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Research Note

Isolation of a cDNA Encoding an Antigenic Polypeptide Containing Repeating Units of *Spirometra erinaceieuropaei*

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Spirometra erinaceieuropaei (*S. erinaceieuropaei*), an intestinal tapeworm of wild or domesticated carnivores, is found commonly in the Orient (Sarma and Weilbaecher, 1986). In this parasitic cestode, final host is usually dog and cat. Humans are infected by the ingestion of the plerocercoid, which is called the sparganum. When the plerocercoid infects human, it migrates to and resides in subcutis, muscle, eye or scrotum (Chi *et al.*, 1980). On rare occasions, the larvae invade vital organs or the central nervous system (Fung *et al.*, 1989; Holodniy *et al.*, 1991). Our study on this parasite has been focused on isolation and gene cloning of the cysteine proteinase with strong antigenicity to the indefinitive host (Liu *et al.*, 1996). In order to study other antigenic proteins in molecular levels, a cDNA library constructed from poly $(A)^+$ RNA (Gubler and Hoffman, 1983) from plerocercoids was immunoscreened using the infected mouse-sera. cDNA inserts extracted from the selected recombinant phages were subcloned into pUC18 and nucleotide sequences of the inserts were determined by dideoxynucleotide chain termination method (Sanger *et al.*, 1977) using Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, INC, USA). In this short article we report the isolation and characterization of cDNA clones encoding antigenic polypeptide containing repetitive unit which represented about 30% of the library as determined by the percentage of positive plaques in immunoscreening experiments (The cDNA library was not amplified). Therefore, the mRNAs corresponded to cDNA clones containing repetitive sequences are likely to be abundant in plerocercoid of *S. erinaceieuropaei*. In total, nucleotide sequences of three cDNA clones containing 123 bp repeating unit were determined (SeRm-1, SeRm-2, SeRm-3). Structures of three clones are shown in Fig. 1a. SeRm-1 is the longest in length (1183 bp) with 8 sets of 123 nucleotides encoding 41 amino acid-tandem repeat which is similar to those in the genomic DNA clones with 43–45 amino acids in size of *Echinococcus granulosus* (Marin *et al.*, 1993). SeRm-2 was exactly the same in structure as SeRm-1 with 7 sets of repeat unit. However, guanine which is a first nucleotide of repeating unit of SeRm-1 and SeRm-2 is replaced by cytosine in SeRm-3. Nucleotide sequences of the repeating unit and nonrepeating regions are shown in Fig. 1b. Single repeat unit within SeRm-1 and SeRm-2 predicts a polypeptide with a molecular weight of 4335 which composed of about 41.5% of hydrophobic amino acid residues containing 5 leucines and 14.6% of hydrophilic residues. Computer search in homology of the nucleotide sequences found that only 42 nucleotides in 123 bp repeat unit in the SeRm cDNA have 76% homology with repeat of *Grus americana* (GenBank accession number X54176). (non-

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Nucleotide sequence data reported in this paper are available in EMBL, GenBank™ and DDJB databases under the accession number U50190.

b

123 bp repeating unit

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GTCTTGGAATCTTGGTCACATTTGAAGAGTTCTGGTTCGGGGCTGGCAGGTTCTCCTTGGCAGCTGTCTTCGCAAGTGGATAAGTCGGAA
V  L  E  S  W  S  H  L  K  S  S  G  S  G  L  A  G  S  P  W  Q  L  S  S  Q  V  D  K  S  E
(G→C: V→L)

CAGTTGTCATCTTCCTCAGTGCAGGCTATGCAG
Q  L  S  S  S  S  V  Q  A  M  Q
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nonrepeating region

SeRm-1, SeRm-2 (199 bp)

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CTCTTGCAATCCCCAAACAAAAACCACAAAAAAGCAGAGAAAACAGAATAACCGCGGATGCTGGCACTGTGCCTGATCCCCTCTATATTA
AGGCCCTTAATAAAGCTTACGCTTAAATAAAATATTAATCGCTAATCAAATAGAGCACTCATGATGCCCAAAGAATAACAAACCAAATTT
ATGCGATTCACCTTAAACC
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SeRm-3 (252 bp)

