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Migration Kinetics of Ultraviolet-attenuated *Schistosoma mansoni* in ICR Mice

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Abstract

The migration of naive and ultraviolet (UV)-attenuated *Schistosoma mansoni* in the skin and the lungs of naive ICR mice has been followed by means of the tissue mincing and incubation technique (TM) and by the indirect immunoperoxidase staining technique (IP). The worm number in the hepatic portal system was also determined by retrograde portal perfusion 21 days after infection. The number of worms recovered from the lungs and hepatic portal system decreased with increasing UV-irradiation doses. Few worms irradiated with 18 mJ/cm^2 (300 μW min/cm^2) were recovered from lungs, and no worms with 15 mJ/cm^2 (250 μW min/cm^2) as well as 18 mJ/cm^2 were recovered from liver. In the skin on day 1, 18 mJ/cm^2-irradiated worms recovered by TM were significantly smaller in number than naive worms, whereas 18 mJ/cm^2-irradiated worms detected *in situ* by IP were comparable to naive worms. Thus UV-irradiation may affect in a dose dependent manner both the migration potential of the worms from the skin through the lungs to the liver and the motility of the worms which crawled out of the minced tissue into the incubation medium.

Key words: migration kinetics, ultraviolet attenuation, cercariae, *Schistosoma mansoni,* vaccination

Introduction

In the mouse model, vaccination with ultraviolet (UV)-irradiated cercariae or schistosomula of *Schistosoma mansoni* (Murrell *et al.*, 1975; Ghandour and Magid, 1978; Murrell *et al.*, 1979; Dean *et al.*, 1983; Ruppel *et al.*, 1990) and *S. japonicum* (Moloney *et al.*, 1985a; Moloney *et al.*, 1985b; Ruppel *et al.*, 1990) induces partial resistance against a challenge infection with normal cercariae.

As compared with gamma- or X-irradiation, which had been widely used to attenuate schistosomes, UV-irradiation is cheaper and more convenient to perform. This appears particularly relevant, if domestic animals were to be vaccinated under field conditions (Shi *et al.*, 1990).

The migration kinetics of the worms appear to be important in the relationship between the immunogenic potential of attenuated parasites and the possible pathologic sequelae of a living vaccine (Kamiya and McLaren, 1987). The migration of UV-irradiated worms which are used for vaccination, however, has not yet been well elucidated. In this study, the migration of naive and UV-attenuated schistosomula of *S. mansoni* in ICR mice has been followed by means of the tissue mincing and incubation technique, the indirect immunoperoxidase staining technique, and by retrograde portal perfusion.

Materials and Methods

Male ICR mice were purchased from a commercial breeder (Funabashi Farm, Funabashi, Japan) and were used throughout the experiments. The life cycle of a Puerto Rican strain of *Schistosoma mansoni* was maintained through *Biomphalaria glabrata* snails and ICR mice. Snails were strongly illuminated for 2 hours to release cercariae. Cercariae were irradiated immediately prior to infection at 254 nm (UV lamp

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from Ultraviolet-Products, San Gabriel, U.S.A.) at the intensity of 455 $\mu W/cm^2$ for a time determined to provide the indicated UV dose. Dosage was calculated from the equation: Dosage = Intensity × Time. The energy output was measured with an UVX Digital Radiometer (Ultraviolet-Products).

Mice, 5-week-old, were anaesthetized by intraperitoneal injection of 45 mg/kg of Nembutal® (pentobarbital sodium, Abbott Laboratories, North Chicago, U.S.A.), shaved on the abdomen and exposed to about 500 cercariae by the 'ring method' (Smithers and Terry, 1965) using a metal ring with an inner diameter of 11 mm. The mean number of infecting cercariae was calculated from 6 random aliquots of larvae. One hour was allowed for cercarial penetration, after which time the water in the ring was examined for nonpenetrating cercariae. Almost all cercariae, both naive and irradiated, could penetrate. However, the number of non-penetrating parasites, if any, was substracted from the number of infecting cercariae.

