A COMPARATIVE STUDY ON MOUSE MHC CLASS I SEQUENCES DETECTED IN *SCHISTOSOMA JAPONICUM* RECOVERED FROM BALB/C (H-2^d) AND C57BL/6 (H-2^b) MICE

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Abstract. The mouse major histocompatibility complex (MHC) class I sequence was detected in all the 8-week-old *Schistosoma japonicum* recovered from BALB/c (H-2^d) and C57BL/6 (H-2^b) mice by *in situ* polymerase chain reaction (*in situ* PCR). The signals of the mouse class I MHC sequence were observed in the nuclei of the mesenchymal and reproductive cells of 8-week-old *S.japonicum*. Furthermore, the class I MHC sequence was detected in each DNA extracted from *S. japonicu*m cercariae maintained in BALB/c and C57BL/6 mice by nested PCR. To prove both horizontal and vertical transmission of this sequence in schistosomes, we have used cercariae obtained from parasites maintained in BALB/c mice to infect C57BL/6 and BALB/c mice, and *vice versa*. The MHC sequences from adult worms were compared to the cercarial MHC and host MHC sequences. Nucleotide sequence comparisons between adult worm DNA, host (H-2^d and H-2^b mice) DNA and cercarial DNA used for the infection suggested that the sequence of mouse class I MHC was incorporated into schistosome adults and inherited throughout their life-cycle.

INTRODUCTION

We have previously reported on the existence of host DNA sequences in schistosomes (Irie *et al*, 1993; Iwamura *et al*, 1991, 1995; Nara *et al*, 1990; Tanaka *et al*, 1989). Recently, our *in situ* PCR results showed evidence of horizontal gene transfer from the host to schistsomes. The mouse type 2 *Alu* sequence (Schmid *et al*, 1982; Kominami *et al*, 1983), the type A (Cole *et al*, 1981) and type C (Khan et *al*, 1982) retroviral sequences were detected in the schistosome bodies (Imase *et al*, 1999, 2000). The type A retroviral sequence was detected in the cercarial DNA of *S. mansoni* and *S. japonicum* (Imase *et al*, 2000). Our previous work indicated that the horizontal and vertical transmission of host sequences might selectively occur in schistosomes.

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In the present study, we detected mouse major histocompatibility complex (MHC) class I sequence, known to be related to the immune evasion of the parasites, in *S. mansoni* adults and cercariae (Imase *et al*, 2001). Since the mouse MHC gene is one of the most polymorphic gene families (Pullen *et al*, 1992), we wanted to demonstrate whether the MHC sequence detected in *S. mansoni* adults and cercariae, was host-derived or not.

In this paper, we have attempted further work on the detection of the class I MHC in male and female worms recovered from BALB/c (H-2^d) and C57BL/6 (H-2^b) mice infected with cercariae maintained in BALB/c mice and recovered from mice of the two strains infected with cercariae maintained in C57BL/6 mice.

Here we show the comparison of the nucleotide sequences of class I MHC detected in the adult worms, the cercariae used for the infection and the host (the mice of the two strains) DNA**.** It was hoped this work could provide the key to explain the origins of the MHC sequence detected in *S. japonicum.*

MATERIALS AND METHODS

Parasites

A Japanese strain of *S. japonicu*m was maintained by standard laboratory procedures in male BALB/c (H-2^d) mice and C57BL/6 (H-2^b) mice and its snail host, *Oncomelania nosophora*. Schistosome was recovered by perfusion (Smithers and Terry, 1965) with RPMI 1640 solution (pH 7.4) from the hepatic portal system of male BALB/c and C57BL/6 mice at 8 weeks after percutaneous infection with 30 cercariae/mouse.

The strategy employed in the recovery of schistosomes is shown in Fig1. Cercariae obtained from parasites maintained in BALB/c mice (described as cercaria d in Fig1) were used to infect BALB/c and C57BL/6 mice. Cercariae obtained from parasites maintained in C57BL/6 mice (described as cercaria b in Fig1) were also used to infect mice of the two strains. The BALB/c (H-2^d) mouse was abbreviated as d and the C57BL/6 (H-2^b) mouse was abbreviated as b in Fig1. Male and female *S. japonicu*m from the d→d, d→b, b→d and b→b groups were recovered at 8 weeks after exposure of cercariae.

Preparation of DNA

DNA was extracted from 8-week-old male and female *S. japonicu*m from the d→d, d→b, b→d and b→b groups, *S. japonicu*m cercaria d and cercaria b and the liver of BALB/c and C57BL/6 mice. DNA extraction was performed by using the WB DNA Extractor Kit (Wako) with the sodium iodide method.

In situ PCR

Frozen tissue sections were prepared as described previously (Imase *et* al, 2000).

