Horizontal and vertical transfer of mouse endogenous retroviral DNA sequences in schistosomes

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SUMMARY

The *in situ* polymerase chain reaction (PCR) results revealed that mouse type A and type C retroviral sequences were transmitted horizontally from the host to schistosomes. The signals to these retroviral sequences were observed in the nuclei of the mesenchymal and reproductive cells of 8-week *Schistosoma japonicum*. These signals were also detected in the nuclei of the mesenchymal and reproductive cells and in the cytoplasm of the tegumental tubercles of 24-week *S. mansoni*. Furthermore, mouse type A retroviral sequence was detected in the DNA extracted from the cercariae of both species. However, mouse type C retroviral sequence and mouse type 2 *Alu* sequence (B2) were difficult to detect in the cercarial DNA of either species. These findings may indicate that some host sequences are propagated in the schistosome progeny, that is to say, not only horizontal but also vertical transfer of the host gene may occur in schistosomes.

Key words: *Schistosoma japonicum*, *Schistosoma mansoni*, mouse type A retrovirus, mouse type C retrovirus, *in situ* PCR.

INTRODUCTION

The existence of common or host antigens between *Schistosoma mansoni* and laboratory hosts has been previously demonstrated (Damian, 1964, 1967; Smithers, Terry & Hockley, 1968; Smithers & Terry, 1969). Since that time some hypotheses have been presented to explain the mechanism of schistosome–host antigen sharing (Smyth, 1973; Capron *et al*. 1976; Smithers & Terry, 1976; Damian, 1979, 1987). Antigenic similarity between *S. mansoni* and intermediate snail hosts has also been described (Dissous, Grzych & Capron, 1986).

We reported that DNA sequences homologous to repetitive sequences in the host genome such as mouse type 1 *Alu* sequences (B1) (Krayev *et al*. 1980), type 2 *Alu* sequence (B2) (Schmid & Jelinek, 1982; Kominami, Muramatsu & Moriwaki, 1983) and mo-2 sequence (Kominami, Takahashi & Mitani, 1989), and the mouse retrovirus related sequences such as mouse intracisternal A-particle (Cole, Ono & Huang, 1981) and endogenous type C retrovirus (A-1) sequence (Khan, Rowe & Martin, 1982) were detected in the DNA of *Schistosoma japonicum* (Iwamura *et al*. 1991). DNA sequences homologous to the *env* region of the mouse ecotropic and xenotropic retroviruses (Chan *et al*. 1980; Buckler *et al*. 1982) were detected in the DNA of male *S. mansoni* by blot hybridization (Tanaka *et al*. 1989; Iwamura & Irie, 1992). In addition, the signals to the sequences of the mouse type C and type A retroviruses were observed in the body of *S. japonicum* and the signals to the *env*-specific region of the mouse ecotropic type C retrovirus were detected in the body of *S. mansoni* by *in situ* hybridization. All of these signals were localized in the subtegumental layer and inner tissues of schistosomes (Irie & Iwamura, 1993). By using polymerase chain reaction (PCR), we dicovered that the DNA extracted from *S. japonicum* and *S. mansoni* contained the H-2 locus of the major histocompatibility complex of the mouse (Iwamura, Yonekawa & Irie, 1995). The presence of host DNA sequences in the schistosome genome has been the subject of some discussion and speculation (Simpson & Pena, 1991; Clough, Drew & Brindley, 1996; Damian, 1997). We recently reported that the B2 sequence, a highly repetitive DNA sequence in the mouse, was localized in the schistosome bodies by using *in situ* polymerase chain reaction (PCR) (Imase *et al*. 1999). The signals were observed in the nuclei of the mesenchymal and reproductive cells. They were also detected in the cytoplasm of the tegument of both schistosomes.

