THE PROTECTIVE IMMUNITY OF A DNA VACCINE ENCODING SCHISTOSOMA JAPONICUM CHINESE STRAIN TRIOSE-PHOSPHATE ISOMERASE IN INFECTED BALB/C MICE

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Abstract. The development of a DNA vaccine for schistosomiasis japonica and testing the protective efficacy after challenge in BALB/c mice were performed. Thirty-nine female BALB/c mice were divided into three groups. Each mouse of the control group was injected intramuscularly with 100 µg of pcDNA3.1 DNA. In the TPI group, each mouse was injected with 100 µg of pcDNA3.1-SjCTPI DNA. The TPI+IL-12 group was injected with 100 µg of pcDNA3.1-SjCTPI DNA and 100 µg of the mixture of pcDNA3.1-P35 and pcDNA3.1-P40 DNA. Each mouse was immunized three times at two-week intervals and challenged with 45 cercariae of Schistosoma japonicum Chinese strain four weeks post-immunization. Then the mice were sacrificed and perfused at 45 days after challenge; the recovered worms and hepatic eggs were counted. Cytotoxic T lymphocyte (CTL) activity mediated by SjCTPI was detected with the ^3Cr release assay. ELISA was performed for the detection of anti-rTPI antibodies. The CTL activity of the control group was 9.1%, while CTL activities of the TPI group and the TPI+IL-12 group were 27.6% and 54.4%, respectively. The worm and egg reduction rates of TPI group and the TPI+IL-12 group were 30.2%, 52.9%, 32.7%, and 47.0%, respectively in comparison with the control group. This study further proved the possibility of the SjCTPI DNA vaccine as a potential DNA vaccine for schistosomiasis.

INTRODUCTION

Schistosomiasis is a serious parasitic disease. Six hundred and fifty-two million people in 76 countries and territories in the world are at the risk for infection, and 193 million people are infected (Chitsulo et al, 2000). With more than 50 years effort, the disease has been controlled in the most of endemic areas of China, but along the Yangtze River and in some mountain areas the disease can not be controlled since the intermediate host (snails), and animal reservoir hosts of schistosomiasis japonica are still existing in the endemic areas and re-infection occurs rapidly (Chen et al, 2002). Vaccines need to be developed as a complementary method to chemotherapy for schistosomiasis control.

The enzymatic antigens, such as triose-phosphate isomerase (TPI) and glutathione-S-transferase (GST) as candidates for schistosomiasis vaccines, have been studied for decades. Their protective effects have been demonstrated by the WHO (Bergquist and Colley, 1998).

The dimeric glycolytic enzyme TPI is a key enzyme in glycolysis. TPI distributes over all the tissues of the schistosome. Harn and his colleagues (1992) demonstrated that the protective McAb M.1 passively transferred partial resistance (41-49%) to naive mice. The glycolytic enzyme TPI can be recognized by McAb M.1. In our laboratory 21.4-27.8% worm reduction rates were induced in mice by immunization of recombinant TPI for Schistosoma japonicum Chinese strain (Yu et al, 1999). The DNA vaccines of SjCTPI and IL-12 plasmid DNA have successfully been constructed in our laboratory. The protective efficacy of the DNA vaccine in inbred C57BL/6 mice can induce 27.9-31.9% worm reduction. T-cell re-
sponse results show that IL-2 was increasing in the groups vaccinated with DNA vaccine for SjCTPI (TPI and TPI+IL-12 groups) before or post challenge. IL-2 was secreted by CD4+ Th1 cells with the stimulation of antigens (SWAP, rTPI and Sm-MAP4). It may be that the cellular immunity induced by Th1 cell was predominant after immunization with the DNA vaccine for pcDNA3.1-SjCTPI, although an IFN-γ could not be detected in that study (Zhu et al., 2002). Since the same antigen can induce different immune responses in different kinds of animals, it may be gene restricted. In order to investigate the protective immunity of the SjCTPI DNA vaccine in different kinds of mice, we choose another inbred mouse, the BALB/c mouse, to be the experimental animal in this study.

