Comparative Study on the Immunogenicity between Hsp70 DNA Vaccine and Hsp65 DNA Vaccine in Human Mycobacterium Tuberculosis*

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Summary: The BALB/c mice were immunized with Hsp70 DNA and Hsp65 DNA vaccines in human Mycobacterium tuberculosis. Eight weeks after immunization, the eyeballs were removed, blood and spleen taken, and intraperitoneal macrophages were harvested. The lymphocytic stimulating index (SI) was used to measure the cellular proliferating ability and NO release to measure the phagocytic activity of the macrophages. With ELISA kit, the levels of interleukin-2 (IL-2) and interferon-γ (IFN-γ) in serum and the splenic lymphocytic cultured supernatant were detected. The results showed that after the mice were immunized with 100 μg/mouse of Hsp70 DNA vaccine intramuscularly, the splenic lymphocytic proliferating ability in the mice was significantly increased as compared with that in the control group, vector group and Hsp65 DNA vaccine group (P<0.01); The contents of NO in the intraperitoneal macrophages of the mice were significantly lower than in the control group and Hsp65 DNA vaccine group (P<0.01); The levels of serum IL-2 in the mice were significantly higher than in the control group, but there was no statistical difference between Hsp65 DNA group and vector group (P>0.05); The contents of serum IFN-γ in the mice were significantly higher than in the control group, but significantly lower than in the Hsp65 DNA vaccine group (P<0.05). It was indicated that immunization with Hsp70 DNA vaccine could obviously enhance the immune response, but its intensity seemed inferior to Hsp65 DNA vaccine. The anti-infection mechanisms and clinical use in the future of the vaccines of Hsp70 DNA and Hsp65 DNA are worth further studying.

Key words: Hsp70 DNA vaccine; Hsp65 DNA vaccine; immunogenicity

There are 3 million deaths per annum worldwide due to tuberculosis, and AIDS is compounding the problem. A better vaccine than the live mycobacterium currently in use, Bacillus Calmette-Guerin (BCG), is needed. Mycobacterium heat shock protein (HSP) can not only enhance the immune response, but also keep the antigen characteristics of mycobacterium itself. Our cooperator, Silva from Lowrie group of British National Medical Academy, cloned the mycobacterium Hsp65 gene into a eukaryotic expression vector pCMV to construct the naked DNA vaccine (DNA65), which was used to immunize the mice. He found that the in vivo specific killing activity of CD4+ CD8+ lymphocytes was obviously increased and DNA65 could prevent the mice from tuberculosis challenge[1]. In this study, by using molecular biological techniques, the Mycobacterium Hsp70 gene was cloned into a eukaryotic expression vector pcDNA3 to construct the naked DNA vaccine (DNA70), which was used to immunize the mice. He found that the in vivo the specific killing activity of CD4+ CD8+ lymphocytes was obviously increased and DNA65 could prevent the mice from tuberculosis challenge[1]. In this study, by using molecular biological techniques, the Mycobacterium Hsp70 gene was cloned into a eukaryotic expression vector pcDNA3 to construct the naked DNA vaccine (DNA70). The mice were immunized with both the vaccines of Hsp65 DNA and Hsp70 DNA to observe the effect of them on the mouse immune response, investigate the anti-infection mechanism of the two vaccines and compare the immunogenicities of the two vaccines.

1 MATERIALS AND METHODS

1.1 Materials

Male BALB/c mice purchased from Department of Experimental Zoology, Tongji Medical College; Hsp70 DNA vaccine constructed by our institute; Hsp65 DNA vaccine supplied by D. B. Lowrie (England); RPMI-1640 purchased from Sigma (USA); IL-2 and IFN-γ ELISA detection kits purchased from Endogen Company (USA); ConA, bovine serum from Beijing Huamei Biological Engineering Company; MTT purchased from Fluka company.

1.2 Methods

1.2.1 Preparation of Plasmids Referred to the reference[2].

1.2.2 Animal Immunization Thirty-two male BALB/c mice, weighing 18±1 g, were randomly divided into 4 group (n=8 in each group); group 1 as blank control group receiving intramuscular (i. m.) injection of 0.1 ml normal saline in each mouse; group 2 as blank vector group receiving i. m. injection of 0.1 ml (100 μg) pcDNA3 in each mouse; group 3 as Hsp65 DNA vaccine group receiving i. m. injection of 0.1 ml (100 μg) Hsp65 DNA vaccine in each mouse; group 4 as Hsp70 vaccine group receiving i. m. injection of 0.1 ml (100 μg) Hsp70 DNA vaccine in each mouse. After immunization for 8 weeks, eyeballs were removed and blood taken. After kill the mice, intraperitoneal macrophages were

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harvested and spleens taken for the experiment.

1.2.3 Lymphocyte Transformation Test

Spleenic lymphocyte suspension was prepared by routine methods and the cell concentration was adjusted to $2 \times 10^6/L$ with RPMI-1640 medium. The suspension was added into 96-well plates. Each sample was added into 6 wells with 100 µl in each well. Among the 6 wells, 3 wells served as controls with addition of 100 µl RPMI-1640 containing 100 µl/L bovine serum in each well and the remaining 3 wells as experimental ones with addition of 100 µl Con A (20 µg/ml). After culturing at 37°C with 5% CO$_2$ for 48 h, 100 µl supernatant in each well was discarded. Five mg/ml MTT 20 µl was re-added into each well, mixed, re-cultured for 6 h, centrifuged and the supernatant was discarded. DMSO 100 µl was added into each well again and placed into an incubator at 37°C for 20 min. Absorbent (A) value was measured by using ELX-800 type enzyme labeling device (BIO-TEK company, USA) at 490 nm. Stimulating index was used to evaluate the lymphocyte transformation (SI=A value in the experimental well/A value in the control well).