Our present research showed the localization of mouse endogenous type A and type C retroviral sequences in both schistosomes by *in situ* PCR. Our work indicated that the horizontal transfer of host sequences occurred in schistosomes. We then

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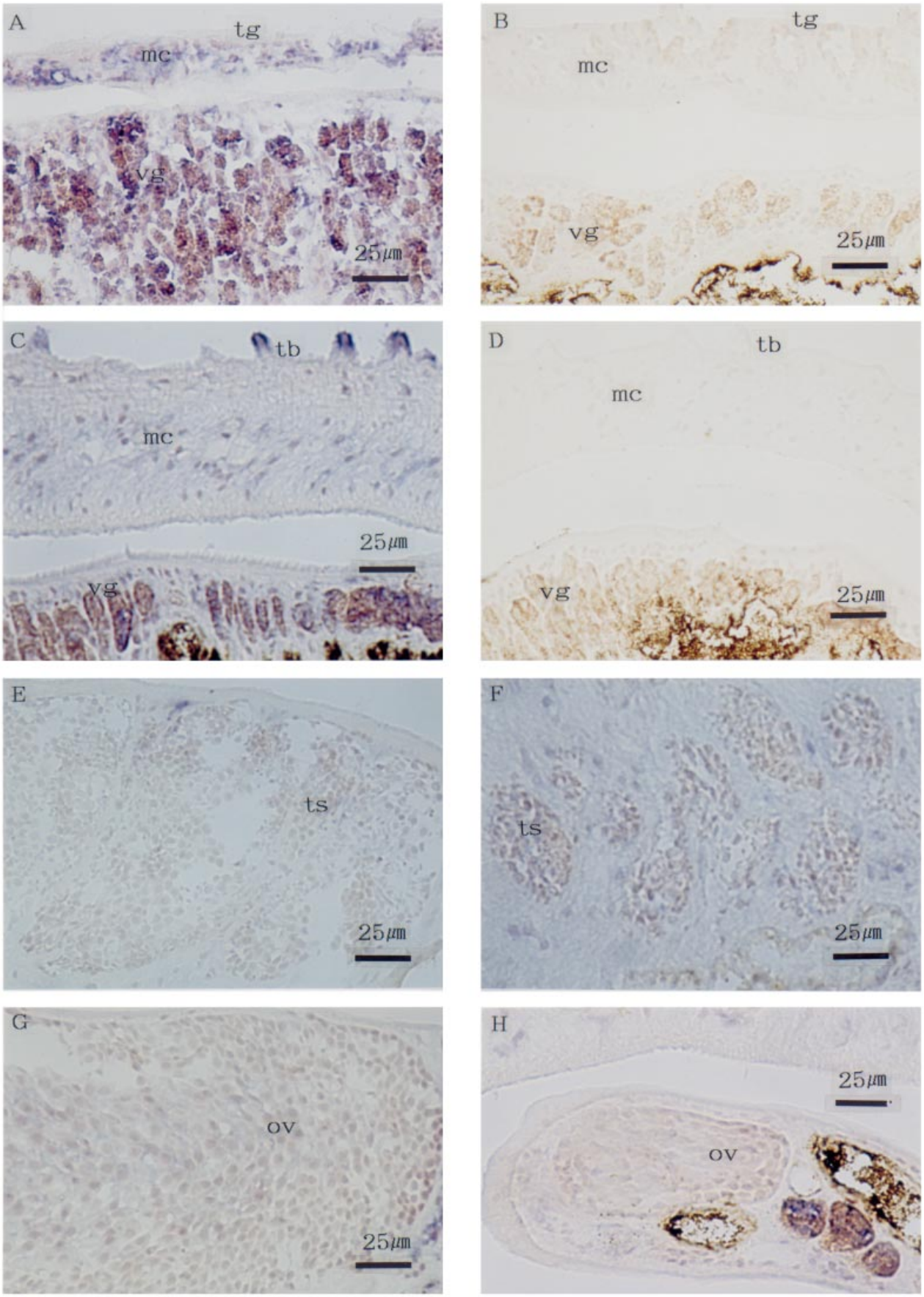

Fig. 1. Localization of mouse type A retroviral sequence in schistosomes. (A) Middle portion of male and female *Schistosoma japonicum*. (B) Middle portion of male and female *S. japonicum* which performed *in situ* PCR without *Taq* DNA ploymerase as a negative control. (C) Middle portion of male and female *S. mansoni*. (D) Middle portion

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attempted to detect host sequences in cercariae to determine the vertical transfer of the sequences.

MATERIALS AND METHODS

Parasites

A Japanese strain of *Schistosoma japonicum* and a Puerto Rican strain of *S. mansoni* were maintained by standard laboratory procedures in male C57BL/6 or ICR mice and their snail hosts, *Oncomelania nosophora* and *Biomphalaria glabrata*, respectively. Schistosomes were recovered by perfusion (Smithers & Terry, 1965) with RPMI 1640 solution (pH 7·4) from the hepatic portal system of male C57BL/6 mice at 8 weeks after percutaneous infection with 50 cercariae/mouse of *S. japonicum* and at 24 weeks after infection with 30 cercariae/mouse of *S. mansoni*.