MATERIALS AND METHODS

Female BALB/c mice (14-16g, 5-6 week-old) were purchased from the Shanghai Experimental Animal Center of the Chinese Academy. Cercaria of Schistosoma japonicum were provided by the snail laboratory of the Jiangsu Institute of Parasitic diseases.

51Cr (Chinese Isotope Company Product), YAC1 cells (provided by Suzhou Medical College), RPMI-1640 culture medium (Shanghai Sagon Biotech), QIAGEN Plasmid Mega-2500 kit (Qiagen company).

Large-scale preparation of pcDNA3.1, pcDNA3.1-SjCTPI, pcDNA3.1-P35 and pcDNA3.1-P40

Plasmid DNA of pcDNA3.1-SjCTPI, pcDNA3.1-P35, and pcDNA3.1-P40 were constructed as mentioned before (Zhu et al., 2002) and prepared for large-scale according to the manufacture’s instructions for the Qiagen Plasmid Mega 2500 kits (Qiagen company), and dissolved in aseptic 0.9% NaCl. The concentration of plasmid DNA was detected in a Nucleic Acid and Protein Analyser.

Protective trails

Thirty-nine of the female BALB/c mice were divided into three groups. Each mouse of the control group was injected with 100 µg pcDNA3.1 into both quadriceps muscles (50 µg each). In the TPI group each mouse was immunized with 100 µg pcDNA3.1-SjCTPI DNA. In the TPI+IL-12 group each mouse was injected with 100 µg pcDNA3.1-SjCTPI DNA in the left leg and 100 µg of the mixture of pcDNA3.1-P35 and pcDNA3.1-P40 in the right leg. Each mouse of the three groups was immunized three times at a two-week intervals with the same doses, using the same method described above.

Each mouse was challenged with 45 Schistosoma japonicum Chinese strain cercariae by the abdomen penetration method four weeks after the last immunization. Forty-five days post-challenge, the mice were sacrificed and perfused and the recovered worms were counted. The liver of the mouse was immersed in 5% KOH for 24 hours, then the eggs were counted. The worm and egg reduction rates were calculated with the following formula.

The worm reduction rate (%) is the mean number of adult worms per mouse in the control group minus the mean number of adult worms per mouse in the experimental group divided by the mean number of adult worms in the control group.

The egg reduction rate (%) is the mean number of eggs per mouse in the control group minus the mean number of eggs per mouse in the experimental group divided by the mean number of eggs per mouse in the control group.

Detection of CTL mediated by the SjCTPI DNA vaccine (51Cr release assay)

Spleen cell culture. A single spleen cell suspension was prepared according to a routine method with two mice each group one week before the challenge. The spleen cells were re-suspended with 10 ml of 5% FCS RPMI-1640. The rSjCTPI was added to 20 µg/ml of final concentration, then the spleen cells were cultured in 5% CO2 at 37ºC for six days. After washing with 5% FCS RPMI-1640, the cells were adjusted to 2x10⁶/ml final concentration as effector cells. Labeling of target cells: 0.5 ml of YAC1 cells (1x10⁷/ml) were piped, 3.7x10⁶ Bq 51Cr were added, then incubated in 5% CO2 at 37ºC for 2 days, washed with 5% FCS RPMI-1640 and centrifuged three times, the cells were adjusted to 2x10⁹/ml as a final concentration of the target cells.
Assaulting assay. The spleen cells of each group were divided into three test groups (experimental group, spontaneous release group, maximum release group), three wells for each test group. To each well of the experimental group was added 0.1 ml target cells and 0.1 ml effector cells. To the spontaneous release group was added 0.1 ml target cells and 0.1 ml RPMI-1640. To the maximum release group was added 0.1 ml target cell and 0.1 ml 1% triton. The final concentration of effector cells and target cells were 1x10^7/ml and 1x10^5/ml, respectively. The mixtures were incubated in 5% CO_2 at 37ºC for 4 hours, then centrifuged and scintillation solution was added to each well, the cpm for each well was counted with the scintillation counter.