The primer set of mouse class I MHC was designed from the nucleotide sequences of H-$2L^d$ genes determining the Ag binding domain (α1) (Evans *et al*, 1982) and from our sequencing data from C57BL/6 mice. The sequences were; GGAGTTCGTGCGCTTCG and CTGCTCTT GGCCCTTGG. Fifty μl of the amplifying solution containing 4.5 mM $MgCl_2$, 200 mM dNTPs, 0.5 μM of each primer and 2.5 U of *Taq* DNA polymerase (TaKaRa EX-*Taq*) was placed over the tissue section (Nuovo, 1994; 1995). The slide glass was then placed directly on the sample block of a thermal cycler (Perkin Elmer). The PCR conditions was as follows : Initial denaturation at 94ºC for 150 seconds followed by 40 cycles of 94ºC denaturation for 3 seconds, 50ºC annealing for 20 seconds, and 72ºC extension for 15 seconds.

After *in situ* PCR, *in situ* hybridization with a digoxigenin-11-dUTP (DIG) labeled probe was performed as described previously (Imase *et al*, 2000).

Polymerase chain reaction (PCR)

The sequences of the primers used were as follows: outer primers; CACACTCGATGCGG TATTTCG and TCTGGTTGTAGTAGCCGA GC, inner primers; CATCTCTGTCGGCTA TGTGG and AGGGTCCTCAGGTTCACTCG.

The first PCR was done with the target DNA described below in a solution of ten μl volume containing 0.5 μM of each outer primer and 0.25 U of *Taq* DNA polymerase (TaKaRa EX-*Taq*). The target DNA was as follows; 20 ng adult worm DNA, 30 ng cercarial DNA, 0.3 ng mouse DNA (a positive control), and 30 ng planaria DNA (a negative control). After a 3-minute incubation, at 94ºC for complete denaturation of the DNA, 40 cycles for cercarial DNA, adult worm DNA and planaria DNA and 30 cycles for mouse DNA, at 94ºC for 30 seconds, 55ºC for 25 seconds and 72ºC for 20 seconds were done in a DNA thermal cycler (Thermal sequencer TSR-300, Iwaki Garasu).

For the nested PCR, 1 μl of the first PCR product was amplified further for 30 cycles using the inner pair of primers as described by Iwamura *et al* (1995). The annealing temperature was 55ºC. Ten microliters of the nested PCR reaction mixture was electrophoresed on a 2.5% agarose gel.

Sequencing of the PCR products

Sequencing was performed by dye terminator cycle-sequencing method in an ABI 377 automated DNA sequencer (PE Biosystems). Each of the nested PCR products was sequenced using the primers for the nested PCR described before. DNA samples used were as follows: DNA of 8-week-old male and female *S. japonicum* from d→d, d→b, b→d and b→b groups, DNA of cercaria d and cercaria b, and DNA of BALB/c and

C57BL/6 mice liver.

RESULTS

Localization of the sequence of mouse class I MHC in *S.japonicum*

The localization of the mouse class I MHC sequence was detected by *in situ* PCR and hybridization with a DIG labeled probe. The signal of the class I MHC sequence, a purple-blue precipitate, was detected in the nuclei of the mesenchymal cells and in the nuclei of the testicular, ovarian and vitelline cells of both 8-week-old *S. japonicu*m recovered from BALB/c and C57BL/6 mice. Fig 2-A, 2-C, and 2-D showed the localization of the class I MHC sequence in *S. japonicu*m recovered from BALB/c mice. Fig 3-A, 3-C and 3-D showed the localization of the class I MHC sequence in *S. japonicum* recovered from C57BL/6 mice. We performed *in situ* PCR on *S. japonicum* without *Taq* DNA polymerase to compare the negative and positive cells. The negative cells are shown in Fig 2-B and 3-B.

The BALB/c and C57BL/6 mice liver tissue sections were used as positive controls. The signals of the mouse class I MHC sequence were detected in the nuclei of the liver cells. The free-living planaria tissue section was used as a negative control. No signal of this sequence was detected in this worm .

Comparison of the nucleotide sequences of the mouse class I MHC detected in amplified DNA

The nucleotide sequence of mouse class I MHC detected in amplified products from 8-week-old male and female *S. japonicu*m DNA from the d→d, d→b, b→d and b→b groups was compared with that of BALB/c and C57BL/6 mice DNA and that of cercaria d or cercaria b DNA used for the infection. The sequence homology between them is shown in Table 1. In this experiment, the sequence homology of class I MHC between BALB/c mice encoding H-2^d gene and C57BL/6 mice encoding H-2^b gene was 90.7%.