Preparation of DNA

DNA was extracted from *S. japonicum* and *S. mansoni* cercariae respectively, and from the liver of male ICR mice. DNA extraction was performed by using the WB DNA Extractor Kit (Wako) with the sodium iodide method.

Preparation of tissue sections

After worm recovery by perfusion, schistosomes were fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS), pH 7·2, containing 3·4% sucrose at 4 °C for 2 h. They were then washed 3 times with PBS (pH 7·2) containing 3·4% sucrose and retained in PBS (pH 7·2) containing 20% sucrose.

After the fixation, schistosomes were frozen in OCT compound (Miles) using crushed dry ice and ethanol. Frozen sections were cut with a cryostat (Tissue-Tek II) at a thickness of 6 μm, and mounted onto silane-coated slides (Perkin–Elmer). Proteinase K (Wako) digestion at 10 ng/ml for 20 min at 37 °C was necessary before *in situ* PCR (Nuovo, 1994, 1995).

C57BL/6 mouse liver as a positive control and planaria as a negative control for this experiment were fixed, frozen and sectioned in the same manner. The tissue section of C57BL/6 mouse liver was digested by proteinase K at 30 μg/ml and planaria at 10 ng/ml before *in situ* PCR.

In situ *PCR*

The mouse type A retroviral specific primers were designed from the sequence of the *gag* region of a mouse genomic intracisternal A-particle (IAP) element, MZA 14 (Mietz *et al*. 1987). The sequences were GGAATCCTTCTCAGATTTTGTGG and GAGCCAGTCTTGTAAGCCTTTG respectively. The mouse type C retroviral specific primers were designed from the envelope gene of leukemia virus-induced C57BL/ka mouse thymic lymphoma (Merregaert, Nuyten & Janowski, 1985). The sequences were CCATCAGGCCCTGTGTAATACCA and CTCAACCAGGACACAGTAATCGG respectively. 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 *Taq* DNA polymerase (TaKaRa EX-*Taq*) were placed over the tissue section (Nuovo, 1994, 1995). The glass slide was covered with a plastic cover-slip (Perkin–Elmer) and placed directly on the sample block of a thermal cycler (Perkin–Elmer).

The C57BL/6 mouse liver tissue section as a positive control and the planaria tissue section as a negative control were used throughout this experiment.

The PCR conditions were as follows. Initial denaturation at 95 °C for 150 sec followed by 30 cycles of 95 °C denaturation for 3 sec, annealing temperature for 20 sec, and 72 °C extension for 15 sec. The annealing temperatures for type A and type C retroviral sequences were 62 °C and 64 °C respectively.

Detection after in situ *PCR*

The digoxigenin-11-dUTP (DIG)-labelled random primed type A and type C retroviral specific DNA probes were prepared using the DIG DNA Labelling Kit (Boehringer–Mannheim) as per the manufacturer's instructions.

After *in situ* PCR, the sections were treated with acetylation buffer (0·1 M triethanolamine buffer, pH 8·0) at room temperature for 10 min (Hayashi *et al*. 1978). They were then denatured in 70% formamide/2 × SSC at 70 °C for 2 min and soaked in 70% ethanol on ice for 5 min and finally soaked in 100% ethanol for 5 min.

The hybridization buffer contained 1 ng/μl DIG-labelled DNA probe (denatured), 0·1 μg/μl denatured salmon sperm DNA (Sigma), 0·2%

of male and female *S. mansoni* which performed *in situ* PCR without *Taq* DNA polymerase as a negative control. (E) The testis of male *S. japonicum*. (F) The testis of male *S. mansoni*. (G) The ovary of female *S. japonicum*. (H) The ovary of female *S. mansoni*. mc, mesenchymal cells; tg, tegument; vg, vitelline gland; ts, testis; ov, ovary; tb, tubercle.

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bovine serum albumin (BSA), 2 × SSC (pH 7·0), 50 % formamide and 10 % dextran sulphate. A volume of 100 μl of the hybridization buffer, denatured at 85 °C for 10 min, were placed on the tissue section. The sections were covered by Parafilm and hybridized overnight at 42 °C. The slides were then washed in 50 % formamide/2 × SSC, pH 7·0 at 40 °C for 15 min and finally by 2 washes of 15 min in 2 × SSC at room temperature (Weier, Kleine & Gray, 1991).

