A Novel Vaccine Development against Leprosy

M. MAKINO, T. MUKAI, Y. MAEDA, T. TAMURA, Y. TSUKAMOTO, M. MATSUOKA

Leprosy Research Center,
National Institute of Infectious Diseases, JAPAN
Purpose

Production of new recombinant BCG that can overcome the intrinsic defect of BCG to inhibit phagosome maturation
Previous findings (I)

- One of the immunodominant Ag of *M. leprae*: MMP-II (Major Membrane Protein-II)
- MMP-II activates DC through ligation with TLR2
- Production of recombinant BCG that secretes MMP-II (BCG-SM)

**Effect of BCG-SM**

**IFN-γ production from naïve T cells**

![Diagram showing the effect of BCG-SM on IFN-γ production from naïve T cells](image.png)
Previous findings (II)
Up-regulation of the ability to stimulate naïve CD8⁺ T cells

Western blotting analysis of protein secreted from BCG-70M

Antibody to: MMP-II HSP70

<table>
<thead>
<tr>
<th>Lane</th>
<th>Description</th>
<th>MOI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Culture filtrate of vector control BCG (BCG-261H)</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>Recombinant MMP-II protein</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>Culture filtrate of BCG-70M</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Activation of naïve CD8⁺ T cells by BCG-70M

Secretion of HSP70-MMP-II fusion protein was effective in an activation of human naïve CD8⁺ T cells

p<0.001

p<0.005
Previous findings (III)

Production of *ureC* deficient recombinant BCG (BCG-ΔUT-11-3)

Inhibition of acidification of BCG-infected phagosome

- BCG-urease
- Urea → Ammonia
- **Acidification of phagosome** (pH 4.5~5.5)
- Late phagosome
- BCG
- **Fusion**
- Lysosome
- Localization of BCG
- Surface expression of BCG-derived Ag

**Activation of naïve CD4 T cells**

- BCG-Tokyo
- BCG-ΔUT-11-3
- Urease depletion was effective in an activation of human naïve CD4+ T cells

- IFN-γ (pg/ml)
  - MOI 0.063: BCG-Tokyo: 50, BCG-ΔUT-11-3: 300
- p < 0.001
Construction of new recombinant BCG

HSP70-MMP-II fusion gene was introduced into urease-deficient BCG-ΔUT-11-3 (Production of BCG-D70M)

ΔUT-11-3 — Ure C —

D70M — Ure C — HSP60p — HSP70 — MMP-II

Combined the two independent method; (1) activation of naïve CD4⁺ T cells (2) activation of naïve CD8⁺ T cells
Recombinant BCGs used in this study:

1. BCG-261H : Vector control BCG
2. BCG-ΔUT-11-3 : Urease-deficient BCG
3. BCG-70M : Normal BCG introduced with HSP70-MMP-II fusion gene
4. BCG-D70M : Urease-deficient rBCG that secretes HSP70-MMP-II fusion protein
Activation of naïve CD4+ T cells by DC

- None
- BCG-261H
- BCG-ΔUT-11-3
- BCG-70M
- BCG-D70M

IFN-γ production (pg/ml)

MOI: 0.125 (T:DC = 40:1)

 MOI: 0.25 (T:DC = 40:1)

p<0.001

Activation of memory CD4+ T cells by MØ

- IFN-γ production (pg/ml)

MOI: 0.25 (T:MØ = 40:1)

 MOI: 0.50 (T:MØ = 40:1)

p<0.01
Inhibition of CD4⁺ T cell activation by treatment of APC with mAb

IFN-γ production (pg/ml)

Naïve CD4⁺ T cells:
- BCG-D70M-infected DC (MOI:0.25, T:DC=40:1)
- BCG-D70M-infected MØ (MOI:0.5, T:MØ=20:1)

Memory type CD4⁺ T cells:
- BCG-D70M-infected DC (MOI:0.25, T:DC=40:1)
- BCG-D70M-infected MØ (MOI:0.5, T:MØ=20:1)

Resp: None, Normal IgG, anti-HLA-DR mAb, anti-CD86 mAb

p<0.005
p<0.005
p<0.005
p<0.05
p<0.05
p<0.05
Activation of naïve CD8+ T cells by DC infected with recombinant BCG

IFN-γ production (pg/ml)

- None
- BCG-261H
- BCG-ΔUT-11-3
- BCG-70M
- BCG-D70M

MOI:
- 0.25 (T:DC = 40:1)
- 0.25 (T:DC = 20:1)
- 0.5 (T:DC = 20:1)

p<0.01
p<0.05
p<0.05
Inhibition of naïve CD8+ T cells activation by treatment of DC with mAb

IFN-γ production (pg/ml)

None  Normal IgG  anti-HLA-ABC mAb  anti-CD86 mAb

(Treatment of BCG-D70M-infected DC)

: BCG-D70M (MOI:0.5, T:DC=20:1)

p<0.01

p<0.01
IL-12p70 production from DC by stimulation with recombinant BCG

- BCG-261H
- BCG-ΔUT-11-3
- BCG-70M
- BCG-D70M

IL-12p70 production (pg/ml)

MOI: 0.25

p<0.05

MOI: 0.5

p<0.05
Phenotypic change induced by infection with BCG-D70M

Infection (MOI)

None

BCG-261H (0.25)

BCG-D70M (0.25)

HLA-ABC  HLA-DR  CD86  CD83  CD1a

192.1  149.9  40.1  1.6  291.8
235.5  188.7  57.3  2.1  267.9
355.3  259.7  183.4  7.3  175.1
Effect of chloroquinin treatment of APC on T cell activation

Chloroquinin: an inhibitor of phagosomal acidification
Effect of reagent on activation of naïve CD8^+ T cells by DC infected with BCG-D70M

![](image)

IFN-γ production (pg/ml)

- **BCG-D70M** (T:DC=20:1)

<table>
<thead>
<tr>
<th>Brefeldin A (μg/ml)</th>
<th>0.0</th>
<th>2.5</th>
<th>0.0</th>
<th>2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(MOI:0.25)</td>
<td>p&lt;0.005</td>
<td></td>
<td>p&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>(MOI:0.50)</td>
<td></td>
<td>p&lt;0.005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Lactacystin (μMo)**

<table>
<thead>
<tr>
<th>(MOI:0.25)</th>
<th>0.0</th>
<th>50.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>(MOI:0.50)</td>
<td>0.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

- Brefeldin A (an inhibitor of antegrade Golgi transportation and of TAP-dependent transportation)
- Lactacystin (a proteosomal protein degradation blocker)
Production of T cells responsible to secondary stimulation in mice

C57BL/6 mice infected for 12 wks
Inhibition of *M. leprae* multiplication by vaccination with BCG-D70M

C57BL/6

4 weeks

8 months

Footpad

<table>
<thead>
<tr>
<th>Number of <em>M. leprae</em> recovered from footpad (log₁₀/mouse)</th>
<th>PBS</th>
<th>BCG-261H</th>
<th>BCG-D70M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

p<0.005

p<0.01

p<0.05

Enumeration of *M. leprae*
Hypothesis

The two independent methods:
1) Phagosomal acidification by urease-depletion
2) Intracellular secretion of antigenic protein worked synergistically

The secretion of fusion protein in lysosome may induce the strongest activation of naïve T cells.
Effect of BCG-D70M infection on co-localization with lysosome

Magnification

BCG-261H

BCG-70M

BCG-D70M

Low

High

Green: BCG
Red: Lysotracker (Lysosome)