FEATURES OF *SCHISTOSOMA MANSONI* INFECTION IN SCID MICE

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Abstract. Features of *Schistosoma mansoni* infection in SCID mice, which lack functional T- and B-lymphocytes, were investigated. The retarded development of parasites as well as the reduction of liver egg recovery in SCID mice was significantly lower than those in congenic counterpart C.B-17 mice. Furthermore, the rate of parasite recovery from SCID mice with primary infection was always lower than that from C. B-17 mice by 20%, showing the innate resistance to *S. mansoni* infection. SCID mice vaccinated with UV-attenuated *S. mansoni* cercariae did not show protective immunity against a homologous challenge infection. The present innate resistance exhibited in SCID mice is discussed in relation to cell mediated immunity of macrophage activation by IFN-γ which would not involve T-lymphocytes but is initiated by IL-12 and TNF-α cytokines. SCID mice may provide novel information on the host-parasite relationship in schistosome infections.

INTRODUCTION

Immunodeficient animals, such as nude mice (Hsu *et al*, 1976; Sher *et al*, 1982), P strain mice (James *et al*, 1984) and also nude rats (Ford *et al*, 1987; Capron and Capron, 1986) have provided a lot of new facets on the study of protective immunity and pathophysiology of schistosomiasis. It is thought, therefore, that SCID mice which lack functional T- and B-lymphocytes appear an ideal recipient vehicle in *Schistosoma* and other parasitic infections (Stanley and Virgin, 1993).

Furthermore, Harrison and Doenhoff (1983) reported an interesting finding of the retarded development of *S. mansoni* in immunosuppressed mice. Contrary to this, Amiri *et al* (1992) reported that development of *S. mansoni* in SCID mice was comparable to that observed in the control immunocompetent BALB/c mice, although the fecundity of female worms seemed to be much lower in the former than the latter. In the same study, the authors also clarified that TNF-α when introduced externally in SCID mice infected with *S. mansoni* restored liver-egg granulomas and the fecundity of female flukes recovered to same level as that observed in the control mice. From these findings, they suggested that cytokines such as TNF-α might modulate the host-parasite relationship in schistosomiasis mansoni. In the present study, the basic mechanism of innate resistance of SCID mice in *S. mansoni* infection is reported.

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MATERIALS AND METHODS

Parasites

A Puerto Rican strain of *S. mansoni* maintained in *Biomphalaria glabrata* snail and ICR mice was used throughout the experiments. Cercariae were used within one hour of being shed.

Animals

In the present study, 7-8 week-old C. B-17/SCID/SCID and their congenic counterpart, C. b-17 mice were used. These animals were bred at Central Laboratory for Experimental Animals (CLEA), Kawasaki, Japan, and housed in pathogen-free conditions at the Institute for Animal Experimentation, Hirosaki University School of Medicine. Bedding was sterilized. 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 Hirosaki University.

Immunization and challenge infection of animals

Immunization with 18 mJ/cm² UV (254 nm wave length)–attenuated cercariae (Kamiya *et al*, 1993) and the challenge infection with native cercariae were carried out by the "ring method" of Smithers and Terry (1965). UV -attenuated cercariae were applied for immunization on the shaved back of mice, anesthetized by intraperitoneal injection of 30 mg/kg Nembutal (pentobarbital sodium; Abbott Laboratories, North Chicago, USA). Mice vaccinated 5 weeks previously, were challenged with normal cercariae applied on the shaved abdomen to eliminate the effect of local epidermal inflammation.

Recovery of parasites

Two methods were applied to recover and assess the parasite migration; namely the tissue mincing and incubation technic (Kumagai *et al*, 1992) and the retrograde portal-perfusion of mice (Smithers and Terry, 1965). The recovered worms were fixed in the boiled Lillie's buffered 10% formalin solution.

Recovery of eggs

Livers of infected mice were excised and digested with 4% KOH in PBS (pH 7.2) and the number of eggs in the liver was counted (Cheever *et al*, 1984).

Measurements of worm length

Worm length was recorded according to Ozaki and Kamiya (1993). The body profile of fixed schistosomes was traced using a Universal Projector, UP-350 (Olympus, Tokyo), and their length were calculated with Comcurve-5 (Digital curvemeter, Koizumi, Tokyo).

Statistical analysis

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

RESULTS

Worm development

Worm length: The body length of flukes recovered from SCID and C. B-17 mice from 2 to 8 weeks pi is shown in Table 1. The male and female flukes of SCID mice were significantly smaller than those of C. B-17. Furthermore, 7.6% of 5 week-old flukes from SCID mice could not be differentiated by sex, and 1.5% for C. B-17.

Fecundity of parasites: Fecundity of female worms were examined from 5 to 8 weeks pi as

Table 1

Worm length of *Schistosoma mansoni* recovered from SCID and C. B-17 mice.