Detection of DIG was done with an alkaline phosphatase-conjugated anti-digoxigenin-labelled antibody at a 1 : 500 dilution using the DIG Nucleic Acid Detection Kit (Boehringer–Mannheim) as per the manufacturer's protocol. The alkaline phosphatase-based colorimetric detection method used the chromogen nitroblue tetrazolium (NBT) in the presence of 5-bromo-4-chloro-3-idolylphosphate (BCIP) and yielded a purple-blue precipitate as a positive cell marker.

Polymerase chain reaction (*PCR*)

The sequences of type A and type C retroviral specific primers were described in the paragraph of *in situ* PCR. The sequences of the B2 specific primers were described before (Imase *et al*. 1999). Amplification was made with 30 ng DNA in 10 μl volume containing 0·5 μM of each primer and 0·25 U of *Taq* DNA polymerase (TaKaRa EX-*Taq*). In the case of mouse DNA, 0·3 ng of DNA was used for amplification.

After a 3-min incubation at 94 °C for complete denaturation of the DNA, 40 cycles for cercarial DNA and planaria DNA and 30 cycles for ICR mouse DNA, at 94 °C for 30 sec, each annealing temperature for 25 sec and 72 °C for 20 sec were done in a DNA thermal cycler (Thermal sequencer TSR-300, Iwaki Garasu) as described by Iwamura *et al*. (1995).

Analysis of the amplified products by PCR

Ten μl of the PCR reaction mixture was electrophoresed on a 3 % agarose gel. The DNA was transferred onto a nylon membrane (Schleicher & Schuell, Naitran), and was hydridized at 42 °C for 18 h with the oligonucleotide probes labelled by [γ-^{32}P]dATP at the 5′ end, in the buffer containing 5 × SSC, 7 % SDS, 20 mM NaH_2PO_4, 10 × Denhardt's solution (pH 7·0), 10 % dextran sulphate and 20 μg/ml salmon sperm DNA. The membrane was then washed to a stringency of 6 × SSC at 55 °C for 10 min, followed by autoradiography with an intensifying screen at −80 °C for 4 h as described by Iwamura *et al*. (1995). Labelled probes used were as follows: mouse type A retroviral sequence (*gag* locus); GAGCGTATTT-TTGGAGAGTCAGAG, mouse type C retroviral sequence (*env* locus); ACCATTTGGGCTTGCA-ACACCGGG, B2 sequence; AGGGAGTCAGA-TCTTGTTACG.

RESULTS

Localization of mouse type A retroviral sequence in schistosomes

The localization of mouse type A retroviral *gag* sequence was detected by *in situ* PCR and hybridization with a digoxigenin-11-dUTP (DIG) labelled probe. The signal of the type A retroviral 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 *S. japonicum* and *S. mansoni* (Fig. 1). The sequence was also detected in the cytoplasm of the tegumental tubercles of male *S. mansoni* (Fig. 1C).

To compare the positive and negative cells in both schistosomes, *in situ* PCR was done without *Taq* DNA polymerase. The negative cells were shown in Fig. 1B and D.

The positive and negative controls with *in situ* PCR were extremely important. A successful run was defined by a strong signal in the positive control and no signal in the negative control. The C57BL/6 mouse liver tissue section was used as a positive control. The signals of the type A retroviral sequence were clearly detected in the nuclei of the liver cells. The free-living planaria tissue section was used as a negative control, because this organism belonged to the same phylum. As expected, no signal was detected in this worm (data not shown).

Localization of mouse type C retroviral sequence in schistosomes

The signals of type C retroviral *env* sequence were observed in the nuclei of the mesenchymal cells and in the nuclei of the testicular, ovarian and vitelline cells of *S. japonicum* and *S. mansoni* (Fig. 2). In male *S. mansoni* the signals were found in the cytoplasm of the tegumental tubercles (Fig. 2C).

To compare the positive and negative cells in both schistosomes, *in situ* PCR was performed without *Taq* DNA polymerase. The negative cells were shown in Fig. 2B and D.

