hosts. They have established that *Schistosoma mansoni* adults have an exterior coating of host antigens acquired after about seven days in the host (Clegg *et al.*, 1971 a), which is not readily removed by washing (Clegg *et al.*, 1970) and which persists for about seven days following transfer of the worms from mice to monkeys, after which it is replaced by antigens from the new host (Smithers *et al.*, 1969). In monkeys immunized against mouse cells, worms transferred from mice die after 7–25 hours, with a destruction of the worm integument (Smithers *et al.*, 1969). Incorporation of host antigens, such as those of human blood groups, also occurs during *in vitro* worm cultivation, so that monkeys immunized with human A or B blood cells destroy worms cultured in media containing cells of that blood group (Clegg *et al.*, 1971 b). Smithers' group (Smithers and Terry, 1969) propose that these exterior coatings of host antigen enable *Schistosoma mansoni* to survive in the immunologically hostile environment of the host in a manner analogous to the concomitant immunity of tumor cells (Gershon *et al.*, 1967). The importance of these findings to the understanding of host-parasite relationships in schistosomiasis prompted us to try to confirm some of their work and to determine whether this phenomenon also occurs with *S. japonicum*.

Materials and Methods

Duplicate experiments were performed using *S. mansoni* and *S. japonicum*. Parasite strains used were a Puerto Rican strain of *S. mansoni* maintained in laboratory mice and *Biomphalaria glabrata* and a Japanese strain (Yamanashi Prefecture) of *S. japonicum* maintained in mice and *Oncomelania hupensis nosophora*. Rhesus monkeys (*Macaca mulatta*) were obtained through a primate dealer in India. Exposure of mice to *S. mansoni* cercariae was by tail immersion for 30 minutes under restraint in plastic capsules (Broome and Radke, 1971) and to *S. japonicum* cercariae by counting the cercariae with a bacteriological loop onto microslide cover glasses, which were then placed for 15–20 minutes on the shaved and washed skin of mice anesthetized with chloral hydrate (Relaxans —Pitman-Moore, Inc—0.4 mg/g of body weight). Monkeys anesthetized with phen-

cyclidine hydrochloride (Sernylan—Bio-ceutic Laboratories, Inc.—1 mg/kg of body weight) were exposed to *S. mansoni* by pipetting cercariae onto the clipped and washed abdominal skin (30 minutes) and to *S. japonicum* by the same technique as that for mice. Adult worms for surgical transfer to monkeys were collected from the infected mice or monkeys seven weeks after exposure and were transferred with sterile techniques. Before perfusion, heparin (100 units for mice, 1,000 units for monkeys) was injected I. V., and the animals were killed by an I. V. injection of pentobarbital. After placement of appropriate ligatures to confine the flow of perfusion fluid to the mesenteric system and out the portal vein opened close to the liver, sterile Hank's tissue culture medium containing 1 g of glucose per liter was injected into the aorta. The worms flushed from the portal vein were collected on sterile 4-cm square pieces of stainless steel screen (105 mesh, 0.17-mm openings) and transferred to dishes containing Hank's solution with glucose. After being washed in three changes of Hank's solution, the worms were pipetted into the plastic tubing (0.2×31.5 cm) of a disposable-type pediatric intravenous injection set fitted with a 19 ga. needle (Abbott Laboratories). A 5-ml syringe containing Hank's solution was attached to this tubing for injection of the worms. This transparent tubing permitted observation of the worms during the necessary manipulations to distribute the worms evenly along the tubing and during the injection to assure that all worms had been flushed into the vein. Fifty worm pairs were transferred to each recipient monkey for the *S. mansoni* experiment, and 30 pairs for the *S. japonicum*.

