Immunogenicity and therapeutic effects of Ag85A/B chimeric DNA vaccine in mice infected with *M. tuberculosis*

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- In the patient with pulmonary TB
  - MDR-rate is 8.32%
  - XDR-rate is 0.68%
The difficulty of MDR-TB treatment

- The strategy of DOTS is achieving substantial progress in the control of TB worldwide.
- MDR-TB, XDR-TB has emerged as a new challenge, especially in the developing countries.
- Second-line anti-TB drugs are expensive, less effective and more toxic.
- It is mainly due to lacking of the funding to support the treatment of MDR-TB with second line anti-TB drugs.
Immunotherapy

- boost the efficacy of the immune system
- an alternative way for the treatment of tuberculosis
- assist anti-TB chemotherapy to shorten curable duration in patients.
DNA vaccine

- elicit both humoral and cellular immune responses,
- to confer protective and therapeutic effects on TB in animal models.
- a novel and potentially powerful agent to prevent and treat disease

- For the first time used the DNA vaccine in the infected mice
- found that hsp65 DNA could eliminate residual *M. tb* in the organs from infected mice and had remarkable therapeutic actions.
Objective

- compared the therapeutic efficacy of Ag85A DNA, Ag85B DNA and Ag85A/Ag85B DNA singly or in combination with chemotherapy in the animal model.
- To obtain a new therapeutic agents or regimen to treat tuberculosis, especially MDR-TB
1. Immunogenicity and protective efficacy of Ag85A and Ag85B DNA vaccines
C57BL/6 mouse TB model and DNA immunization

1. Saline
2. Vector pVAX1 100 µg
3. BCG vaccine
4. M64 100 µg + E6 100 µg
5. M64 50 µg + E6 50 µg
6. M64 75 µg + E6 25 µg
7. M64 25 µg + E6 75 µg
8. M64 25 µg + E6 25 µg
9. M64 100 µg + IFNγ 100 µg
10. E6 100 µg + IFNγ 100 µg
11. M64 100 µg + IL-12 100 µg
12. E6 100 µg + IL-12 100 µg
13. 85A 100 µg
14. 85B 100 µg
## Antibodies detection in the sera by ELISA

<table>
<thead>
<tr>
<th>Group</th>
<th>Ag85A-specific antibody (X±SD)</th>
<th>Ag85B-specific antibody (X±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgG1/ IgG2a</td>
</tr>
<tr>
<td>saline</td>
<td>0.166±0.05</td>
<td>1.00</td>
</tr>
<tr>
<td>vector pVAX1</td>
<td>0.204±0.05</td>
<td>0.90</td>
</tr>
<tr>
<td>BCG vaccine</td>
<td>0.179±0.09</td>
<td>1.19</td>
</tr>
<tr>
<td>85A DNA</td>
<td>0.382±0.15</td>
<td>1.91</td>
</tr>
<tr>
<td>85B DNA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The numbers of viable bacteria in the lungs at 4 weeks after M.tb infection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Log CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>The saline</td>
<td>2.0</td>
</tr>
<tr>
<td>Vector pVAX1</td>
<td>2.4</td>
</tr>
<tr>
<td>BCG vaccine</td>
<td>2.2</td>
</tr>
<tr>
<td>100 mg M64 + 100 mg r</td>
<td>2.8</td>
</tr>
<tr>
<td>75 mg M64 + 50 mg r</td>
<td>2.4</td>
</tr>
<tr>
<td>25 mg M64 + 25 mg r</td>
<td>2.2</td>
</tr>
<tr>
<td>75 mg M64 + 25 mg r</td>
<td>2.4</td>
</tr>
<tr>
<td>25 mg M64 + 75 mg r</td>
<td>2.6</td>
</tr>
<tr>
<td>100 mg M64 + 100 mg IFp</td>
<td>2.8</td>
</tr>
<tr>
<td>100 mg M64 + 25 mg r</td>
<td>2.4</td>
</tr>
<tr>
<td>100 mg E6 + 100 mg IFp</td>
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<td>2.8</td>
</tr>
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<td>100 mg M64 + 100 mg IFp</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Note: Log CFU represents the log of the number of colony-forming units.
Histopathologies of lungs each group at 4 weeks after M.tb infection

- **生理盐水**
- **pVAX1**
- **卡介苗**
- **M64 100—E6 100**
- **M64 50—E6 50**
- **M64 75—E6 25**
- **M64 25—E6 75**
- **M64 25—E6 25**
- **M64—IFNγ**
- **E6—IFNγ**
- **M64—IL12**
- **E6—IL12**
- **85A**
- **85B**
Conclusion (1)