The number of worms in the skin and lungs was assessed by two methods. The tissue mincing and incubation technique (TM) of Smithers and Gammage (1980) was done with some modifications. Briefly, mice were killed by ether inhalation. The shaved abdominal skin, about 2 cm in diameter, including the site which had been exposed to cercariae and the lungs were removed. Tissues were cut with fine pointed scissors for 5 minutes and suspended in 10 ml of RPMI 1640 medium (Nissui Pharmaceutical, Tokyo, Japan), which was prepared as described by Smithers *et al.* (1980). Minced pieces of skin and lungs were incubated for, respectively, 8 or 18 hours in plastic petri dishes (60 mm diameter; Sumitomo Bakelite, Tokyo) in a humidified atmosphere of 5% CO_2 in air at 37°C in order to allow the worms to migrate into the incubation medium.

The second method used was the indirect immunoperoxidase staining technique (IP) modified from the method of Kamiya *et al.* (1981). The skin, about 2 cm in diameter, including the site of cercarial exposure and the lower lobe of the left lung were fixed in 10% neutral buffered formalin. Each type of tissue was then divided evenly into eight pieces, and embedded in paraffin. Ten sections (5 μm thick) were collected from each paraffin block at intervals of 50 μm. They were deparaffinized, dehydrated and washed for 15 minutes in 0.01 M PBS (pH 7.2). The sections were incubated for 30 minutes at 37°C in a moist chamber with *S. mansoni*-infected rat serum as primary antibody. The serum was diluted 1:100 with PBS containing 10% normal goat serum (Funakoshi Pharmaceutical, Tokyo) in order to block non-specific antibody binding or endogenous peroxidase reaction (Kamiya *et al.*, 1981). After extensive rinsing with PBS, the sections were incubated for 30 minutes at 37°C in a moist chamber with peroxidase conjugated goat anti-rat IgG (Cappel, West Chester, U.S.A.; diluted at 1:500 with PBS containing 10% normal goat serum). The sections were again rinsed with PBS, and the reaction was performed for 10 minutes at room temperature with 0.05% diaminobenzidine tetrahydrochloride in 0.02 M Tris•HCl, pH 7.5, containing 0.03% H_2O_2. The sections were washed with distilled water and counterstained with Mayer's haematoxylin. Schistosomula were stained brown and were counted under a microscope.

Adult parasites were recovered by retrograde portal perfusion of mice (Smithers *et al.*, 1965). In addition, the liver and mesenteries of each perfused mouse were compressed between glass plates and examined under a dissection microscope for any remaining worms.

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

Results

The migration of naive and 18 mJ/cm^2-irradiated *S. mansoni* through the skin, lungs and to the hepatic portal system of mice was assessed by TM and retrograde portal perfusion (Fig. 1). The general pattern of recovery from the skin of irradiated worms was similar to that of naive worms, with a peak on day 2 post-infection (PI), and the number then gradually decreased. Up to day 4 the number of irradiated worms recovered

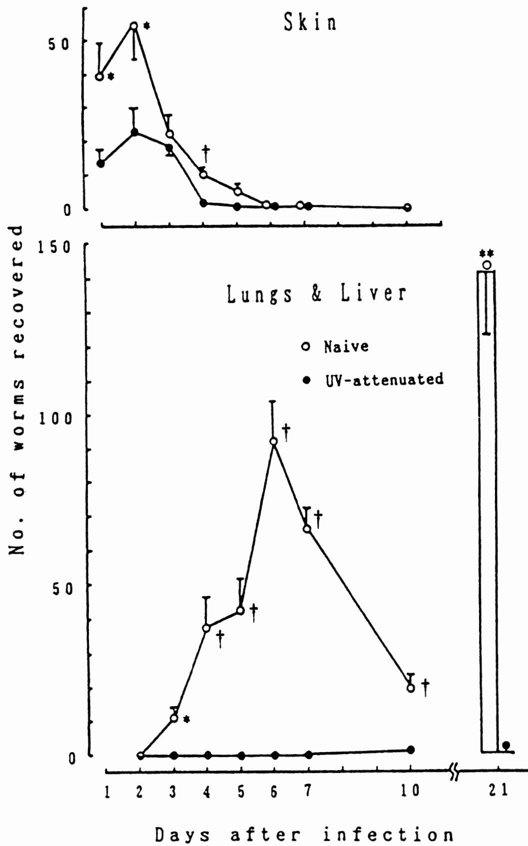

Fig. 1 Migration kinetics of naive and UV-irradiated *Schistosoma mansoni* in ICR mice assessed by tissue mincing and incubation technique (skin and lungs; till day 10) and by retrograde portal perfusion (hepatic portal system; on day 21). Mice were infected with 505.5 ± 9.1 naive cercariae (○) or 503.2 ± 12.1 cercariae irradiated with 18 mJ/cm^2 (●). Values are expressed as mean number ± SE of worms recovered per mouse (n = 5). The levels of significance for the difference between the naive and irradiated parasite numbers are $P<0.05$ (*), $P<0.01$ (†).

