Prospects for Vaccines and Immunotherapies against TB

October 2006
Why do we need new TB vaccines?

• 2 million people die every year
• 8 million new infections every year
• 2 billion people are infected
• HIV and multi-drug resistant (MDR) TB are worsening the situation
• BCG vaccination gives limited protection.
The Problem

• Treatment takes too long.

A Consequence

• TB is increasingly Multi-Drug-Resistant

The Reality

• There will always be new resistance to new drugs.

The Solution

• New Vaccines and Immunotherapy
Why does BCG fail?

• Protection ranges from 80% to zero in different parts of the world.
• Environmental influences.
• Vaccination is given to infants but protection only lasts about 15 years.
  - strong protection against childhood TB
  - little against the adult infectious form.
Spectrum of protective efficacies of BCG

<table>
<thead>
<tr>
<th>Population</th>
<th>Protective Efficacy %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controlled Trials</strong></td>
<td></td>
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<tr>
<td>Haiti</td>
<td></td>
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<tr>
<td>British School Children</td>
<td></td>
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<tr>
<td>N. American Indians</td>
<td></td>
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<tr>
<td>Chicago Infants</td>
<td></td>
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<tr>
<td>Puerto Rico-Gen. Pop.</td>
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<tr>
<td>S. Indian-Bangalore</td>
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<tr>
<td>Georgia+Alabama</td>
<td></td>
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<tr>
<td>S. Indian-Chingleput</td>
<td></td>
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<tr>
<td>Illinois Children</td>
<td></td>
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<tr>
<td>Georgia School Children</td>
<td></td>
</tr>
<tr>
<td><strong>Observational Studies</strong></td>
<td></td>
</tr>
<tr>
<td>Brazil (São Paulo)(^1)</td>
<td></td>
</tr>
<tr>
<td>India (Delhi)(^1)</td>
<td></td>
</tr>
<tr>
<td>Thailand (Bangkok)</td>
<td></td>
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<tr>
<td>Korea (Seoul)</td>
<td></td>
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<tr>
<td>Argentina (Buenos Aires)</td>
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<tr>
<td>Cameroon (Yaoundé)</td>
<td></td>
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<tr>
<td>Togo (Lomé)(^2)</td>
<td></td>
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<tr>
<td>England (Birmingham Asians)</td>
<td></td>
</tr>
<tr>
<td>Canada (Manitoba Indians)</td>
<td></td>
</tr>
<tr>
<td>Thailand (Bangkok)(^2)</td>
<td></td>
</tr>
<tr>
<td>Canada (Treaty Indians)</td>
<td></td>
</tr>
<tr>
<td>England (Asians)</td>
<td></td>
</tr>
<tr>
<td>Pápua New Guinea</td>
<td></td>
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<tr>
<td>Burma (Rangoon)</td>
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<tr>
<td>Indonesia (Jakarta)</td>
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<tr>
<td>Sri Lanka (Colombo)</td>
<td></td>
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<tr>
<td>Colombia (Cali)</td>
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<tr>
<td>Malawi (Karonga District)(^3)</td>
<td></td>
</tr>
</tbody>
</table>

**Immunization by environment?**
**BCG protection is probably gone after 15 years**

### Annual incidence of tuberculosis per 1000 participants and protective efficacy of vaccination according to interval since vaccination.

<table>
<thead>
<tr>
<th>Trial Group</th>
<th>Annual incidence per 1000 participants(^1) in each interval (in years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-20</td>
</tr>
<tr>
<td>Negative, Unvaccinated</td>
<td>0.98</td>
</tr>
<tr>
<td>Negative, BCG Vaccinated</td>
<td>0.23</td>
</tr>
<tr>
<td>Negative, Vole bacillus Vaccinated</td>
<td>0.23</td>
</tr>
<tr>
<td>Positive-to 3 TU:</td>
<td></td>
</tr>
<tr>
<td>Induration ≥ 15mm</td>
<td>1.04</td>
</tr>
<tr>
<td>Induration 5-14mm</td>
<td>0.45</td>
</tr>
<tr>
<td>Positive only to 100 TU</td>
<td>0.45</td>
</tr>
<tr>
<td>Protective Efficacy (%)</td>
<td></td>
</tr>
<tr>
<td>BCG</td>
<td>77</td>
</tr>
<tr>
<td>Vole Bacillus</td>
<td>77</td>
</tr>
<tr>
<td>Both</td>
<td>77</td>
</tr>
</tbody>
</table>

\(^1\) After allowing for removals from population at risk due to death or contracting tuberculosis.

