RESEARCH NOTE

IMMUNIZATION OF LABORATORY ANIMALS WITH ULTRAVIOLET-ATTENUATED LARVAE AGAINST HOMOLOGOUS CHALLENGE INFECTION WITH *TRICHINELLA BRITOVI*

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Abstract. Immunogenicity of *Trichinella britovi* (Japanese isolate, cord ISS 408) muscle larvae irradiated with 5 mJ/cm^2 of short wavelength ultraviolet (UV) was studied using homologous challenge infections. SCID mice vaccinated with UV - attenuated larvae showed no resistance, although its congenic CB-17 mice exhibited remarkable immunity. Following challenge vaccinated Mongolian gerbils, *Meriones unguiculatus,* did not show significant immunity. Use of these animal models vaccinated with UV -attenuated larvae will contribute to the investigation of immune mechanisms against *Trichinella* infection and also to the development of a vaccine of trichinellosis.

INTRODUCTION

Trichinellosis is a serious parasitic zoonosis with worldwide distribution (Ancelle *et al,* 1985; Dissamarn and Indrakamhang, 1985; Yamaguchi *et al,* 1985; MacLean *et al,* 1989). Prophylaxis against this parasitic infection is, therefore, very much expected. The larvae of *Trichinella britovi,* suggested by Pozio *et al* (1996), irradiated with UV doses greater than 5 mJ/cm^2 of short wavelength (254 nm) were completely inhibited in their development to the adult stage (Nakayama *et al,* 1996). This approach was applied to immunize animals against homologous challenge infection. The purpose of the work was to determine the availability of animal models vaccinated with UV-attenuated *Trichinella* larvae.

A Japanese isolate of *Trichinella britovi* (isolate cord ISS 408) maintained in ICR mice (Pozio *et al,* 1996) was used throughout the study. Infective muscle-stage larvae (7 weeks post infection , PI) were obtained from ICR mice digested in artificial gastric juice (0.8% pepsin and 0.8% HCl in PBS, pH 7.2) for 3 hours at 37°C.

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In the present study, 10-week-old male CB-17 *scid/scid* (SCID) mice and their congenic counterpart, CB-17 mice, were used. These animals were bred at Central Laboratory for Experimental Animals (CLEA), Kawasaki, Japan, and were housed in specific pathogen free conditions at the Institute for Animal Experiment, Hirosaki University School of Medicine. Ten to 13-week-old male Mongolian gerbils, *Meriones unguiculatus,* bred in the Institute were also used. All animals were fed food pellets (CE-2, CLEA, Japan) and sterilized water *ad libitum.* All animal experiments in this paper followed the Guidelines for Animal Experimentation of the Hirosaki University.

UV-irradiation was carried out according to the method of Nakayama *et al* (1996). Briefly, approximately 200 larvae/ml were suspended in 2mm-deep PBS in plastic dishes measuring 53 mm in diameter and irradiated by UV at 254 nm of wavelength with UV lamp (Ultraviolet-Products Inc, CA, USA) at a dose rate of 455 μm/cm^2 at room-temperature. The lamp was allowed to warm up for more than 20 minutes prior to use to ensure a stable intensity of irradiation. The energy output was measured with a UVX Digital Radiometer (Ultraviolet Products). Animals were infected with 1,000 attenuated larvae by esophageal intubation within 30 minutes after irradiation. Three weeks after inoculation of irradiated larvae, challenge

infection with normal larvae was carried out. Challenge parasites were recovered from intestines 7 days after challenge. The small intestine was slit open and incubated for 4 hours in 50 ml of PBS at 37°C. Adult worms were counted under a dissection microscope. Reduction of the parasite population was calculated using the formula: R=(C-I) x 100/C, where R is the percentage of reduction, C= mean worm recovery from naive and challenge animals, and I= mean worm recovery from previously infected and challenged animals. Statistical analysis of the results was calculated using Student's *t* -test, with $p<0.05$ being taken as the minimal acceptable level of significance.

