Immunization of Rhesus Monkeys against Schistosome Infection by Cercariae Exposed to High Doses of X-radiation* (34057)

S. Y. Li Hsü, H. F. Hsü, and J. W. Osborne

Department of Preventive Medicine and Environmental Health and Radiation Research Laboratory, University of Iowa College of Medicine, Iowa City, Iowa 52240

The knowledge of immunization against schistosome infection has been greatly increased by studies of the acquired resistance obtained in experimental animals through the use of X-irradiated cercariae. The most effective radiation exposures used for cercariae which subsequently immunize the animal range from 2500 to 3000 R (1, 2). Since inflammatory reactions caused by the schistosomula derived from irradiated cercariae exposed to these amounts of X-radiation have been observed in the liver and lungs (3, 4), it will be of interest to investigate the possibility of using a higher X-ray exposure which will make the schistosomula perish in the less critical tissues such as the skin. It has been demonstrated that practically all schistosome cercariae exposed to 24,000, 48,000, or 50,000 R will perish in the dermal tissue during the time of immunization (3, 4). The possibility of inducing acquired resistance by immunizing experimental animals with cercariae exposed to such large amounts of X-radiation needs to be investigated. Further interest in this study lies in the fact that some investigators (5) have recently claimed that the migratory schistosomular stage contributes little to the development of host resistance. Since all immunizing cercariae exposed to such large amounts of X-radiation will perish in their early schistosomular stage (3, 4), the role of schistosomula in the induction of acquired immunity can be ascertained by immunizing hosts with heavily irradiated cercariae.

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Materials and Methods. Male rhesus monkeys (*Macaca mulatta*) weighing approximately 2 kg were used. They were shown to be negative for both tuberculin reactivity and schistosome eggs in the stools. The cercariae of the Japanese strain of *Schistosoma japonicum* were shed from the snail *Oncomelania nosophora* obtained from Kofu, Japan; and the cercariae of *S. mansoni,* from the snail *Australorbis glabratus* originating from Puerto Rico. The cercariae were irradiated in dechlorinated tap water suspensions 10 mm deep and 17 mm in diameter. The vessels containing the liquid were of glass with walls 1.6 mm thick. The X-radiation was delivered by a General Electric Maxitron unit. Radiation factors were 250 kVp., 30 mA, with 0.25 mm copper and 1 mm aluminium filtration. The half-value layer for the beam was 1 mm copper. The distance from the target to the surface of the suspensions was 248 mm, which yielded an exposure rate of 620 R/min. The total exposure given to the cercariae for immunizing the monkeys was either 24,000 or 48,000 R. The irradiated cercariae were transferred to cover glasses for the immunizing application. Only the actively swimming cercariae were used.

Before administering the cercariae, monkeys were anesthetized with nembutal and the abdominal hair was clipped. The cercariae were then applied to the abdominal skin with cover glasses. The immunizing doses for monkeys consisted of cercariae of both sexes shed from 30 to 40 snails. For the challenge exposure of immunized monkeys and for the exposure of control monkeys, only the cercariae of known sexes were used, each monkey receiving 200 males and 200 females. The cercariae for challenging experimental and

control monkeys originated from the same batch of snails and were shed on the same day. From Day 30 of the challenge, the stools of each animal were examined daily by the sedimentation method. After eggs were found in the stool, the number of eggs/g/day was calculated by Stoll's method. For monkeys in the *S. japonicum* series, the mean number of eggs/g/day for the first 30 days in the patent period (MNEPG30) was used as an index for the intensity of the challenge infection and the maximal number of eggs/g/day in the same period (MXEPG30) was used as an additional reference; for monkeys in the *S. mansoni* series, MNEPG40 and MXEPG40 were used. After challenge, each monkey was killed at Day 63–94 of the challenge and the worms were collected by the perfusion method. After perfusion, the liver was sliced into pieces for further worm-searching and the mesenteric veins were examined manually so that the unperfused worms could still be collected.

Results. Four monkeys were given immunizing cercariae of *S. japonicum* which had been irradiated with 48,000 R. The monkeys were immunized 3–8 times. The total number of the irradiated cercariae used varied from 9000 in one monkey and from 18,000–25,100 in three monkeys. After challenge, the MNEPG30 of two monkeys which were immunized with 20,500–25,100 irradiated cercariae varied from 21 to 24 and that of one monkey which was immunized with 9000 irradiated cercariae was 13,621. Owing to its untimely accidental death, the stool examination of monkey EJ297, which was immunized with 18,000 cercariae, was very incomplete. The numbers of worms recovered at autopsy were 272 for the monkey which was immunized with 9000 cercariae and varied from 11 to 62 for the three monkeys which were immunized with 18,000–25,100 cercariae. In the three control monkeys, the MNEPG30 varied from 6500 to 8100 and the numbers of worms recovered at autopsy varied from 230 to 290. It is evident that monkeys immunized 3–8 times with a total of 18,000 to 25,100 *japonicum* cercariae irradiated with 48,000 R manifested a high degree of acquired resistance against the challenge infection while such an acquired resistance against the challenge infection was not shown in monkeys immunized with 9000 cercariae (Table I).

Two monkeys were treated four times with a total of 35,000–40,000 cercariae of *S. mansoni* which had been irradiated with 24,000 R; and four monkeys, 3–5 times with a total of 25,000–27,000 cercariae irradiated with 48,000 R. Three control monkeys were challenged at the same time as the experimental monkeys. After challenge, the MNEPG40 of the two monkeys which were immunized with cercariae irradiated with 24,000 R varied from 0 to 24; that of the four monkeys which were immunized with cercariae irradiated with 48,000 R, from 0 to 87. The numbers of worms recovered at autopsy varied from 6 to 54 in the 24,000 R group and from 11 to 157 in the 48,000 R group. In the three control monkeys, the MNEPG40 varied from 230 to 276, and the numbers of worms recovered at autopsy varied from 220 to 260. It was also evident that monkeys immunized 3 to 5 times with a total of 25,000 to 40,000 *mansoni* cercariae irradiated with 24,000 R or 48,000 R manifested a high degree of acquired resistance against the challenge infection (Table I).