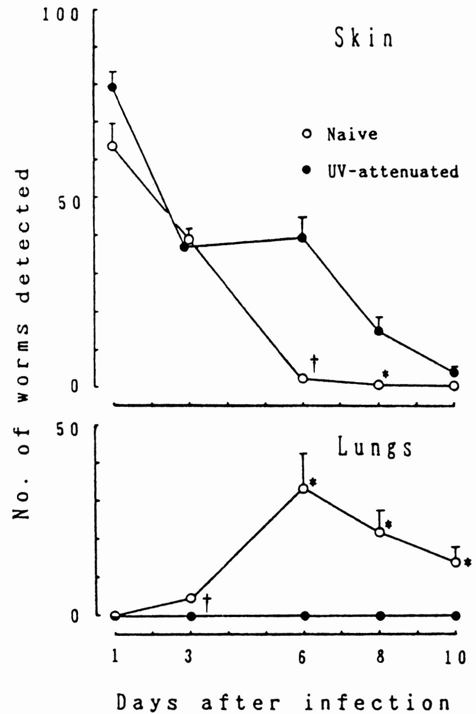

Fig. 2 Migration kinetics of naive and UV-irradiated *Schistosoma mansoni* in ICR mice assessed by the indirect immunoperoxidase staining technique. Mice were infected with 548.8 ± 12.2 naive cercariae (○) or 533.8 ± 1.5 cercariae irradiated with 18 mJ/cm^2 (●). Values are expressed as mean number ± SE of worms recovered per mouse (n = 5). The levels of significance for the difference between the naive and irradiated parasite numbers are $P<0.05$ (*), $P<0.01$ (†).

was significantly smaller than that of naive worms. Naive worms were recovered from the lungs with a peak on day 6, whereas very few of the irradiated worms were recovered (maximum of 1.2 ± 0.6). On day 21, 141.2 ± 18.3 of naive worms were recovered from the hepatic portal system, whereas none of the irradiated worms were recovered.

The migration of the worms *in situ* was examined by means of IP. As shown in Fig. 2, 18 mJ/cm^2-irradiated worms detected in the skin were similar in number to naive worms up to day 3, and were significantly more on days 6 and 8. The number of naive worms in the lungs reached its peak level on day 6, while none of the irradiated worms were detected in the lungs.

The microscopic examination of the skin sections, which were stained by IP and counterstained with haematoxylin and eosin, revealed the inflammatory infiltration, rich in eosinophils, around – and sometimes inside of – the 18 mJ/cm^2-irradiated schistosomula on day 6 and thereafter. Contrary to this, no inflammatory infiltration was detected around the naive worms

Table 1 Effect of various UV-irradiation doses on migration kinetics of *Schistosoma mansoni* in ICR mice assessed by the tissue mincing and incubation technique and by portal perfusion

Irradiation doses (mJ/cm^2)	No. of infecting cercariae	No. of worms recovered by: Tissue mincing on day 6 — skin	Tissue mincing on day 6 — lungs	Portal perfusion on day 21
0	505.5 ± 9.1	1.2 ± 0.5	92.0 ± 11.6 (5)	141.2 ± 18.3 (5)
2	520.8 ± 30.0	2.0 ± 2.0	62.7 ± 5.5 (3)	137.0 ± 20.3 (3)
5	484.8 ± 5.9	2.8 ± 1.0	13.5 ± 4.6 (4)	36.3 ± 12.2 (3)
10	502.0 ± 13.8	0.4 ± 0.2	0.6 ± 0.2 (5)	0.8 ± 0.5 (4)
15	520.8 ± 30.0	0	0 (5)	0 (4)

Data are expressed as mean ± SE. The data of naive worms are from the experiment shown in Fig. 1. The number of mice used is in parentheses.

throughout the experiment.

The relationship between the doses of UV-irradiation and the migration potential of the worms was then examined. As shown in Table 1, the worms recovered by TM from the lungs on day 6, and from the hepatic portal system on day 21, decreased in number with increasing irradiation doses; only very few worms developed from cercariae irradiated with 10 mJ/cm^2 and none from 15 mJ/cm^2-irradiated ones.