The sequence homology between the MHC sequence of male and female worms from the d→d group and that of the host (BALB/c mice) was 100%. The MHC sequence of the male worm of b→d group was identical with that of BALB/c mice from which adult worms were recovered. The MHC sequence of the female worm of b→d group was closely related to that of BALB/c mice (sequence homology; 97.3%). On the other hand, in the case of d→b group, the sequence homo-

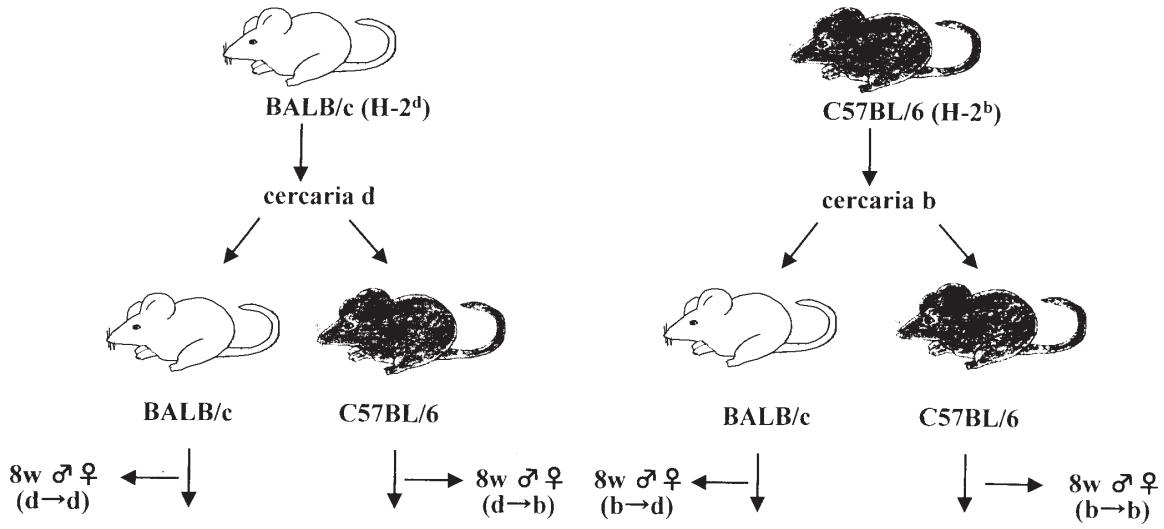

Fig 1-The strategy employed in the recovery of schistosomes. Male and female *S. japonicu*m from d→d, d→b, b→d and b→b groups were recovered at 8 weeks after exposure of cercariae. *S.japonicum*, cercaria d and cercaria b were respectively obtained from parasites maintained in BALB/c and C57BL/6 mice; d: BALB/c mice (H-2^d), b: C57BL/6 mice (H-2^b).

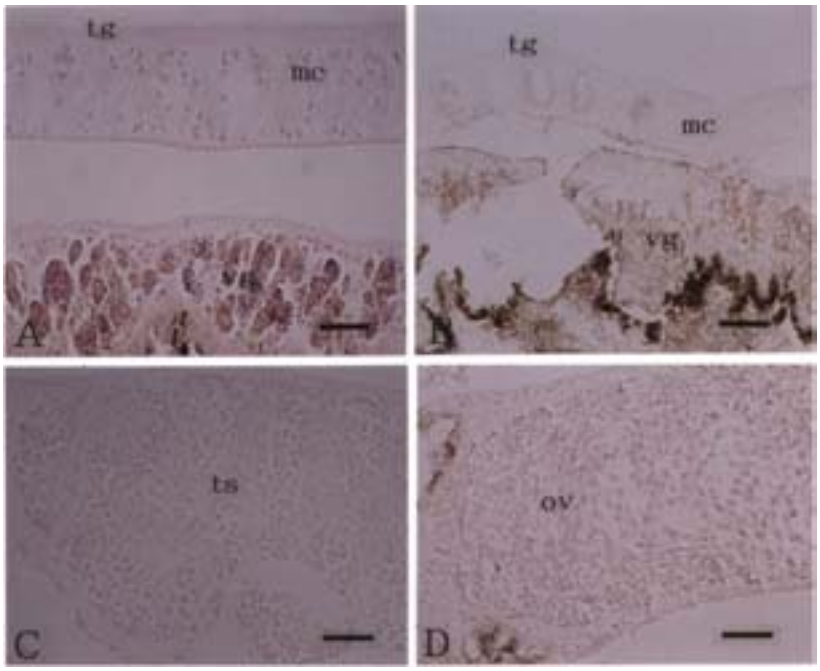

Fig 2-Blight-field micrographs showing the localization of the sequence of mouse class I MHC in *S. japonicum* recovered from BALB/c mice. *In situ* PCR and hybridization was performed on the frozen sections. The positive signals are purple-blue precipitates. Scale bar is 25 μm in all photos. (A) middle portion of male and female worms, (B) middle portion of male and female worms that had *in situ* PCR performed without *Taq* DNA polymerase as a negative control, (C) the testis of male worms, (D) the ovary of female worms. mc, mesenchymal cells; ov, ovary; tg, tegument; ts, testis; vg, vitelline gland.