Detection of the host sequences in cercarial DNA

Type A retroviral sequence was detected in *S. mansoni* and *S. japonicum* cercarial DNA (Fig. 3A). The nucleotide sequences of the type A retrovirus detected in the DNA from cercariae of both species

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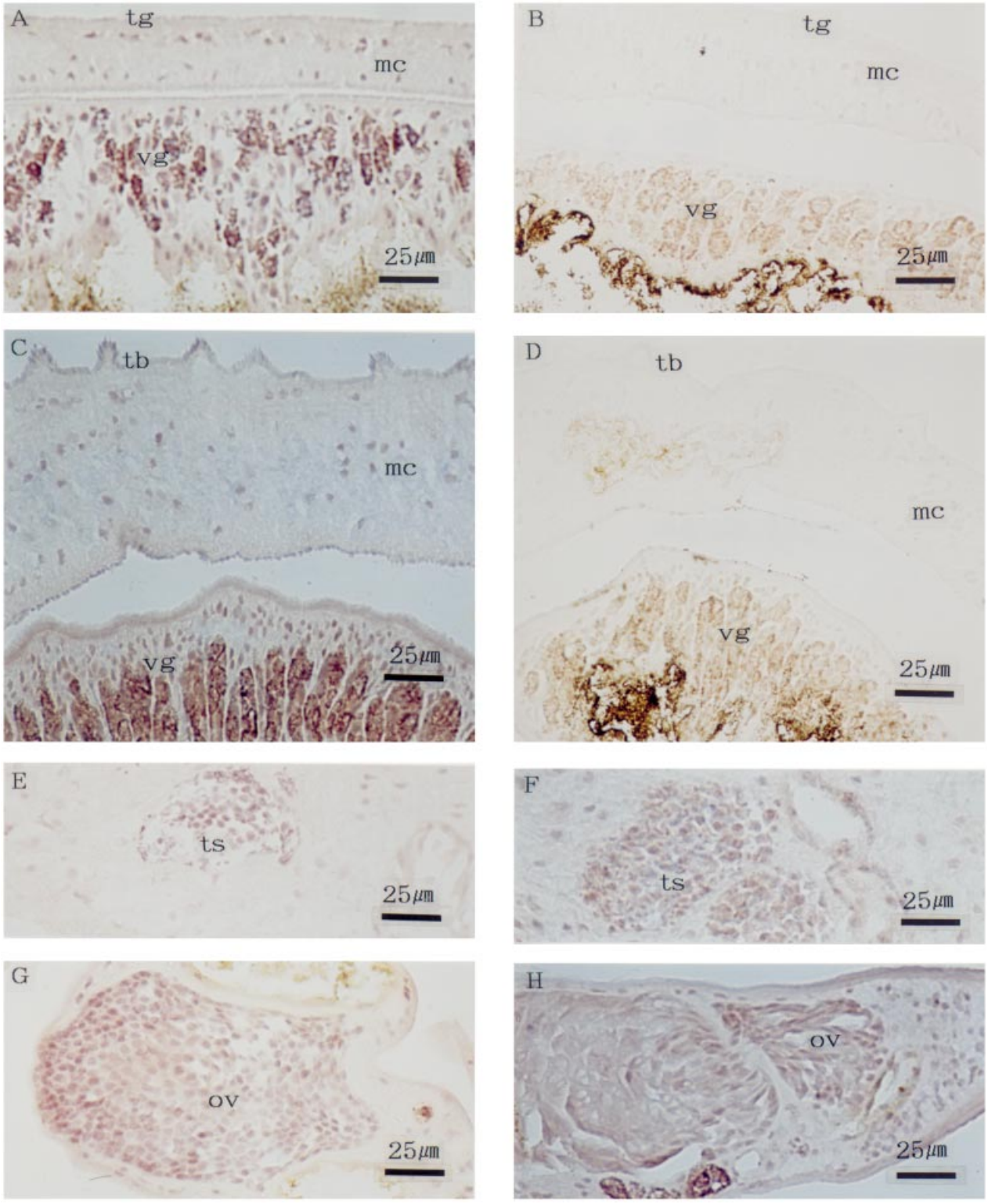

Fig. 2. Localization of mouse type C retroviral sequence in schistosomes. (A) Middle portion of male and female *Schistosoma japonicum*. (B) Middle portion of male and female *S. japonicum* which performed *in situ* PCR without *Taq* DNA polymerase as a negative control. (C) Middle portion of male and female *S. mansoni*. (D) Middle portion of male and female *S. mansoni* which performed *in situ* PCR without *Taq* DNA polymerase as a negative control. (E) The testis of male *S. japonicum*. (F) The testis of male *S. mansoni*. (G) The ovary of female *S. japonicum*. (H) The ovary of female *S. mansoni*. mc, mesenchymal cells; tg, tegument; vg, vitelline gland; ts, testis; ov, ovary; tb, tubercle.

were identical with that of the laboratory host (ICR mouse) DNA (data not shown). Whereas type C retroviral sequence could not be detected in either cercarial DNA (Fig. 3B). Furthermore, the B2 sequence had also disappeared in the cercarial DNA of both species (Fig. 3C).