Discussion

The migration kinetics of *Schistosoma mansoni* attenuated with a dose of 18 mJ/cm^2 (300 $\mu W\ min/cm^2$) UV, conceivable to be an optimal dosage for the parasite attenuation to induce host resistance against challenge infection (Ruppel *et al.*, 1990), was followed in naive ICR mice since the migration potential of gamma-irradiation-attenuated parasites might be closely related to the level of induced immunity (Kamiya *et al.*, 1987). As shown in Fig. 1, 18 mJ/cm^2-irradiated parasites collected from the skin, as assessed by TM, were significantly smaller in number than untreated ones. Very few of the 18 mJ/cm^2-irradiated worms were recovered from the lungs, although from the skin normal and attenuated worms were collected with overall similar kinetics. These results raise two questions: (1) Whether irradiated worms might fail to migrate out of the minced tissues into the incubation medium; (2) Whether irradiated worms might fail to migrate from the skin to lungs.

The numbers of irradiated and normal parasites *in situ* detected on day 1 by IP were similar, implying that similar proportions of naive and irradiated parasites penetrated into the skin (Fig. 2). By TM, however, fewer of the irradiated worms were recovered (Fig. 1), indicating that only a part of the irradiated worms was recovered by this technique. This might suggest a reduced motility of irradiated worms which would enable only a part of them to migrate out of the skin tissues into the incubation medium. The number of both naive and irradiated worms recovered by TM on day 2 was higher than on day 1, although this was not statistically significant. Similar results were reported by Smithers *et al.* (1980) suggesting that on day 2 PI worms might be more mobile than that on day 1. As shown in Fig. 2, irradiated worms resided in the skin for longer time periods than naive ones, and were not detectable in the lungs by IP. Taken together these results suggest that UV-irradiation would reduce also the migration potential of the worms from the skin to the lungs.

UV-irradiation with 18 mJ/cm^2 is equivalent to the dose used by other authors to induce

significant resistance by attenuated cercariae (Dean *et al.*, 1983; Ruppel *et al.*, 1990). Such parasites failed to reach the lungs (Figs. 1 and 2), although worms attenuated with 20 krad gamma-irradiation, and which also induce high levels of resistance, do migrate to the lungs in the *S. mansoni*-guinea pig model (Kamiya *et al.*, 1987).

Although there seems to be no report on the number of UV-irradiated schistosomes in the lungs, Bickle *et al.* (1979) and Minard *et al.* (1978) reported that the number of the gamma-irradiated parasites recovered from the lungs by TM decreased also in an irradiation dose-dependent fashion. Kamiya *et al.* (1987) followed the migration kinetics of naive and gamma-irradiated *S. mansoni in situ* in guinea pigs using compressed tissue autoradiography and found that fewer worms were detectable in the lungs with increasing irradiation doses.

The number of worms recovered on day 21 by portal perfusion also decreased in an irradiation dose-dependent manner, and 15 mJ/cm^2 UV inhibited worm migration to the liver (Table 1). The effect on worm migration of 15 to 18 mJ/cm^2 UV-dose appears equivalent to that of 50 krad gamma-irradiation which blocks the migration of worms to the lungs in guinea pigs (Kamiya *et al.*, 1987). Ariyo and Oyerinde (1990) also reported that the number of adult *S. mansoni* in mice recovered by portal perfusion decreased with increasing UV doses. However, they did not quantify the doses of irradiation. Ruppel *et al.* (1990) showed that the development of irradiated cercariae to worms perfusable at 7 weeks PI was reduced from 50 to 1% with irradiation doses increasing from 3 to 12 mJ/cm^2. The present results are comparable by showing that 26% of 2 mJ/cm^2-irradiated and 0.16% of 10 mJ/cm^2-irradiated parasites were recovered on week 3.

The present UV-attenuation model might be useful to study the mechanism of antigen presentation in the afferent phase of vaccine immunity in schistosomiasis mansoni (Kusel *et al.*, 1989), since vaccination with UV-attenuated parasites induce significant immunity by the parasites (Cohen and Eveland, 1988; Ruppel *et al.*, 1990; Kamiya *et al.*, unpublished) which may reside solely in the skin. It remains to be determined whether higher protection might be induced by more other UV-attenuation protocols and whether differences of antigen expression exist between UV-irradiated and gamma-irradiated parasites.

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