- Ag85A DNA could alleviate lung lesions in the mouse TB model.
- Ag85B DNA could decrease the viable bacteria in the lungs.
- Ag85A and Ag85B DNA all have some protective effects on TB.
2. The immunotherapeutic effects of Ag85A and Ag85B DNA vaccines
Strain

- *M. tuberculosis* isolates HB361
- Conventional drug susceptibility testing: RFP 250 µg/ml
- INH 1µg/ml
- SM 10µg/ml
- Molecular test: gene mutations
Establishment of MDR-TB mouse model

- 80 female Balb/c mice of 6-8 weeks were challenged intravenously by tail vein with 220000 CFU/0.4ml of clinical isolate HB361 suspensions.
Mouse TB model and DNA immunization

(1) Saline
(2) Plasmid vector pVAX1
(3) 0.4 mg RFP
(4) 100 µg hsp65 DNA
(5) 0.4 mg RFP + 100 µg Ag85A DNA
(6) 100 µg Ag85A DNA
(7) 0.4 mg RFP + 100 µg ESAT6/Ag85A DNA
(8) 100 µg ESAT6/Ag85A chimeric DNA
Histopathologies of lungs each group

Saline  
vector  
RFP  
Hsp65  
RFP+85A  
85A DNA  
RFP+85A/E6  
85A/E6
The numbers of viable bacteria

![Bar chart showing the numbers of viable bacteria in lung and spleen across different groups.](chart)

- **Groups**
  - saline
  - vector
  - RFP
  - DNA hsp65
  - RFP-DNA 85A
  - DNA 85A/E6
  - RFP+DNA 85A/E6

- **CFU (Log10)**
  - Lung: 6.2, 6.4, 6.6, 6.8
  - Spleen: 5.4, 5.6, 5.8, 6
Ag85A DNA singly or combined with RFP or PZA had best effect of treatment on MDR-TB infected mice.

The hypothesis is that the immune system was stimulated by Ag85A DNA vaccination and resulted in the enhancement of drug effect of RFP or PZA to kill the MDR-bacteria.
3. The immunotherapeutic effects of Ag85A/B chimeric DNA
Mouse TB model and DNA immunization

(1) Saline;
(2) 25µg vector pVAX1control;
(3) 50µg vector pVAX1;
(4) 100µg vector pVAX1;
(5) M. Phlei F.U.36 injection;
(6) 25µg Ag85A DNA;
(7) 50µg Ag85A DNA;
(8) 100µg Ag85A DNA;
(9) 25µg Ag85A/B DNA;
(10) 50µg Ag85A/B DNA;
(11) 100µg Ag85A/B DNA.
The Cellular immune response in the mice immunized with Ag85A/B chimeric DNA
The numbers of viable bacteria in the lungs and the livers
Immunotherapy with Ag85AB DNA vaccine in Guinea pigs TB model

Weeks

(1) Saline
(2) Vector pVAX1 (150 µg)
(3) chemotherapy: RFP+INH
(4) Ag85A DNA (150 µg)
(5) Ag85AB DNA (150 µg)
Gross pathologies of spleens each group

Saline

150µg vector

RFP+INH

150µg Ag85A DNA

150µg Ag85AB DNA
Conclusion (3)

- Ag85A/B chimeric DNA vaccine had obvious clear immunotherapeutic effects on TB model.
- Inserting Ag85B DNA into Ag85A DNA increased the immunogenicity and immunotherapeutic effect of Ag85A DNA.
- Chimeric DNA is a helpful strategy for the development of better TB vaccines.
- In one word, therapeutic DNA vaccine is a promising and affordable strategies for the treatment of tuberculosis in developing countries.
Acknowledgment

- The Institute for Tuberculosis Research:
  - Yan Liang, Junxian Zhang, Yourong Yang, Chenglong Liu, Yingchang Shi, et al.
- The Department of Pathology:
  - Ning Li, Qi Yu, Xuejuan Bai, et al.
- This work was supported by the grant from the Serious Infectious Diseases Special Foundation of China (2008ZX10003013-2) WHO IVR Steering Committee (V25-181-202) and National Nature and Science Foundation of China (No. 30070730).
Acknowledgement

- Shanghai H&G Biotechnology company, Shanghai, China
  - Qingliang Liu, Pingjing Zhang, Zhongming Li
Animal model in the negative pressure BSL-II Lab.

- **Mouse TB model.** challenged by intravenous injection or aerosol using a Middlebrook Airborne Infection Apparatus.

- **Guinea pig model.** challenged by intraperitoneal injection or injection around inguinal lymph nodes.
Thank you!

Institute of Tuberculosis Research