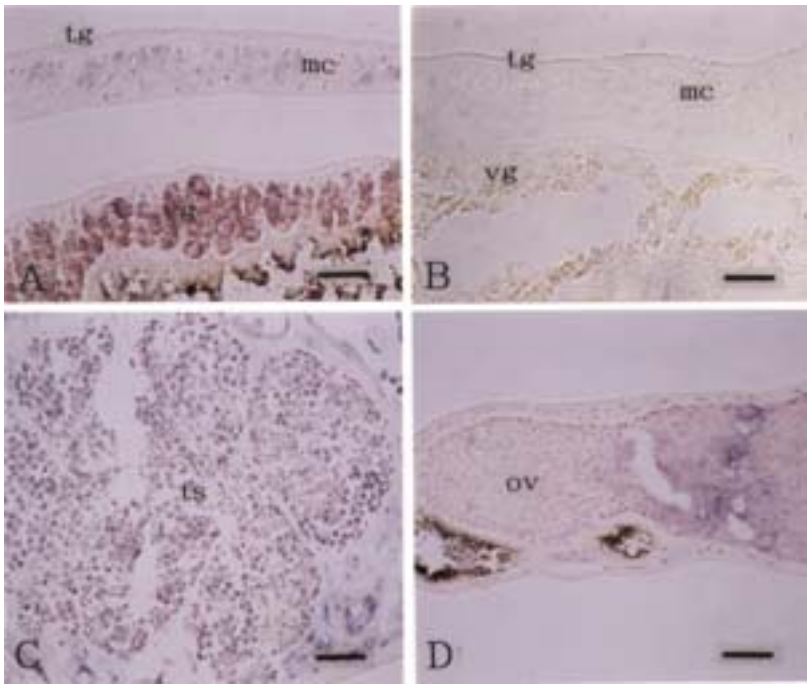

Fig 3-Blight-field micrographs showing the localization of the sequence of mouse class I MHC in *S. japonicum* recovered from C57BL/6 mice. *In situ* PCR and hybridization was performed on the frozen sections. The positive signals are purple-blue precipitates. Scale bar is 25 μm in all photos. (A) middle portion of male and female worms, (B) middle portion of male and female worms that had *in situ* PCR performed without *Taq* DNA polymerase as a negative control, (C) the testis of male worms, (D) the ovary of female worms. mc, mesenchymal cells; ov, ovary; tg, tegument; ts, testis; vg, vitelline gland.

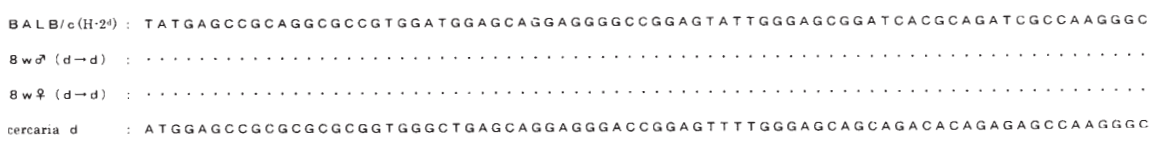

Fig 4-Comparison of the nucleotide sequences of the mouse class I MHC in the d→d group. From the top of the nucleotide sequences, DNA samples: BALB/c mice liver, 8-week-old male and female *S japonicum* (d→d) recovered from BALB/c mice infected with cercaria d and *S. japonicum* cercariae (cercaria d) maintained in BALB/c mice; a dot denotes sequence identity with the host sequence.

logy of adult worms and BALB/c mice, the previous host, was 89.3% and that of adult worms and C57BL/6 mice from which adult worms were recovered, was 92.0%. In the case of the b→b group, the sequence homology of adult worms and BALB/c mice was 88.0% and that of adult worms and C57BL/6 mice was 92.0%.

In addition, the sequence homology of male and female worms and cercariae used for the infection was lower than that of male and female worms and the host from which adult worms were recovered. However, the sequence homology of b→b group adult worms and cercariae b and the sequence homology of b→b group adult worms and C57BL/6 mice were the same (92.0%).

The nucleotide sequence comparisons in each group between the host from which adult worms were recovered, the male and female worms and the cercariae used for the infection are shown in Figs 4-7. Where necessary, the sequencing data of adult worms are attached. As seen in Figs 4-7, 17 nucleotides in italic of male and female worm sequences were not identical with nucleotides of the host sequence. Yet they were identical with nucleotides of cercarial sequence used for the infection. The underlined 9 nucleotides differed from the host sequence and the cercarial sequence. However, they had a minor signal (indicated by an arrow in sequencing data) that represented a nucleotide of host sequence in addition to a major signal.