Worm recipient monkeys were immobilized with phencyclidine during presurgical preparation and were anesthetized by inhalation of methoxyflurane (Penthrane—Abbott Laboratories) during the laparotomies. A loop of small intestine was manipulated through the 5- to 6-cm medial incision, the needle attached to the I.V. set was inserted into a suitable mesenteric vein, and the worms were slowly injected. The vein was flushed with the 1–3 ml of Hank's remaining in the syringe to assure that the worms had passed on toward the portal vein. Bleeding from the vein after withdrawal of the needle was prevented by the application of manual pressure until clotting had closed the opening. The intestine was kept moistened with sterile saline during the injection. No bandaging was required after closure of the incision with gut and polyethylene sutures. Chloromycetin (100 mg/kg) was administered to each monkey before being returned to its cage. Blood for bacterial cultures was drawn from a femoral vein of recipient monkeys just before surgery, at one and five days thereafter, and at the time of necropsies. A sample of the fluid remaining in the tubing after injection of the worms was also taken for bacterial culture.

Four weeks after the worms had been injected, all monkeys were injected with heparin and killed by injection of pentobarbital. The mesenteric circulation and liver were perfused with physiological saline, and the worms were collected on stainless steel screens (105 mesh, 0.17– mm openings) in plastic capsules (Radke *et al.*, 1962) and counted. Samples of liver from different lobes totaling about 50 g for each monkey were removed, weighed, and frozen for subsequent egg counts after digestion with 4% KOH (Cheever, 1968). Red blood cells from normal laboratory mice were washed three times in Hank's solution; 0.4 ml of packed cells was emulsified with an equal volume of Freund's complete adjuvant (FCA-Difco); and 0.2 ml of the mixture was injected into each of four sites (I.M. in each thigh, S.C. into each axilla-Smithers *et al.*, 1969) on each of six monkeys. Six additional monkeys were similarly injected with FCA emulsified with equal volumes of Hank's (0.2 ml in each of the four sites). Monkeys were injected with mouse cells with FCA or with adjuvant alone twice (at three weeks and one week for the *S. mansoni* experiment and at four weeks and two weeks for the *S. japonicum* experiment)

before worm transfers were made. Just before the worm transfers, blood was collected for determination of titers to antigens. Sera from all monkeys were tested using a passive hemagglutination in microtiter plates against washed mouse erythrocytes. In conducting the research described here, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, U.S. National Academy of Sciences, U.S. National Research Council.

Results

S. mansoni experiment

In preliminary experiments designed to standardize surgical and other techniques required, we added sodium barbital (200 mg/liter) to the first wash in Hank's solution in order to relax the worms for ease of handling during subsequent injections. In monkeys and hamsters perfused 72 hours after having been injected with *S. mansoni* adults from mice or hamsters, most worms recovered were surrounded by massive clots of blood cells and appeared to be dead. In monkeys perfused five weeks after receiving worms from mice, only two stunted living worms were recovered from each of two monkeys out of twelve examined. In subsequent transfers, barbital was not used, and we were able to confirm the work of the British group with *S. mansoni*.

No worms were recovered from monkeys immunized with mouse RBCs with FCA and injected with *S. mansoni* adults from mice (Table 1, group II). The 44 % mean percent recovery of worms from monkeys receiving mouse worms after being immunized with FCA alone (Table 1, group I) was considerably lower than that (84 %) reported by Smithers *et al.* (1969). Injection of mouse RBCs with FCA did not decrease the percent recovery of monkey derived worms as compared with the recovery of monkey worms

Table 1 Worm recoveries and eggs per gram of liver per female worm in monkeys 4 weeks after implantation of *Schistosoni mansoni* adults and hemagglutination titers at time of surgery

Group	Monkey no.	Worm donor	Immunization*	% worm recovery		EGLF†	Titer‡
				Individual	Group mean		
I	25	mouse	FCA	61	44	11	—
	31	mouse	FCA	32		10	—
	35	mouse	FCA	40		5	—
II	1	mouse	RBC+FCA	0	0	0	4,096
	12	mouse	RBC+FCA	0		+	512
	20	mouse	RBC+FCA	0		0	1,024
III	15	monkey	FCA	55	54	13	—
	16	monkey	FCA	32		18	—
	30	monkey	FCA	76		16	—
IV	2	monkey	RBC+FCA	78	66	6	256
	4	monkey	RBC+FCA	39		25	16,384
	11	monkey	RBC+FCA	82		7	1,024