CTL activity (%) is (cpm in the experimental group minus cpm in the spontaneous release group) divided by (cpm in the maximum release group minus cpm in the spontaneous release group).

Detection of anti-SjCTPI antibodies

Mice sera were collected from each group before immunization, at challenge and two weeks post-challenge. ELISA plates were coated with rSjCTPI (20 µg/ml) and SWAP (20 µg/ml) overnight at 4°C, blocked with 1% BSA-PBST for 1 hour at 37°C, washed three times with PBST, incubated with serum diluted 1:50 with PBST for 1 hour at 37°C, washed three times with PBST and incubated with sheep-anti mouse IgG-HRP (1:1000) for 1 hour at 37°C, TMB was added for 5 minutes, then 2M H_2SO_4 was added to stop the reaction, then the OD value was read at 450 nm.

RESULTS

Preparation of pcDNA3.1, pcDNA3.1-SjCTPI, pcDNA3.1-P35, and pcDNA3.1-P40 DNA

The concentration of pcDNA3.1-SjCTPI prepared with Qiagen plasmid Mega Kit was 4.0 mg/ml, OD260/280:1.83; the concentration of pcDNA3.1-P35 was 3.2 mg/ml, OD260/280:1.89; pcDNA3.1-P40, 3.2 mg/ml, OD260/280:1.89; pcDNA3.1, 3.2 mg/ml, OD260/280:1.85.

Detection of CTL mediated by SjCTPI

The CTL activities in the control, TPI and TPI+IL-12 groups were 9.1%, 27.6%, and 54.4% respectively (Table 1).

Detection of anti-SjCTPI antibodies

Anti-SjCTPI antibodies in the control group before challenge were negative, five of ten mice serum samples from the TPI group were weakly positive, and six of ten from TPI+IL12 group were weakly positive.

Protective effects

Adult worm reduction rate. The mice were perfused 45 days after challenge. Collection and calculation of the recovered worms after portal perfusion six weeks post cercariae challenge were performed. The worm reduction rates in the TPI and TPI+IL-12 groups were 30.2% and 32.7%, respectively, compared with the control group (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Experimental group (cmp)</th>
<th>Spontaneous release group (cmp)</th>
<th>Maximum release group (cmp)</th>
<th>Cytotoxic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6,877</td>
<td>6,296</td>
<td>12,681</td>
<td>9.1%</td>
</tr>
<tr>
<td>TPI</td>
<td>7,534</td>
<td>7,129</td>
<td>8,599</td>
<td>27.6%</td>
</tr>
<tr>
<td>TPI +IL-12</td>
<td>8,437</td>
<td>6,792</td>
<td>9,816</td>
<td>54.4%</td>
</tr>
</tbody>
</table>

Table 1

Detection results of CTL activity in the three groups mediated by SjCTPI.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of mice</th>
<th>Mean no. of adult worms</th>
<th>Worm reduction rate (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPI</td>
<td>11</td>
<td>23.30±4.51</td>
<td>30.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TPI +IL-12</td>
<td>10</td>
<td>22.44±4.81</td>
<td>32.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>33.36±7.01</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2

Comparison of the adult worms recovered from the three groups of mice.
The numbers of adult worms recovered from the TPI and TPI+IL-12 groups were significantly fewer than the control group (both p<0.01) by variance analysis.

**Egg reduction rate.** In the calculation of the mean number of liver eggs per mouse, the egg reduction rate for the TPI and TPI+IL-12 groups were 52.9% and 47.0%, respectively, compared with the control group (Table 3).

By variance analysis, the liver eggs per mouse in TPI and TPI+IL-12 groups were significantly fewer than the control group (both p<0.01).