DISCUSSION

Although it has been well known that mouse MHC antigens are expressed on the surface of schistosomes (Gitter *et al*, 1982; Simpson *et al*, 1983; Sher *et al*, 1987), the origin of these MHC antigens is still unclear. Our previous work involved the finding of host DNA sequences including the class I MHC loci in schistosome DNA by blot hybridization and PCR analysis (Iwamura *et al*, 1995).

In this current work, we used the *in situ* PCR and hybridization method to determine the localization of the sequence of mouse class I MHC in *S.japonicum*. The sequence of mouse class I MHC was localized in the nuclei of the mesenchymal and reproductive cells of both 8-week-old *S. japonicum* recovered from BALB/c and C57BL/6 mice. The signals of mouse class I MHC sequence showed similar localization to those of the repetitive sequences such as mouse type 2 *Alu* sequence and mouse endogenous type A and type C retroviral sequences as previously reported (Imase *et al*, 1999; 2000). These findings supported the existence of DNA homologies between host and schistosomes. The host DNA sequences may have originated from viral infection (Howell, 1985) or the induction of a transposon (Laski *et al*, 1986). It might be that DNA homologies between host and parasite point to direct incorporation of host genetic material into the parasite genome (Salzet *et al*, 2000).

Furthermore, the mouse class I MHC sequence was detected in *S. japonicum* cercarial DNA. It supports the idea that the MHC gene was vertically transmitted in *S. japonicum* as reported previously on *S.mansoni* (Imase *et al*, 2001). As the mouse class I MHC gene is well known for its high level of genetic polymorphism (Pullen *et al*, 1992), the comparison between the nucleotide sequences of class I MHC detected in the DNA of male and female worms, the DNA of hosts (BALB/c and C57BL/6 mice) and the DNA of cercariae used for the infection could shed some light on the origin of this sequence existing in schistosomes.

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BALB/c(H-2d) : TATGAGCCGCAGGCGCCGTGGATGGAGCAGGAGGGGCCGGAGTATTGGGAGCGGATCACGCAGATCGCCAAGGGC
8w♂ (b→d)    : ·····························································
8w♀ (b→d)    : ··········G·····G···························································
cercaria b   : TTGGAGCCGCGGGCGCCGTGGATGGAGCAGGAGGGGCCGGAGTATTGGGAGCGGATCACGCAGATCGCCAAGGGA
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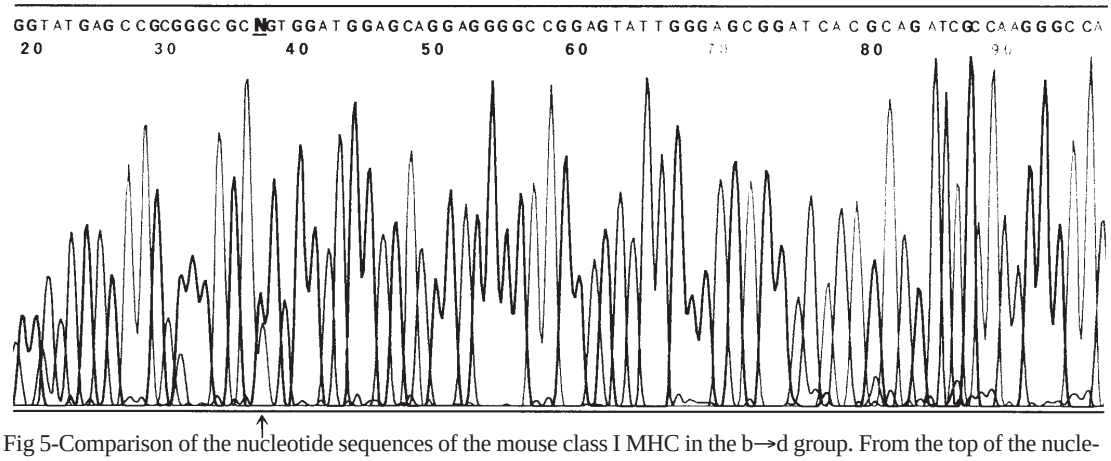

Fig 5-Comparison of the nucleotide sequences of the mouse class I MHC in the b→d group. From the top of the nucleotide sequences, DNA samples: BALB/c mice liver, 8-week-old male and female *S japonicum* (b→d) recovered from BALB/c mice infected with cercaria b and *S. japonicum* cercariae (cercaria b) maintained in C57BL/6 mice. A dot denotes sequence identity with the host sequence. The nucleotide in italic indicates the nucleotide identical to the cercarial sequence and different from that of the host MHC sequence. The underlined nucleotide differed from both the host MHC sequence and the cercarial MHC sequence. It had a minor signal indicated by an arrow in the sequencing data in addition to a major signal. The sequencing data of 8-week-old female *S. japonicum* of b→d group is attached.