1.2.4 Determination of NO in Macrophages

The intraperitoneal macrophages in the mice were taken, washed with RPMI-1640 for 3 times and centrifuged at 1000 r/min for 10 min. Two ml RPMI-1640 containing 100 µl/L bovine serum was added into the 24-well plate to culture about 2 h. After adherent to the wall, the cells were re-washed for 3 times to remove the non-adherent cells. Two ml RPMI-1640 containing 100 µl/L bovine serum was added to culture for 48 h. The supernatant 0.1 ml was taken with addition of 0.1 ml Griess agents (A solution: 1.0 g/L N-1-Naphthatenyl-1,2-ethanediamine, B solution: 50 ml/L phosphate supplemented with 20 g sulfonamide, A:B=1:1) and placed at room temperature for 20 min. The culture medium served as controls. The positive result was cherry-like red. By using an enzyme labeling photometer, colorimetric analysis was performed at 550 nm. Sodium nitrite was taken as standard to draw a standard curve to calculate the NO contents.

1.2.5 Determination of IL-2 and IFN-γ

The eyeballs were removed to take blood for separating serum. The induced culture of splenic lymphocytes IL-2 and IFN-γ in the mice was carried out according to the routine methods. By using ELISA method, the levels of IL-2 and IFN-γ were detected by the instruction of the detection kits. At 490 nm of ELX-800 enzyme labeling device, A value was measured. Standard product, serum and the samples of lymphocyte culture supernatant were monitored. Taking the A value of the standard product drew a standard curve to the concentration to calculate the levels of IL-2 and IFN-γ in serum and the culture supernatant of the lymphocytes.

1.2.6 Statistical Analysis

The data were expressed as $\bar{x} \pm s$. t-test was applied to compare the data.

2 RESULTS

2.1 Effects of Hsp70 DNA and Hsp65 DNA Vaccines on the Lymphocyte Transformation Function and Macrophage Phagocytic Activity in the Mice

From the table 1, it was found that after immunization of mice with Hsp70 DNA vaccine i.m., the splenic lymphocytic proliferating activity was significantly increased as compared with that in the blank control group, vector group and Hsp65 DNA vaccine group ($P < 0.01$). The NO release from the macrophages was obvious decreased as compared with that in the blank control group and Hsp65 DNA vaccine group ($P < 0.01$), but there was no statistical difference as compared with that in the vector group ($P > 0.05$).

Table 1 Effects of Hsp70 DNA and Hsp65 DNA vaccines on the lymphocyte transformation function and macrophage phagocytic activity ($\bar{x} \pm s, n=8$)

<table>
<thead>
<tr>
<th>Groups</th>
<th>SI</th>
<th>NO (nm/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.39±0.23</td>
<td>145.14±8.12</td>
</tr>
<tr>
<td>Vector</td>
<td>1.89±0.21</td>
<td>93.32±8.98</td>
</tr>
<tr>
<td>Hsp65 DNA</td>
<td>1.67±0.4</td>
<td>178.75±29.54</td>
</tr>
<tr>
<td>Hsp70 DNA</td>
<td>2.18±0.19*</td>
<td>87.69±9.53**</td>
</tr>
</tbody>
</table>

* $P < 0.01$, ** $P < 0.05$ as compared with control group and Hsp65 DNA group

2.2 Effects of Hsp70 DNA and Hsp65 DNA vaccine on the serum IL-2 levels in the mice

The results were shown in the table 2.

Table 2 Effects of Hsp70 DNA and Hsp65 DNA vaccine on the IL-2 levels in the serum in the mice ($\bar{x} \pm s, n=8$)

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-2 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>58.94±28.57</td>
</tr>
<tr>
<td>Vector</td>
<td>95.20±23.33</td>
</tr>
<tr>
<td>Hsp65 DNA</td>
<td>175.67±29.54</td>
</tr>
<tr>
<td>Hsp70 DNA</td>
<td>128.79±68.52*</td>
</tr>
</tbody>
</table>

* $P < 0.01$ as compared with vector group

2.3 Effects of Hsp70 DNA and Hsp65 DNA Vaccine on the IFN-γ Levels in the Serum and Splenic Lymphocyte Culture Supernatant in the Mice

The serum IFN-γ concentration in the mice immunized with Hsp70 DNA vaccine was significantly higher than in the blank control group ($P < 0.05$), but significantly lower than in the Hsp65 DNA group ($P < 0.05$), but there was no significant difference between the Hsp70 DNA vaccine group and vector group ($P > 0.05$). The concentration of IFN-γ in the splenic lymphocyte culture supernatant in the mice immunized with Hsp70 DNA vaccine was significantly lower than in the blank control group ($P < 0.05$), but there was no significant difference between Hsp70 DNA vaccine group and vector groups or