* FCA=Freund's complete adjuvant; RBC+FCA=red blood cells plus FCA.
† EGLF=eggs per gram of liver per female worm recovered; + indicates positive, but less than 1 EGLF.
‡ Reciprocal of hemagglutination titer; — indicates negative.

from monkeys immunized with FCA alone (group III). As reported by the British investigators, somewhat fewer female than male *S. mansoni* were recovered from monkeys receiving mouse worms (a mean ratio of 1.8 male to 1 female); in both groups (II and IV) which received monkey worms, the male-to-female ratio was 1.1 : 1. The data on eggs per gram of liver per female recovered indicate that egg production was occurring (Table 1). The variability was too great for valid generalization, but it appeared that the mouse-derived *S. mansoni* were producing eggs at a rate somewhat lower than that of monkey-donor worms. A few eggs (about two per gram of liver) were found in the liver of monkey No. 12, although no worms were recovered at necropsy. Serum hemagglutination titers to mouse RBCs (Table 1) for monkeys injected with mouse cells ranged from 1 : 256 to 1 : 16, 384. This demonstrated that antibodies to mouse cells were present; however, these apparently did not affect the survival and recovery of transferred monkey worms.

S. japonicum experiment

In the experiment utilizing *S. japonicum* worms, no worms were recovered from monkeys immunized with mouse RBCs with FCA before receiving mouse schistosomes (Table 2, group II). With this species a low recovery of mouse-derived worms was observed in monkeys immunized with FCA (group I), but survival of transferred monkey worms (groups III and IV) was comparable to that seen with *S. mansoni*. More female worms (male-to-female ratio 1 : 1.4) than males were recovered in all groups. A greater accummulation of eggs in the liver per *S. japonicum* female was observed in monkeys injected with monkey-derived worms than in those with mouse worms. A mean value of 42 *S. japonicum* eggs per gram of liver was found for monkeys (group II) from which no worms were recovered. One

Table 2 Worm recoveries and eggs per gram of liver per female worm in monkeys 4 weeks after implantation of *Schistosoma japonicum* adults and hemagglutination titers at time of surgery

Group*	Monkey no.	Worm donor	Immunization†	% worm recovery		EGLF‡	Titer§
				Individual	Group mean		
I	5	mouse	FCA	12	15	19	—
	22	mouse	FCA	17		17	—
II	7	mouse	RBC+FCA	0	0	+	256
	18	mouse	RBC+FCA	0		+	256
	21	mouse	RBC+FCA	0		+	512
III	23	monkey	FCA	58	66	60	—
	28	monkey	FCA	72		95	—
	36	monkey	FCA	68		35	—
IV	6	monkey	RBC+FCA	57	51	48	1,024
	19	monkey	RBC+FCA	45		64	256

* One monkey designated for group I died during surgery; data for one monkey in group IV not obtained because of technical failure during worm injection.

† FCA=Freund's complete adjuvant; RBC+FCA=red blood cells plus FCA.

‡ EGLF =eggs per gram of liver per female worm recovered; + indicates positive, but less than 1 EGLF.

§ Reciprocal of hemagglutination titer; — indicates negative.

monkey in group I died during surgery because of respiratory failure, and data on one monkey in group IV were not obtained owing to technical failure during injection of the worms. Serum hemagglutination titers (Table 2) to mouse RBCs were lower than in the *S. mansoni* experiment (range 1 : 256 to 1 : 1,024). These antibodies did not appear to affect the recovery of monkey worms.

In both experiments blood cultures taken before and after surgery were negative for growth after seven days' incubation for all monkeys. In three cases the Hank's suspension fluid remaining after injection of the worms was positive for bacteria by culture (two with *Staphylococcus epidermidis* and one with *Alcaligenes denitrificans*), but no bacteria were cultured from the blood of these three monkeys one day later, or subsequently. These tests were performed to detect any possible erratic results which might be attributable to worm destruction during concomitant bacteremias resulting from contamination during worm transfers (Ottens and Dickerson, 1972).