Discussion. Although the results of the present study indicate that a strong acquired resistance against schistosome infection can generally be induced by previous immunizations with cercariae exposed to 24,000 or 48,000 R, it failed in one monkey immunized with 9,000 *japonicum* cercariae exposed to 48,000 R. The failure in this case may be either due to an especially high individual susceptibility of this monkey to infection or due to the comparatively smaller number of the cercariae which have been used in the immunization of this monkey compared to the other monkeys. It is reasonable to assume that while the life span for schistosomula derived from cercariae exposed to large amounts of X-radiation is shorter than those derived from cercariae exposed to much lower amounts, a larger number of cercariae is needed to furnish sufficient functional antigen to induce enough functional antibody in the former case than in the latter case.

TABLE I. Stool Examination of Experimental and Control Monkeys.

Monkey no.	Immunizing cercariae: X-ray exposure (R)	Immunizing cercariae: No. of immunizing applications	Immunizing cercariae: Intervals between immunizing applications (days)	Immunizing cercariae: Total no. immunizing cercariae	Challenge: No. of cercariae	Challenge: Days after immunizing application: 1st	Challenge: Days after immunizing application: Last	Eggs/g of stool/day (1st 30 or 40 days): Maximum	Eggs/g of stool/day (1st 30 or 40 days): Mean	Autopsy: days after challenge	Worms recovered: Adult	Worms recovered: Immature	Worms recovered: Total
S. japonicum													
EJ179	48,000	3	30–34	9000	400	108	44	29,526	13,621	68	272	0	272
EJ297	48,000	6[a]	32	18,000	400	1007	14	Inc[b]	Inc[b]	63	10	1	11
EJ230	48,000	8[a]	29–33	20,500	400	1424	21	32	21	74	52	7	59
EJ186	48,000	7[a]	29–171	25,100	400	1558	26	32	24	68	57	5	62
CJ101					400			17,000	8100	74	290	0	290
CJ102					400			15,200	6500	68	232	0	232
CJ103					400			18,800	7415	63	280	0	280
S. mansoni													
EM159	24,000	4	33–52	35,000	400	271	145	0	0	71	6	0	6
EM160	24,000	4	33–52	40,000	400	320	194	48	24	90	54	0	54
EM46	48,000	5[a]	54–124	27,000	400	801	17	0	0	94	11	0	11
EM174	48,000	3	34–43	27,600	400	201	124	64	26	94	29	38	67
EM167	48,000	4	37–64	27,000	400	265	124	48	11	89	54	0	54
EM161	48,000	3	34–42	25,000	400	306	194	280	87	86	157	0	157
CM201					400			450	230	86	220	0	220
CM202					400			610	276	89	260	0	260
CM203					400			585	255	94	256	0	256

[a] In monkeys EJ297, EJ230, and EJ186, the last two immunizing applications were boosters. The interval between the first booster and the previous immunizing application ranged from 219 to 374 days; the interval between the two boosters ranged from 568 to 927 days; in monkey EM46, the last immunizing application was a booster and it was performed 231 days after the previous immunizing application.

[b] Incomplete due to untimely death of the monkey by accident at Day 9 of the patent period.

Previous investigators have reported that one prior exposure of albino mice to *mansoni* cercariae irradiated with 7500 R or irradiated with from 12,500 to 40,000 R failed to produce a significant degree of acquired resistance (6, 7). On the other hand, it has been reported that rhesus monkeys previously exposed for four times to *japonicum* cercariae irradiated with 12,000 R demonstrated very strong resistance against the challenge infection (7), and that albino mice and albino rats previously exposed for three to four times to *mansoni* cercariae irradiated with 8000 R showed significant resistance against challenge (9). These facts show that for the induction of an effective acquired resistance with the administration of heavily irradiated cercariae, several immunizing applications at different times may be necessary.

It becomes evident that immunization with schistosome cercariae treated with such high X-ray exposures has the advantage of avoiding tissue damages in the vital organs, such as lungs and liver, but has the disadvantage of requiring a large number of cercariae and several immunizing applications separated by time. However, the end result of immunization with heavily irradiated cercariae approaches that induced by cercariae subjected to much lower exposures.

Although the success of immunization against schistosome infection with X-irradiated cercariae in rhesus monkeys may not have the same success in humans, the results obtained in the present study increase greatly our basic knowledge for the understanding of the mechanism of schistosome immunity and of the method of immunization. It indicates that the functional antibody of schistosome immunity can be formed by schistosomular contact or deterioration in the dermal tissues, and that the schistosomular migration in the lungs and liver is not necessary for inducing the development of sufficient functional antibody against the schistosome. It shows furthermore that strong acquired resistance can be induced exclusively from functional antigen in the early schistosomular stage. If the theory of dual mechanisms of schistosome immunity–antischistosomulum immunity and anti-adult worm immunity–is to be accepted (10), the results of the present study demonstrate clearly the effect of the function of the antischistosomulum immunity.

Summary. Strong acquired resistance against schistosome infection in rhesus monkeys can be induced by previous immunizations with cercariae exposed to large amounts of X-radiation; *i.e.*, 24,000 or 48,000 R. This indicates that acquired resistance against schistosome infection can be induced exclusively from functional antigen produced after contact or deterioration of the schistosomular stage in the dermal tissues.

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