CB-17 mice vaccinated with attenuated larvae exhibited significant immunity, showing 98% worm reduction of challenge infection, compared to data from SCID mice which showed no immunity (Table 1). Almost all parasites failed to establish in the small intestine of vaccinated CB-17 mice following challenge although the parasites in vaccinated CB-17 mice still retained some worms in the cecum 7 days after challenge. No muscle stage larvae were detected in either group of vaccinated mice at 8 weeks of vaccination.

Attenuated cercariae of *Schistosoma mansoni* were extensively applied to immunize animals for the investigation of mechanisms in protective immunity of schistosomiasis (Dean, 1983; Kamiya, 1986; Kamiya and McLaren, 1987; James and Sher, 1990). On the other hand, no studies have been conducted on this aspect of trichinellosis using UV-attenuated larvae. Expulsion of intestinal nematodes may involve goblet cells and/or mast cells as well as IgA (Brown and Bruce, 1989; Holmgren *et al,* 1992; Muller, 1994; Nawa *et al,* 1994). In the present experiments, SCID mice did not show any significant immunity, although CB-17 mice showed remarkable resistance against challenge infection. These findings suggest initiation of immune mechanisms induced by the inoculation of UV- irradiated larvae (Table 1). Vaccinated gerbils did not exhibit the same significant immunity. This latter finding might be due to the nature of mast cells in intestinal tissues of gerbils, so-called connective tissue mast cells, which do not activate in the intestine (Nawa *et al,* 1994).

UV-irradiation doses as high as 24 mJ/cm^2 have been shown to inhibit glycoprotein synthesis in cercariae of *S. mansoni* (Wales *et al,* 1992), and

Table 1

Worm recovery from mice vaccinated with 5mJ/cm^2 UV -irradiated muscle larvae of Japanese isolate of *Trichinella britovi* on 7 days of challenge.

Mouse (n=6,♂)	Worm recovery				Worm reduction from small intestine (%)
	Naive		Vaccinated		
	Small intestine	Cecum	Small intestine	Cecum	
SCID	79±14	7±10	74±14	6±6	6
CB-17	81±9	8±5	2±3	56±11	98*

No. of challenged larvae; 162±12.
* : $p<0.001$

Mongolian gerbils vaccinated with UV-attenuated larvae showed no significant immunity against challenge infection. Vaccinated gerbils showed only a 14% reduction in adult worm numbers on 7 days post-challenge (Table 2). No muscle stage larvae were recovered from animals inoculated with UV-attenuated larvae at 7 and 26 weeks previously.

gamma-irradiation (Kamiya, 1986) or UV -irradiation (Wales *et al,* 1993) dramatically influences surface-antigen expression by *S.mansoni* schistosomula and/or 5-day-old lung worms. Furthermore, UV-irradiation affects this parasite's migration (Kumagai *et al,* 1992). These observations may be relevant to the analysis of the afferent phase of

Table 2

Immunity of Mongolian gerbils, *Meriones unguiculatus,* vaccinated with UV-attenuated Japanese isolate of *Trichinella britovi* larvae.

Gerbils (n=6,o)	Worm recovery of challenge population*	Worm reduction (%)
Naive	113±15	-
Vaccinated	97±20	14

No. of challenged parasites; 157±27.
*Challenged parasites were recovered from small intestine on 7 days of challenge.

immunity in trichinellosis. Parasites attenuated with 5 mJ/cm^2 UV dose could reside in the intestinal tissues of the inoculated mice (Nakayama *et al,* 1996) and might secrete protective antigens (Wales and Kusel, 1992; Robinson *et al,* 1995) *in situ.* These antigens would then be processed by antigen presenting cells, resulting in the induction of immunity (Walker, 1994). This notion remains to be investigated, and may play a role in the development of an effective vaccine against trichinellosis in the future.

ACKNOWLEDGEMENTS

We would like to thank Dr T Nomura for providing SCID mice, which enabled us to conduct the present project. This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

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