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C57BL/6 (H-2b) : TATGAGCCGCGGGCGCCGTGGATGGAGCAGGAGGGGCCGGAGTATTGGGAGCGGGAAACACAGAAAGCCAAGGGC
8w♂ (d→b)      : ·TG···········G·····························G·····TC·····
8w♀ (d→b)      : ·TG···········G·····························G·····TC·····
cercaria d     : ATGGAGCCGCGCGCGCGGTGGGCTGAGCAGGAGGGACCGGAGTTTTGGGAGCAGCAGACACAGAGAGCCAAGGGC
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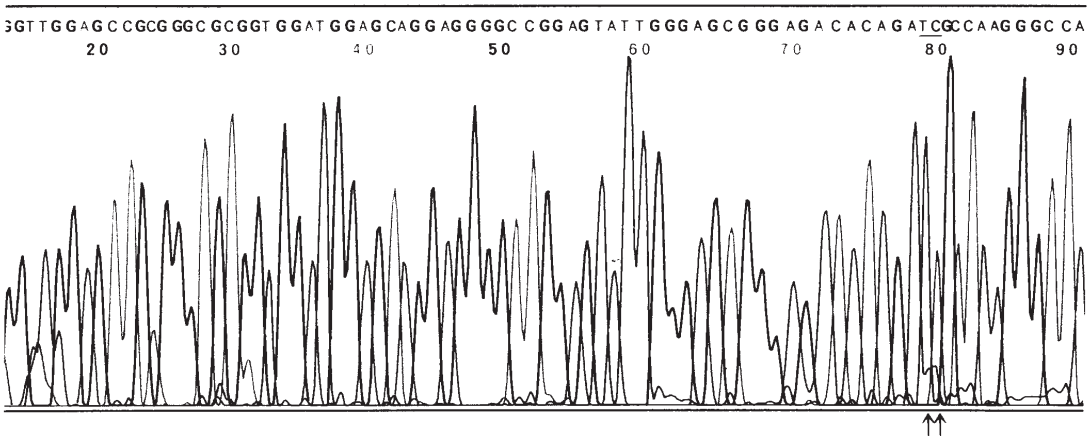

Fig 6-Comparison of the nucleotide sequences of the mouse class I MHC in the d→b group. From the top of the nucleotide sequences, DNA samples: C57BL/6 mice liver, 8-week-old male and female *S japonicum* (d→b) recovered from C57BL/6 mice infected with cercaria d and *S. japonicum* cercariae (cercaria d) maintained in BALB/c mice. A dot denotes sequence identity with the host sequence. The nucleotide in italic indicates the nucleotide identical to the cercarial sequence and different from that of the host MHC sequence. The underlined nucleotide differed from both the host MHC sequence and the cercarial MHC sequence. It had a minor signal indicated by an arrow in the sequencing data in addition to a major signal. The sequencing data of 8-week-old male *S. japonicu*m of d→b group is attached.

