FUNDAMENTAL STUDY OF EXPRESSION-LIBRARY IMMUNIZATION AGAINST *TAENIA TAENIAEFORMIS* IN RATS

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Abstract. Expression-library immunization has been proposed as an effective means to screen a large number of genes of the pathogen as candidate protective molecules. In this study, we examined the efficacy of expression-library immunization using a *T. taeniaeformis* rat model system. Total RNAs were isolated from the last 15 segments of adult *T. taeniaeformis* and poly A RNA was purified. cDNA library was produced using SuperScript Plasmid System, which contains a mammalian expression vector, pCMV·SPORT6. From about 3,500 clones examined, more than 800 clones were found to contain DNA fragments. About 200 clones were sequenced and the homology search was carried out. The blast search revealed that 29% of the expression genes were mitochondrial genes (rRNA; 17%, protein; 12%). Nuclear rRNA genes (10%), nuclear protein (9%) and genes from *Escherichia coli* were also detected. Forty-two percent of sequences did not show a significant similarity to any genes deposited in the public database. Rats were immunized with expression-library and injected orally with 1,000 *T. taeniaeformis* eggs. However the protective effect of expression-library vaccine was not confirmed.

INTRODUCTION

Cysticercosis, one of the most serious larval cestodes of medical importance in humans and economic importance in pigs, is spreading worldwide, especially in Asia, Africa and Latin America. The trial of the vaccine development against *Taenia solium* cysticercosis has been attempted in many countries, and actually some candidate molecules have been reported. However, the effects of the vaccine that used those molecules have not been perfect. Moreover, it was reported recently that there were intra-specific variations within *T. solium* (Nakao *et al*, 2002). Thus, it is necessary to carry out further vaccine development.

Expression-library immunization has been proposed as a means to screen a large number of genes of the pathogen as candidate protective molecules. Expression-library immunization was first reported in a study in which an expression library made from the genomic DNA of the rodent pathogen *Mycoplasma pulmonis* was used as a DNA vaccine and shown to protect mice from challenge infection (Barry *et al*, 1995). Recently, expression-library immunization against murine malaria was also reported (Shibui *et al*, 2001).

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In this study, we examined the efficacy of expression-library immunization for vaccine development against *T. solium* cysticercosis using a *T. taeniaeformis* rat model system. The expression-library was constructed from cDNA because the screening efficiency should be better than that obtained from attempts which used genomic DNAs.

MATERIALS AND METHODS

Parasite materials

The KRN strain of *T. taeniaeformis* was used (Azuma *et al*, 1995). The cat was orally inoculated with metacestodes from the rat. After several months, the cat was sacrificed under anesthesia, and adult worms were collected from the small intestine. Immediately worms were washed by physiological saline with antibiotics in several times. Then, worms were frozen in liquid nitrogen and stored in -80˚C until use.

Construction for cDNA library

Total RNAs were isolated from the last 15 segments of adult *T. taeniaeformis* by ISOGEN LS (Nippon Gene), and poly A RNA was purified using oligotex-dT30 (TaKaRa). cDNA library was produced using SuperScript Plasmid System (Inbitorogen), which contain a mammalian expression vector, pCMV·SPORT6 (Fig 1). Transformed *Escherichia coli* was plated onto LB-plate containing ampicillin, and the cells that grew on the plate were re-suspended in TYGPN medium. PCR amplification was performed

to evaluate the size of insertion DNA fragments. A part of a clone was sequenced by the Model 3100 Sequencer, and the property of the insert was examined.

Cells were propagated in LB medium containing ampicillin. Plasmid DNAs were isolated using an EndoFree Plasmid Maxi Kit (QIAGEN) according to the manufacturer's protocol.

Immunization with cDNA library

Six-week-old F344/Jcl female rats were immunized. For the first immunization, 100 μg of purified cDNAs in 100 μl of sterile saline was injected directly into the spleen or muscle, while the two subsequent injections were intramuscular.

For the spleen injection, mice or rats were anesthetized with xylazin and ketamine. A small incision was made and the spleen was gently pulled out. DNA was directly injected using a 26-gauge needle. For the muscle injection, the skin on the quadriceps muscle was incised to visualize the muscle.

Challenge infection to immunized rat

Twenty rats were divided into 4 groups (A-D). Group A and B were immunized with expression-library. For immunization into the spleen, HVJ Envelope VECTOR Kit "GenomOne" (ISHIHARA SANGYO, Japan) was used for enhancing a trapping efficacy of DNA (Kaneda *et al*, 2002). Group C was subcutaneously inoculated with 300 eggs of *T. taeniaeformis*. Rats were immunized three times. At the time of the last immunization, rats were injected orally with 1,000 *T. taeniaeformis* eggs.

Fourteen days after challenge infection, rats were sacrificed under anesthesia and examined for the number of cysticerci. Schedules of the immunizations and the challenge infection are shown in Fig 2.