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CCGAGTGCAACTGTTGCTCTACCTGTGCAAATGGAGGCAAATGCAACTGTTCTGACTGCAAGAGTTGCAGTAAATAATGTGCAGAGGGCG
AAAGTCTCCGAAATCGGACCACTCTGGGCCATGAACTATTGAATAATGTGGAGTTGACACATTTCCTCCAGAAGTTTCGATTTATTTGT
TCGACGCTATTAATTAATAAAGAACTTGGTGATATGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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Fig. 1 a. Structure of three cDNA clones containing 123 bp repeating units from plerocercoid of *S. erinaceieuropaei*.
b. Nucleotide and predicted amino acid sequence of 123 bp repeating unit and nucleotide sequences of nonrepeating regions. Stop codon and polyadenylation signal are underlined.

repeating regions of SeRm-1, SeRm-2 and SeRm-3 have no homology in nucleotide levels with cDNA reported previously.)

Southern and Northern blot analysis were carried out using 984 bp repeating sequence in SeRm cDNA as a probe. Genomic DNA digested with restriction enzymes showed the same patterns in plerocercoids and adult worms in Southern blot assay. There are two and three bands in *Eco* R I and *Hae* III digestion, respectively (Fig. 2a). In Northern blot assay, a smear hybridization signals from 2 to 10 kb in size was shown in plerocercoids, while no clear band was detected in adult worms. The control probe derived from Se16 encoding an antigenic polypeptide which was immunoscreened from the same cDNA library as that SeRm was done from revealed a clear band both in plerocercoid and adult worm indicating that mRNA was not destroyed (Fig. 2b). The result indicates that the mRNAs containing the repetitive sequence are abundant in number and in size in plerocercoid. Also, repeat units with abundant messages and high percentage of hydrophobic amino acids suggest that they could encode some membranous proteins with strong antigenicity.

The repeating element derived from plerocercoids may be associated with migration of the plerocercoids in host tissue. The polypeptides with repeating element may act as evasive antigens because many mRNAs encoding repeating element are transcribed from genomic DNA of plerocercoid and no such a mRNA was detected in adult worm. It has been demonstrated that repeat structure has a strong anti-