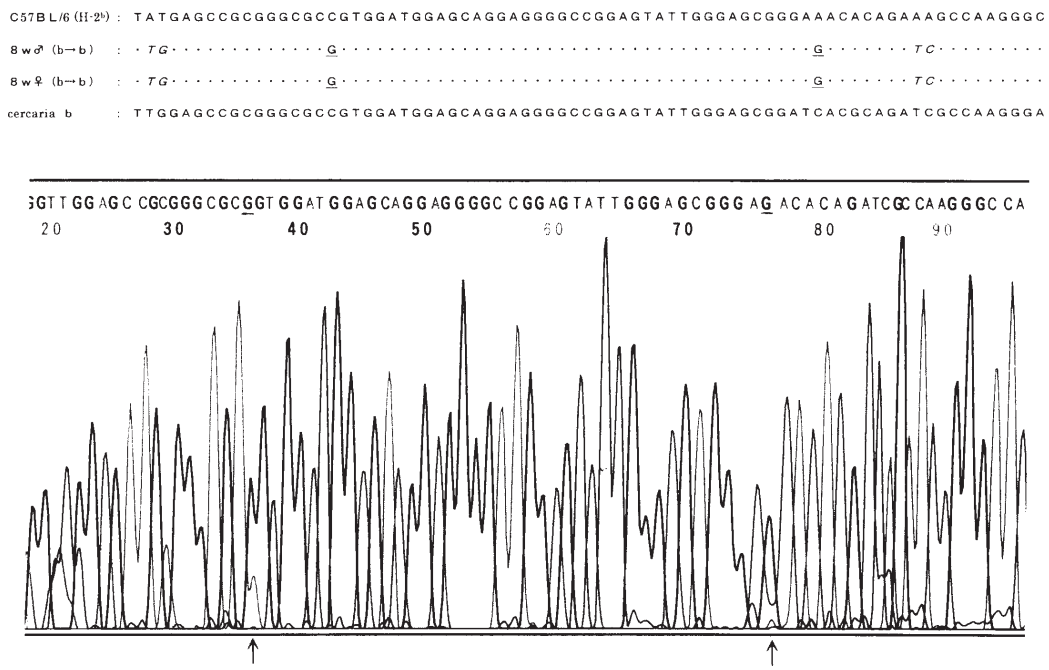

Fig 7-Comparison of the nucleotide sequences of the mouse class I MHC in the b→b group. From the top of the nucleotide sequences, DNA samples: C57BL/6 mice liver, 8-week-old male and female *S japonicum* (b→b) recovered from C57BL/6 mice infected with cercaria b and *S. japonicum* cercariae (cercaria b) maintained in C57BL/6 mice. A dot denotes sequence identity with the host sequence. The nucleotide in italic indicates the nucleotide identical to the cercarial sequence and different from that of the host MHC sequence. The underlined nucleotide differed from both the host MHC sequence and the cercarial MHC sequence. It had a minor signal indicated by an arrow in the sequencing data in addition to a major signal. The sequencing data of 8-week-old male *S. japonicum* of b→b group is attached.

As seen in Table 1, the nucleotide sequences of class I MHC detected in male and female worm DNA recovered from BALB/c mice (d→d and b→d groups) were entirely identical with a host MHC sequence except for a female worm sequence of the b→d group. This female worm sequence resembled a host MHC sequence (sequence homology; 97.3%). On the other hand, the nucleotide sequences of class I MHC detected in male and female worm DNA recovered from C57BL/6 mice (d→b and b→b groups) differed from a host MHC sequence in several nucleotides. We may link these results with the evidence that the BALB/c mouse is the preferred host for *S.japonicum*.

As shown in Figs 4-7, 17 nucleotides in italic and 9 underlined nucleotides of adult worm sequences differed from the host nucleotide sequence. Seventeen nucleotides in italic were identical with the cercarial nucleotide sequence. The 9 underlined nucleotides had a minor signal which showed the host nucleotide sequence. It seems that the class I MHC detected in adult worm DNA could possibly be derived from cercariae and the host respectively. Moreover, the nucleotide sequences of class I MHC detected in adult worm DNA are more closely related to the host sequence than to the cercarial sequence. It should be kept in mind that adult worm DNA has a much larger amount of the horizontal transmitted (host) sequence than the vertical transmitted (cercaria) sequence.

In our previous papers, we reported that the type A retroviral sequence was selectively propagated in schistosome progeny (Imase *et al*, 2000), and the mouse class I MHC sequence was horizontally and vertically transmitted in *S.mansoni* (Imase *et al*, 2001). In this paper, we present evi-

Table 1
Sequence homology of mouse class I MHC sequence detected in amplified DNA. DNA sources: 8-week-old male and female *S. japonicum* from the d→d, b→d, d→b and b→b groups, cercaria d and cercaria b, BALB/c and C57BL/6 mice liver.

	Mouse		Cercaria	
	BALB/c	C57BL/6	Cercaria d	Cercaria b
8w ♂ (d→d)	100.0%	90.7%	85.3%	
8w ♀ (d→d)	100.0%	90.7%	85.3%	
8w ♂ (b→d)	100.0%	90.7%		95.9%
8w ♀ (b→d)	97.3%	90.7%		95.9%
8w ♂ (d→b)	89.3%	92.0%	85.3%	
8w ♀ (d→b)	89.3%	92.0%	85.3%	
8w ♂ (b→b)	88.0%	92.0%		92.0%
8w ♀ (b→b)	88.0%	92.0%		92.0%

*BALB/c : C57BL/6 = 90.7%

dence of the horizontal and vertical transmission of the mouse class I MHC in *S.japonicum*. The existence of such class I MHC sequences in schistosome may be correlated to the evolutionary origin of parasite molecular mimicry (Damian *et al*, 1973; Damian, 1979; 1987; 1997). The host DNA sequences selectively retained in cercarial genome may play an important role in evading host immune attack. These findings may help throw some light on the study of molecular mimicry and host-parasite co-evolution.

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REFERENCES

Cole MD, Ono M, Huang RCC. Terminally redundant sequences in cellular intracisternal A-particle genes. *J Virol* 1981; 38: 680-7.

Damian RT. Molecular mimicry in biological adaptation. In: Nickol BB, ed. Host-parasite interfaces. New York: Academic Press, 1979: 103-26.

Damian RT. Molecular mimicry revised. *Parasitol Today* 1987; 3: 263-6.