Weeks post-infection	C. B-17		SCID	
	Male worm	Female worm	Male worm	Female worm
2	330 ± 70 μm*		270 ± 40	
3	900 ± 350		460 ± 140	
4	3.5 ± 1.0 mm	2.4 ± 0.8	1.9 ± 1.0	
5	4.9 ± 0.6	4.7 ± 0.2	3.4 ± 0.2	3.7 ± 0.2
6	5.8 ± 0.8	7.2 ± 1.3	4.8 ± 0.9	6.2 ± 1.6
8	6.8 ± 1.0	7.6 ± 1.8	5.7 ± 1.0	7.1 ± 1.7

Each group of measured flukes comprised 20-100 worms. The most of worms recovered from C. B-17 and SCID mice 2 and 3 weeks pi and many worms of SCID 4 weeks pi were not differentiated by its sexes. Length of male and female flukes recovered from SCID mice were significantly smaller than those from C. B-17 mice ($p < 0.01$) except 6-and 8-weeks female worms of SCID mice ($p < 0.2$).

* Mean length of worm ± SD

shown in Table 2. By 5 weeks pi, nearly half the number of females recovered from C. B-17 mice had eggs already, but no flukes of SCID mice had eggs. Six weeks pi, the percentage of gravid *S. mansoni* in SCID mice was still lower than in the control C. B-17 mice. However, by the 7th and 8th weeks the percentage increased to 60~73%. The number of eggs collected from liver was significantly larger in C. B-17 hosts than those in SCID hosts.

Protective immunity

Mice were vaccinated with cercariae attenuated by 18 mJ/cm^2 of UV-irradiation 5 weeks previously, then submitted the challenge infection. Vaccinated SCID mice did not exhibit the immunity against a homologous challenge infection. However, it should be stressed that the numbers of parasites recovered from naive SCID mice 5 weeks pi were lower by 20% than the numbers from naive congenic counterpart C. B-17 mice (Table 3).

Parasite migration

It was speculated that the present attrition exhibited in naive SCID mice might occur somewhere on the route of parasite migration. To know the time and/or the site of attrition in the course of primary infection of SCID mice, parasite migration was traced by tissue mincing and incubation technic, showing that the kenetics of parasite migration in both strains of mice was almost the same from skin to lungs, up to 10 days pi (Fig 1).

Table 2

Fecundity of *Schistosoma mansoni* flukes yielded in SCID and C. B-17 mice.

Weeks post-infection	No. of flukes examined	Percentage of female flukes with egg		No. of livers examined	No. of eggs recovered from liver		Significance
		C. B-17	SCID		C. B-17	SCID	
5	50	45.6	0	ND	ND	ND	ND
6	20	48.5	20.0	5	15.2 ± 4.2*	2.3 ± 0.3	$p < 0.05$
7	30	60.6	60.6	5	38.6 ± 7.4	17.1 ± 2.7	$p < 0.05$
8	30	60.0	73.3	8	45.8 ± 7.3	28.0 ± 2.5	$p < 0.05$

* Mean ± SE × 10^3/mouse
ND : not done

Table 3

Protective immunity of SCID and C. B-17 mice vaccinated with UV -attenuated *Schistosoma mansoni* cercariae against a homologous challenge infection.

No. of experiment	Mouse group	Weeks of vaccination	Mean no. ±SD of challenge parasites	Mean no. ± SD of challenge worms recovered		Reduction*	Significance
				Naive	Vaccinated		
I	C. B-17	5	206 ± 15	64 ± 9	34 ± 16	47	$p < 0.001$
	SCID			49 ± 6	54 ± 19	0	NS
II	C. B-17	5	232 ± 6	69 ± 13	37 ± 13	46	$p < 0.01$
	SCID			58 ± 11	59 ± 14	0	NS

Mice were vaccinated with approximately 500 UV -attenuated cercariae. Each experiment comprised 5 naive and 5 vaccinated female animals. All mice were perfused by 5 weeks post challenge infection.
* R = (C-V) × 100/C, where R = percent reduction, C = mean recovery from naive and challenged mice, and V = mean recovery from previously vaccinated and challenged animals.