\(^2\) Parentheses indicate a protective efficacy based on a total of 20 cases of tuberculosis or less.
TB; Age Distribution by Patient Origin

Number of cases

Age group (years)

Foreign

Swiss
The Challenge

• BCG can not ethically be discontinued or withheld for trial purposes
• A new vaccine will have to give a substantially better protection on top of BCG and environment to be measurable.
The Disease Process
Fig. 1A—Location of the single calcified primary lesions in 105 individuals. From American Review of Tuberculosis.
Fig. 1B—Location of single cavitary lesions in 204 individuals with TB. From the American Review of Tuberculosis.
Primary lesion

Haematogenous spread

Latent TB

In situ PCR

Reactivation (HIV Stress Poverty Smoking)

Progression

Cavities open into bronchi

Cough!

Cavitary TB

Borrowed from G.A.W. Rook
Pre- and Post-Exposure Vaccines

• The disease is due to the immune response - hence we have to be careful that vaccines do not make the response worse.
Types of New Candidate

• Attenuated M. tuberculosis
• Recombinant BCG
• Recombinant virus vector
• Subunit plus adjuvant
• DNA/RNA
Attenuated M. tuberculosis

• RD1 deletion in M. tuberculosis – still pathogenic
• Combined leucine & pantothenate k/o – similar to BCG
• Combined lysine & pantothenate k/o – similar to BCG
Recombinant BCG

• BCG over-expressing Ag85A – protection better than the parent BCG. Phase 1 pending.
• BCG or M. microti expressing CFP-10 & ESAT-6 of RD1 – little better than parents
• BCG expressing listeriolysin, urease k/o – better protection than by parent? Phase 1 pending.
Recombinant Virus Vector

- Adenovirus expressing Ag85A
- Modified Vaccinia Ankara (MVA) expressing Ag85A – modest on its own, but boosts protection after BCG. Into Phase 1
Subunit plus Adjuvant

• Heparin binding Haemagglutinin (HBHA) in MPL/DDA, similar to BCG
• Mtb72f fusion protein in AS01/AS02 boosts BCG – into Phase 1
• ESAT6/Ag85B fusion protein in MPL/DDA, similar to BCG – starting Phase- 1
DNA/RNA

- hsp65DNA or Ag85DNA similar to BCG
- hsp65RNA or Ag85RNA delivered from Sindbis vectors similar to BCG
- Hsp65/Apa/hsp70DNA enhances BCG protection in cattle (naturally exposed to environmental mycobacteria)
- hsp65DNA plus IL-12 DNA in HVJ-liposome, better than BCG
Prime Boost Strategy for Protection of Infants Against Adult TB

• Prime with BCG (or a recombinant BCG or a live TB variant)
• Boost with recombinant fusion protein in adjuvant
• Boost with a vectored vaccine
  - recombinant MVA
  - recombinant Adenovirus
  - packaged DNA
<table>
<thead>
<tr>
<th>Candidate - vaccines</th>
<th>Developer</th>
<th>Initial cGMP Production</th>
<th>Regulatory Assessm.</th>
<th>Phase I Clinical Trials</th>
<th>Clinical Trials in PPD+</th>
<th>Other Clinical Trials in PPD+ &amp; HIV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVA-Ag85A, [VV, Pr-1]</td>
<td>UOXF</td>
<td>Done (lot1)</td>
<td>Done (lot1)</td>
<td>On-going 2003-4 UOXF</td>
<td>2004-5 UOXF</td>
<td>2005 UOXF</td>
</tr>
<tr>
<td>Mtb72f hybrid recAg + AS02</td>
<td>GSKbio</td>
<td>Done (lot1)</td>
<td>Done (lot1)</td>
<td>Initiated Feb 2004 USA</td>
<td>09-2005 Lausanne</td>
<td>2006-7 Lausanne</td>
</tr>
<tr>
<td>Ag85B-ESAT6 recAg + IC31</td>
<td>SSI</td>
<td>Done 2004</td>
<td>May 2005</td>
<td>09/ 2005 Leiden</td>
<td>2006 Leiden</td>
<td>2006-7 Ethiopia?</td>
</tr>
</tbody>
</table>
Correlates of Protection are Critically Needed