Damian RT. Parasite immune evasion and exploitation: reflections and projections. *Parasitology* 1997; 115: s169-s175.

Damian RT, Greene ND, Hubbard WJ. Occurrence of mouse α_2-macroglobulin antigenic determinants on *Schistosoma mansoni* adults, with evidence on their nature. *J Parasitol* 1973; 59: 64-73.

Evans GA, Margulies DH, Camerini-Otero RD, Ozato K, Seidman JG. Structure and expression of a mouse major histocompatibility antigen gene, H-2L^d. *Proc Nat Acad Sci USA* 1982; 79: 1994-8.

Gitter BD, McCormick SL, Damian RT. Murine alloantigen acquisition by *Schistosoma mansoni*: presence of H-2K determinants on adult worms and failure of allogeneic lymphocytes to recognize acquired MHC gene products on schistosomula. *J Parasitol* 1982; 68: 513-8.

Howell MJ. Gene exchange between hosts and parasites. *Int J Parasitol* 1985; 15: 597-600.

Imase A, Kumagai T, Ohmae H, Irei Y, Iwamura Y. Localization of mouse type 2 *Alu* sequence in schistosomes. *Parasitology* 1999; 119: 315-21.

Imase A, Kobayashi K, Ohmae H, Irie Y, Iwamura Y. Horizontal and vertical transfer of mouse endogenous retroviral DNA sequences in schistosomes. *Parasitology* 2000; 121: 155-62.

Imase A, Kobayashi K, Ohmae H, Matsuda H, Iwamura Y. Horizontal and vertical transmission of mouse class I MHC sequence in *Schistosoma mansoni*. *Parasitology* 2001; 123: 163-8.

Irie Y, Iwamura Y. Host-related DNA sequences are

localized in the body of schistosome adults. *Parasitology* 1993; 107: 519-28.

Iwamura Y, Irie Y, Kominami R, Nara T, Yasuraoka K. Existence of host-related DNA sequences in the schistosome genome. *Parasitology* 1991; 102: 397-403.

Iwamura Y, Yonekawa H, Irie Y. Detection of host DNA sequences including the H-2 locus of the major histocompatibility complex in schistosomes. *Parasitology* 1995; 110: 163-70.

Khan AS, Rowe WP, Martin MA. Cloning of endogenous murine leukemia virus-related sequences from chromosomal DNA of BALB/c and AKR/J mice: identification of an *env* progenitor of AKR-247 mink cell focus-forming proviral DNA. *J Virol* 1982; 44: 625-36.

Kominami R, Muramatsu M, Moriwaki K. A mouse type 2 *Alu* sequence (M2) is mobile in the genome. *Nature* 1983; 301: 87-9.

Laski FA, Rio DC, Rubin GM. Tissue specificity of *Drosophia* P element transposition is regulated at the level of mRNA splicing. *Cell* 1986; 44: 7-19.

Nara T, Iwamura Y, Tanaka M, Irie Y, Yasuraoka K. Dynamic changes of DNA sequences in *Schistosoma mansoni* in their course of development. *Parasitology* 1990; 100: 241-5.

Nuovo GJ. PCR *in situ* hybridization. In: Nuovo GJ, ed. PCR *in situ* hybridization, protocols and applications. New York: Raven Press, 1994: 193-244.

Nuovo GJ. *In situ* PCR: protocols and applications. In: Dieffenbach CW, Dveksler GS, Cuddihy J, eds. PCR methods and applications. New York: Cold Spring Harbor Laboratory Press, 1995: s151-s167.

Pullen JK, Horton RM, Cai Z, Pease LR. Structural diversity of the classical H-2 genes: K, D and L. *J Immunol* 1992; 148: 953-67.

Salzet M, Capron A, Stefano GB. Molecular crosstalk in host-parasite relationship: Schistosome-and leech-host interactions. *Parasitol Today* 2000; 16: 536-40.

Schmid CW, Jelinek WR. The *Alu* family of dispersed repetitive sequences. *Science* 1982; 216: 1065-70.

Sher A, Hall BF, Vadas MA. Acquisition of murine major histocompatibility complex gene products by schistosomula of *Schistosoma mansoni*. *J Exp Med* 1978; 148: 46-57.

Simpson AJG, Singer D, McCutchan TF, Sacks DL, Sher A. Evidence that schistosome MHC antigens are not synthesized by the parasite but are acquired from the host as intact glycoproteins. *J Immunol* 1983; 131: 962-5.

Smithers SR, Terry RJ. The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of the adult worms. *Parasitology* 1965; 55: 695-700.

Tanaka M, Iwamura Y, Amanuma H, *et al*. Integration and expression of murine retrovirus-related sequences in schistosomes. *Parasitology* 1989; 99: 31-8.