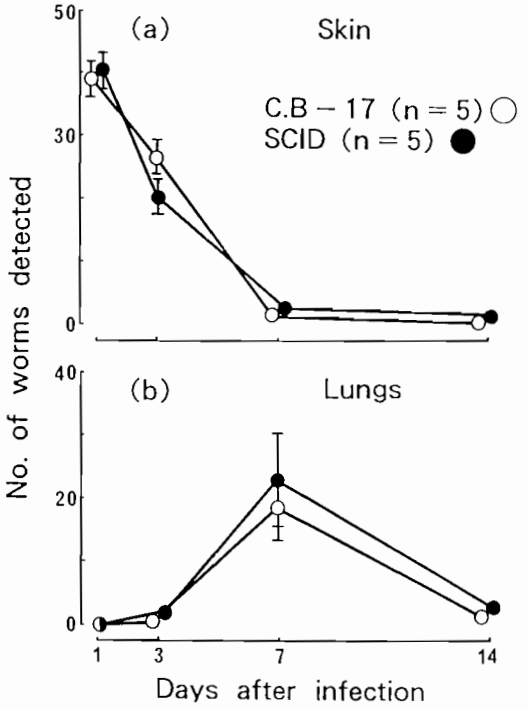

Fig 1–Migration kinetics of *S. mansoni* in naive C. B-17 and SCID mice assessed by tissue mincing and incubation technic. Values are mean ± SD of five mice. (a) migration in skin (b) migration in lungs. Mean number ± SD of infecting cercariae was 242 ± 32.

DISCUSSION

Protective immunity and immunopathology of schistosomiasis has been investigated intensively in relation to host-parasite relationships of schistosomiasis. Immunodeficient animals, nude mice (Hsu *et al*, 1976; Sher *et al*, 1982), P strain mice (James *et al*, 1984) and also nude rats (Ford *et al*, 1987; Capron and Capron, 1986) have provided extensive information on the critical involvement of cell and/or humoral mediated immunity for attrition of *Schistosoma mansoni* infection. However, not much work has been done on the detailed primary infection of *Schistosoma* spp. in immunosuppressed animals. It is reasonable to consider that SCID mice which are deficient in functional T- and B-lymphocytes, might be the most ideal animals to investigate the features of *S. mansoni* infection under immunocompromised *in vivo* conditions. It was surprising, however, that the development of flukes in SCID mice was delayed and the fecundity of the parasites retarded, compared with those in C. B-17 mice. Recently, Amiri *et al* (1992) showed that the parasites in SCID mice developed normally, but their fecundity was apparently lower than that in immunocompetent BALB/c mice. The later evidence was consistent with the present results (Table 2). This is implied by the present investigation showing the retarded development of worms in SCID mice (Table 1), comparative with the result of Harrison and Doenhoff (1983) who reported retarded development of *S. mansoni* in mice immunosuppressed by corticosteroid treatment.

The present experiment showed that SCID mice harbored a 20% lower worm-burden than that in C. B-17 mice. This was contrary to the results of Amiri *et al* (1992) who reported that schistosome infected SCID hosts had more adult worms than those in control BALB/c hosts. The present findings could indicate that SCID mice have significant innate resistance against the primary infection of *S. mansoni* on their migration routes after lungs (Fig 1). The nature of innate resistance generated in SCID mice is not well understood, although the SCID mice have normal macrophage population or serum content as the immunocompetent mice exhibited. Natural killer (NK) cell activity is also intact in SCID mice as those in congenic counterpart, C. B-17 mice (Dorshkind *et al*, 1985), although the cellular function of NK cells was not determined in the precise association of immune response in schistosomiasis (Newport and Colley, 1993). On the other hand, fascinating evidence was proposed on IL-12 and TNF-α cytokines which could induce cell mediated immunity, independent of T-lymphocytes in immunocompromized host defence against microbial pathogens, such as *Toxoplasma gondii* or *Listeria* infection (Locksley, 1993; Scott, 1993; Gazzinelli *et al*, 1993). Macrophages interacting with those pathogens released IL-12 and TNF-α. Then, those cytokines could stimulate NK cells to release INF-γ which activates macrophages (Locksley, 1993; Tripp *et al*, 1993, 1994). The activated macrophage is one of the effector cells involved in protective immunity of schistosomiasis (James *et al*, 1984; McLaren and James, 1985; Xu and Xu, 1991). This notion might provide a possible solution to explain the present innate resistance to *S. mansoni* infection in naive SCID mice.

Moreover, it has been reported that antibodies against *S. mansoni* tegumental components accelerated the surface shedding of the parasites to evade the host immune attack (Perez and Terry, 1973; McLaren, 1980). From this observation, it could be speculated on the other hand that these stimuli which accelerate the surface-turnover of parasite tegument might be necessary for the parasite development itself (Hockley and McLaren, 1973). The retarded development of worms in SCID mice would be partially explained by this possible speculation, since SCID mice do not produce any antibodies against *S. mansoni*. These suppositions remain to be investigated.

SCID mice reconstituted with human peripheral blood mononuclear cells can exhibit the human antibody response to *S. mansoni* infection (Mazinque *et al*, 1991). SCID mice, therefore, could be the appropriate vehicle to establish alternative animal models to humans, although the present innate resistance in SCID mice should be taken into consideration.

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