Discussion

Although *in vitro* experiments (Sell and Dean, 1972; Dean and Sell, 1972; Damian *et al.*, 1973) have demonstrated the presence of host or host-like antigens associated with the tegument of *Schistosoma mansoni*, to our knowledge this is the first report confirming the Smithers' group findings on the destruction of mouse worms in monkeys immunized with mouse cells. Our experiments have shown that this rhesus-schistosome phenomenon also occurs with *S. japonicum*.

In our preliminary experiment we found that worms exposed to sodium barbital during the washing in Hank's solution became surrounded by host cells and died after transfer to normal, as well as immunized, rhesus. It seems likely that successful transfers of schistosomes require that the worms remain active after placement into the blood stream of the new host in order to remain free of clots and attack by nonspecific cellular defense mechanisms. It has become standard practice in many laboratories working with schistosomes to kill animals before perfusion by an overdose injection of pentobarbital which relaxes the worms and facilitates perfusion. This anesthetic apparently does not produce the effect we observed with barbital, either because the dose to which the worms are exposed is less or because of the shorter-action characteristics of pentobarbital.

The liver egg count data revealed that schistosomes transferred from mice had resumed egg laying by the time of necropsy four weeks after their transfer to rhesus. Because we did not study fecal egg passage and a rapid destruction of schistosome eggs occurs in tissues in rhesus monkeys (Cheever and Powers, 1971), no conclusions can be formed on differences in the rate of egg production by worms from mice or monkeys. However, it is noteworthy that in monkeys receiving monkey-*S. mansoni* after being immunized with FCA alone, the number of eggs per gram of liver per female was about one-fourth that in similarly treated rhesus which received *S. japonicum*. A ratio of 1 : 10 in egg production capacity of *S. mansoni*, compared to that of *S. japonicum*, has been reported (Moore and Sandground, 1956; Moore and Warren, 1967). The disparity in our results may be the consequence of a greater proportion of *S. japonicum* eggs being passed in the feces rather than being carried to the liver, or of a faster rate of destruction of *S. japonicum* eggs in tissues. The presence of a few eggs in the liver of one monkey in group II (Table 1) in the *S. mansoni* experiment and a few eggs in all three in group II of the *S. japonicum* experiment (Table 2) suggests that mouse worms may have survived and produced eggs for some time in the anti-mouse monkeys, although all worms had disappeared by four weeks after the transfer. However, it is likely that these few eggs still remained in the tissues from the first few days after entering the new host, since Cheever and Powers (1971) found that the rate of egg destruction in previously unin-

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リーサス種モンキーにおけるマンソン住血吸虫および日本住血吸虫の Host-Parasite Relationships：宿主間の虫体移植について

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この一連の実験は Smithers らにより報告されたマウスより得られたマンソン住血吸虫をマウス細胞に対して免疫化されたリーサス種モンキーに移注した際にみられた虫体破壊の現象が，マンソン住血吸虫のそれらと同じく，日本住血吸虫にもみられるかを，目的としたものであつた．初めに，プエルトリコ株のマンソン住血吸虫を用いて，次いで山梨株の日本住血吸虫を用いて実験を行つた．この結果，マンソン住血吸虫がマウスから，事前にマウスの赤血球に対して免疫化されたリーサス種モンキーに移植せられた場合，宿主内で破壊されることが認められた．他方，モンキーから，同様に処置せられたモンキーに移植された虫体は，生存することが明らかにされた．そしてこの現象は，リーサス種モンキーに移植された日本住血吸虫についてもみられることが明らかにされた．

更に，この虫体破壊は，宿主内の随伴性の菌血症とは関連のないことも明らかにされた．そして，この現象を説明する諸問題についての，考察を行つた．