RESULTS

Property of the inserts

Among about 3,500 clones examined, more than 800 clones were found to contain DNA fragments. As a result of the sequencing, partial sequences of the insert from 116 clones were determined. The blast search revealed that 29% of the expression genes were mitochondrial genes (rRNA; 17%, protein; 12%) and 42% of sequences did not show a significant similarity to any genes deposited in the public database. Nuclear rRNA genes (10%), nuclear protein genes (9%) and genes from *E. coli* were also detected (10%). In nuclear protein genes, homologues of actin (2 clones), phosphoglycerate mutase (2 clones), cyclophilin (1 clone), glutatione-S-transferase (1clone), lactate dehydrogenase (1 clone), malate dehydrogenase (1 clone), paramyosin (1clone), polyubiquitin (1 clone) and topomyosin (1 clone) genes were detected. However the homologue of the protective antigen gene of taeniid cestodes, such as To45W and TSOL18, were not detected.

Challenge infection to immunized rat

The results of challenge infection are shown in Table 1. The number of recovered cysticerci considerably varied on each group. A small number of cycticerci were recovered from the livers of rats in

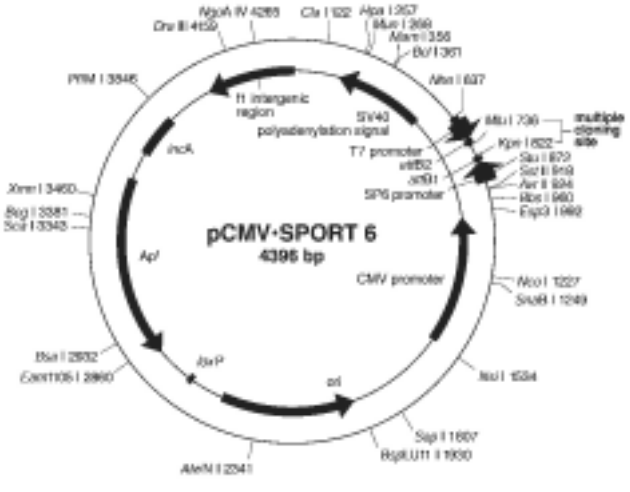

Fig 1- Schematic representation of the plasmid vector pCMV·SPORT6. The plasmid vector pCMV·SPORT6 is included in SuperScript Plasmid System (Inbitorogen). This vector has a CMV promoter which shows high promoter activity in the mammalian cell.

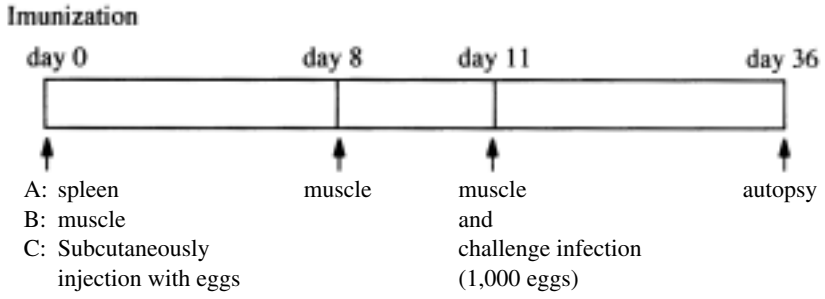

Fig 2- Schematic representation of immunization and challenge infection schedules.

Table 1
Numbers of *T. taeniaeformis* cycticerci in rats immunized with expression-library.

Group	Number of cycticercus in individual rat					Mean
	1	2	3	4	5	
Group A	93	148	157	180	210	157.6
Group B	85	131	183	229	-	157.0
Group C	3	3	6	12	-	4.8
Group D	77	106	129	177	207	139.2

Each rat was orally inoculated with 1000 eggs of *T. taeniaeformis* and examined 14 days post-inoculation.
Group A: muscle + muscle + muscle (immunized with expression-library); group B: spleen + muscle + muscle (immunized with expression-library); group C : subcutaneously inoculated with eggs (positive control); group D: non-treated (negative control).

group C, which were immunized with viable eggs. In group A, which was immunized with expression-library, however, no effect was observed. Equally, no effect was recognized in group C, which was immunized using HVJ Envelope VECTOR Kit.

DISCUSSION

The adult worm of taeniid cestodes parasitizes the host in the inside of the small intestine. The environment in the intestine is anaerobiotic. Thus, it is considered that mitochondria are not so important for adult worms. It was reported that the number of mitochondria in adult taeniid cestodes was not so many (Yap *et al*, 1987). In the present study, however, about 30% of the sequenced clones had mitochondrial genes. This fact indicates that the mitochondrion has an important role in the adult worm.

The host-protective antigen against taeniid cestodes is present in non-living parasite extracts and the oncosphere is a particularly rich source of protective antigens (Lightowlers and Gauci, 2001). The last 15 fragments of adult *T. taeniaeformis*, which were sources of RNA, contained plenty of eggs, which included oncospheres. In the Western blot analysis, several weak bands were detected in mice immunized with the expression-library (data not shown). Judging form this, injection of the library vaccine could induce an immune response against *T. taeniaeformis*. In the present study, however, the protective effect of expression-library vaccine was not confirmed.

As a primary cause of this, it is considered that the quality of the library is not so good. In this study, RNA was extracted from adult worms. As mentioned above, the adult worm should have expressed oncosphere antigens. However, many other proteins were also expressed. Thus, the proportion of nuclear protein was very low. In future studies, the oncosphere should be used as a source of RNA.

There is another factor: that the expressions of plasmid inoculated were considered to be fairly low. In the Western blot analysis, only very few and weak bands were detected. In order to increase the expression, we used the HVJ Envelope VECTOR Kit.

But the efficacy of expression has not been improved. It is necessary to examine the use of electroporation or a gene gun.

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