**DISCUSSION**

It has been reported that six vaccine candidates, paramyosin (Sm97), IrV5, SmGST, Sm14, Sm23, and SmTPI have been chosen for further development by the WHO. These peptide vaccines have been shown in mice to induce partial protection from schistosome infection, at rates of 30%, 50-70%, 40-60%, 65%, 40-50% and 30-60% (Bergquist and Colley, 1998). DNA vaccination is a novel technique developed during the last decade. DNA vaccines have several advantages over conventional vaccines (Robinson and Torres, 1997; Tighe et al., 1998). DNA vaccines have several advantages over conventional vaccines (Robinson and Torres, 1997; Tighe et al., 1998), for example, the delivery route of DNA vaccines is simpler, injection of the plasmid DNA is usually into the muscle and/or skin. The direct delivery of genetic antigens to host cells results in subsequent in vivo production of the desired antigen and persistent low level expression of antigens over a long period of time. The protein produced in the cells may be more like the native molecule. Other advantages are that it is economical and simple to prepare. Since DNA is heat stable, there is no need to have a cold-chain in delivery. In our study, we developed a SjCTPI DNA vaccine for protection against *Schistosoma japonicum* infection.

In this study, the SjCTPI DNA vaccine (pcDNA3.1-SjCTPI) (TPI group) or co-immunized with pcDNA3.1-IL-12 plasmid (TPI+IL-12 group) induced 30.2% and 32.7% worm reduction rates and 52.9% and 47.0% of liver egg reduction rates, respectively, in infected BALB/c mice. This indicates that the SjCTPI DNA plasmid can induce partial protection against *Schistosoma japonicum* infection in BALB/c mice. The worm reduction rate in BALB/c was similar to that in C57BL/6 mice (27.9% and 31.9%) (Zhu et al., 2002), but the liver egg reduction rate in BALB/c infected mice was much higher than that in C57BL/6 infected mice (13.7% and 18.6%) (Zhu et al., 2002). It may be there are different immune responses to SjCTPI plasmid DNA in different strains of mice.

It has been reported that the DNA vaccine can induce both cellular and humoral responses, and induce MHC class 1-restricted cytotoxic T lymphocytes (CTLs). CD8+ CTLs generally recognize peptides derived from endogenous proteins presented in the context of MHC class1 molecules (Robinson and Torres, 1997; Tighe et al., 1998). The CTLs can kill target cells specifically. This is a major mechanism of action for the DNA vaccine, and the main difference with peptide vaccine. In our previous study, SjCTPI DNA vaccine induced both cellular (Th1) and humoral responses, but CTL activity was not detected (Zhu et al., 2002). In this study we detected CTL activity, mediated by SjCTPI, with the ⁵¹Cr release assay. The results show that in the SjCTPI and SjCTPI+IL-12 groups the CTL activities were 27.6% and 54.4%, respectively, significantly

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of mice</th>
<th>Mean no. of eggs per mouse</th>
<th>Egg reduction rate (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPI</td>
<td>11</td>
<td>61,597.4±28,132.7</td>
<td>52.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TPI +IL-12</td>
<td>10</td>
<td>69,244.2±22,743.9</td>
<td>47.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>130,707.5±46,107.5</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
higher than 9.1% in the control groups. The SjCTPI DNA vaccine may induce not only a Th1 response (CD4+), but CTL activity (CD8+) as a protective effect of immunization with the SjCTPI DNA vaccine. The SjCTPI plasmid DNA co-stimulated by the IL-12 plasmid can increase the cytotoxic activity. We did not find that the IL-12 plasmid DNA could increase the worm reduction rate and egg reduction rate in this study. Further study is needed to discover the reason for this.

In the TPI and TPI+IL-12 groups, anti-TPI antibody was detected though the response is not strong. SjCTPI plasmid DNA can induce both cellular and humoral immune responses in infected BALB/c mice. In a previous study, we found the SjCTPI DNA vaccine induce an increase in IL-2 (Zhu et al, 2002). The cellular immunity of the SjCTPI DNA vaccine may have a major effect against schistosome infection. This study indicates the SjCTPI DNA vaccine is a potential vaccine for schistosomiasis japonica.

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