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 polymerase chain reaction (in situ PCR) results showed evidence of horizontal gene transfer from the host to schistosomes. 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.

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. japonicum* 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. japonicum* 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. japonicum* from the d→d, d→b, b→d and b→b groups, *S. japonicum* 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 CTGCTCTTGGCCCTTGG. Fifty µl of the amplifying solution containing 0.5 mM 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 94°C for complete denaturation of the DNA, 40 cycles for cercarial DNA, adult worm DNA and planaria DNA and 30 cycles for mouse DNA, at 94°C for 30 seconds, 55°C for 25 seconds and 72°C 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 55°C. 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 of a thermal cycler (Perkin Elmer). The PCR conditions was as follows: Initial denaturation at 94°C for 150 seconds followed by 40 cycles of 94°C denaturation for 3 seconds, 50°C annealing for 20 seconds, and 72°C 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;CACACTCGATGCCGTTATTTCGTCTGGTTGTAGTAGC GGAC, inner primers; CATCTCTGTCGGCTACTGGAGGCCTCAGGTTCACTCG.

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 94°C for complete denaturation of the DNA, 40 cycles for cercarial DNA, adult worm DNA and planaria DNA and 30 cycles for mouse DNA, at 94°C for 30 seconds, 55°C for 25 seconds and 72°C 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 55°C. Ten microliters of the nested PCR reaction mixture was electrophoresed on a 2.5% agarose gel.
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. japonicum* 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. japonicum* 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. japonicum* 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 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.
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.
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.

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. japonicum of d→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 evo-
Detection of Mouse MHC Sequence in *S. japonicum*

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.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Cercaria d</th>
<th>Cercaria b</th>
</tr>
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<tbody>
<tr>
<td>BALB/c</td>
<td>85.3%</td>
<td>85.3%</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>90.7%</td>
<td>95.9%</td>
</tr>
</tbody>
</table>

*BALB/c : C57BL/6 = 90.7%

Evidence 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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