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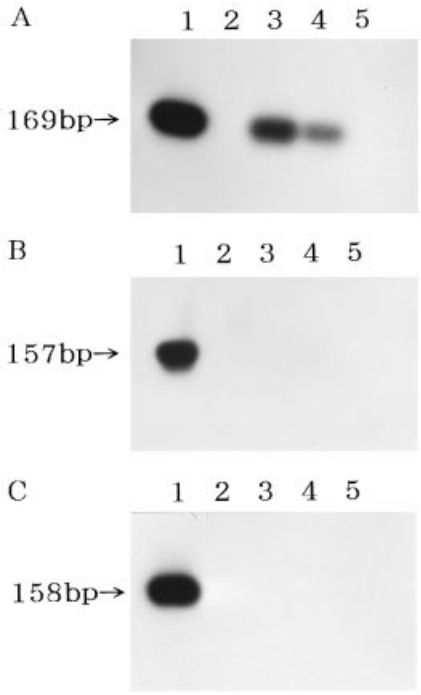

Fig. 3. Detection of the host sequence in cercarial DNA. [γ-^{32}P]dATP-labelled probes. (A) Mouse type A retroviral sequence, (B) mouse type C retroviral sequence, (C) B2 sequence. The sources of the DNA samples: Lane 1, ICR mouse liver cells DNA; Lane 2, no DNA; Lane 3, *Schistosoma mansoni* cercarial DNA; Lane 4, *S. japonicum* cercarial DNA; Lane 5, planaria DNA.

DISCUSSION

The *in situ* PCR and hybridization method provides direct histological evidence of the existence of host sequences in schistosomes. We previously reported the localization of the B2 sequence in both schistosomes. This highly repetitive sequence was known to have about 300000 copies in the mouse genome. Therefore, this sequence was a good marker to investigate the existence of host sequence in schistosomes. The present study demonstrated the localization of mouse endogenous type A and type C retroviral sequences in schistosomes. These endogenous retroviral sequences are middle repetitive in the mouse genome. There are approximately 1000 copies of the type A retroviral sequence (Lueders & Kuff, 1977; Ono *et al*. 1980) and about 40 copies of the type C retroviral sequence (Jaenisch *et al*. 1975; Bacheler & Fan, 1980) in the haploid mouse genome. The localizations of the type A retroviral sequence and B2 sequence in schistosomes resembled each other. Furthermore, the signals of the type C retroviral sequence also showed similar localization to those of the type A retroviral sequence and B2 sequence in both schistosomes.

These observations would suggest that the host sequences examined here were incorporated into the restricted cells of schistosomes from the host. Some of these incorporated host sequences may code certain host-like masking antigens in schistosomes. These sequences including the H-2 locus might be expressed for immune evasion. As pointed out by Howell (1985), a part of the gene exchange between hosts and parasites might be mediated by retroviruses.

In addition, retroviruses have the ability to enter the germ line genes of their host as endogenous proviruses and be transmitted vertically as Mendelian elements from parent to offspring. Our present *in situ* PCR results in which type A and type C retroviral sequences were detected in the nuclei of the germ cells suggested vertical transmission of these sequences in schistosomes. In fact, the type A retroviral sequence was detected in the DNA of cercariae of both schistosomes. However, the type C retroviral sequence and the B2 sequence could not be detected in either cercarial DNA. Considering that the highly repetitive B2 sequence and the middle repetitive type C retroviral sequence could not be detected in the cercarial DNA of either species, schistosomes might be selecting the sequences to propagate in their progeny. However, the mechanism by which this is done, is still unknown. These data showed that the incorporated type A retroviral sequence was more long-lived in schistosome progeny than type C retroviral sequence or B2 sequence. The products of type A retroviral sequence may play an important role in evading host immune attack. Our present data suggest that not only horizontal, but also vertical transfer of the host gene may occur in schistosomes. These findings may help shed some light on the study of molecular mimicry and host–parasite co-evolution.

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