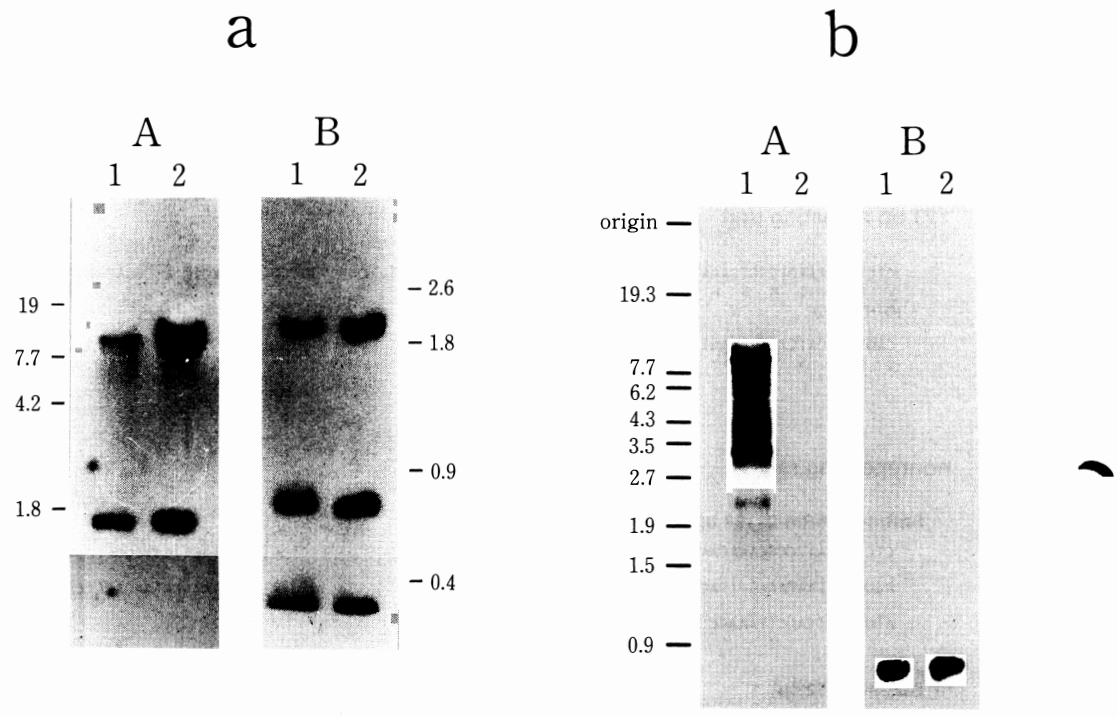

Fig. 2 a. Southern blot analysis of the genomic DNA from plerocercoids and adult worms of *S. erinaceieuropaei*. Two micrograms of genomic DNA digested with *Eco* R I (A) and *Hae* III (B) was electrophoresed on a 0.8% agarose gel, transferred to Hybond N^+ membrane and hybridized with ^{32}P-labeled SeRm-1 cDNA probe. Lane 1, plerocercoid; Lane 2, adult worm. Figures (kb) indicate the positions of markers.

b. Northern blot analysis of mRNA from plerocercoids and adult worms of *S. erinaceieuropaei*. Five hundred nanograms of poly (A)-rich mRNA was electrophoresed on a 1.2% agarose gel, transferred to Hybond N^+ membrane and hybridized with ^{32}P-labeled cDNA probe derived from SeRm-1 (A) and Se16 (B). Lane 1, plerocercoid; Lane 2, adult worm. Figures (kb) indicate the positions of markers.

genicity and that dominant antibody response is directed against repeat epitopes (Nussenzweig and Nussenzweig, 1985; Marin *et al.*, 1992), which compete against host protective immune responses. Ycas (1972) and Ohno (1984) have concluded that repeat sequence may have a major role in protein evolution. Rapid evolution of polypeptides is presumably important for parasites to evade from protective immune responses of the host. However, localization of the antigenic polypeptide encoded by SeRm in plerocercoid has not been studied.

The SeRm reported here has interesting features. It contains a long tandem repeat with stage-specific transcription.

References

1) Chi, J. G., Chi, H. S. and Lee, S. H. (1980): Histopathological study on human sparganosis in Korea. Korean J. Parasitol., 18, 15–23.
2) Fung, C. F., Ng, T. H. and Wong, W. T. (1989): Sparganosis of the spinal cord. Case report. J. Neurosurgery, 71, 290–302.
3) Gubler, J. and Hoffman, G. R. (1983): A simple and very efficient method for generating cDNA libraries. Gene, 25, 263–269.
4) Holodniy, M., Almonoff, J., Loutit, J. and Steinberg, G. K. (1991): Cerebral sparganosis: case report and review. Rev. Inf. Dis., 13, 155–159.
5) Liu, D-W., Kato, H., Nakamura, T. and Sugane, K. (1996): Molecular cloning and expression of the gene encoding a cysteine proteinase of *Spirometra erinacei*. Mol. Biochem. Parasitol., 76, 11–21.
6) Marin, M., Garat, B., Pettersson, U. and Ehrlich, R. (1993): Isolation and characterization of a middle repetitive DNA element from *Echinococcus granulosus*. Mol. Biochem. Parasitol., 59, 335–338.
7) Marin, M. S., Prieto, M., Marin, J. M., Casais, R., Boga, J. A. and Parra, F. (1992): Identification and expression of a *Fasciola hepatica* gene encoding a gut antigen protein bearing repetitive sequences. Mol. Biochem. Parasitol., 55, 155–166.
8) Nussenzweig, V. and Nussenzweig, R. S. (1985): Circumsporozoite proteins of malaria parasites. Cell, 42, 401–403.
9) Ohno, S. (1984): Birth of a unique enzyme from an alternative reading frame of the preexisted, internally repetitious coding sequence. Proc. Natl. Acad. Sci. USA, 81, 2421–2425.
10) Sanger, F., Nicklen, S. and Coulson, A. K. (1977): DNA sequencing with chain-terminating inhibitor. Proc. Natl. Acad. Sci. USA, 74, 5463–5467.
11) Sarma, D. P. and Weilbaecher, T. G. (1986): Human sparganosis. J. Am. Acad. Dermatol., 15, 1145–1148.
12) Ycas, M. (1972): De novo origin of periodic protein. J. Mol. Evol., 2, 17–27.