- Delayed-type hypersensitivity?
- Frequency of circulating IFN-γ producing T cells?
- Frequency of granule-mediated cytotoxic T cells?
Killing and Cytotoxic Granules

Viable M. tuberculosis after 24 h (log$_{10}$ CFU)

BLT-esterase (Log$_{10}$ $A_{405}$ x $10^3$)
Protection *In Vivo*

![Graph showing the relationship between BLT-esterase activity and M. tuberculosis in spleen (log10 CFU). The x-axis represents BLT-esterase activity in terms of \( \log_{10} A_{405} \times 10^3 \), while the y-axis shows M. tuberculosis in spleen. The graph indicates a negative correlation, suggesting increased BLT-esterase activity leads to a decrease in M. tuberculosis in spleen.]
Post-exposure (Immunotherapy)
One approach is immuno-suppression

- HIV infection accelerates sputum conversion rate during chemotherapy.
- In non-HIV patients, in combination with effective chemotherapy, immuno-suppression can reduce pathology and enhance cure: prednisolone (Whalen JID 2005), etanercept (anti-TNF, Wallis AIDS 2004) – but beneficial effects have been small, adverse effects outweigh benefits.
Another Approach is Immune ‘Re-inforcement’

• Four trials of IFN-γ by aerosol or intramuscular delivery, one of IFN-α by aerosol; all had negligible benefit.

• Two trials of IL-2 sub-cutaneously; one suggested small benefit, one (with non-MDR patients) significantly delayed sputum conversion.
Animal Experiments Now Suggest Immune ‘Re-alignment’ is the Way Forward

- Multiple approaches now show the potential:
  1. DNA expressing TB antigen
  2. DNA expressing IL-12
  3. Immunoglobulin (IvIg)
  4. Antibody against IL-4.
  5. M. vaccae
  6. RUTI
Applications of Post-infection (Immune Re-alignment) Vaccines

- Therapy of MDR TB
- Shortening of standard DOTS
- Replacement of chemoprophylaxis
Therapy by DNA Vaccination

M.Tb

DNA x3

6 weeks

several months
Therapy of Chronic TB by DNA Vaccination

• 2 months after i.v. infection, 4 doses of DNA were given at 2-week intervals.

• Numbers of live bacteria at 2 months (open bars) and 5 months (red bars) after start of treatment are shown.
DNA therapy switches immunity from IL-4- to IFN-γ-dominated.

- IFN-γ: tan (2 Month) / red (5 Month) = Th1 immune response
- IL-4: blue (2 Month) / green (5 Month) = Th2 immune response

**Frequency of T cells producing IFN-γ or IL-4**

- Untreated
- Saline
- BCG
- Hsp65 DNA
- Hsp70 DNA
- ESAT6 DNA
- Empty DNA
- Treatment

Phenotype frequency spots/10³ cells
DNA Vaccination Re-aligns the Immune Response During Chemotherapy

<table>
<thead>
<tr>
<th>Infection</th>
<th>Treatment</th>
<th>IL-4 producing T-cell frequency (×10³)</th>
<th>Reactivation by dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time 0</td>
<td>6 months</td>
</tr>
<tr>
<td>H37Rv</td>
<td>Untreated</td>
<td>1.9 ± 0.04</td>
<td>1.7 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>DNA-hsp65</td>
<td>2.0 ± 0.05</td>
<td>0.2 ± 0.01*</td>
</tr>
<tr>
<td></td>
<td>Drug</td>
<td>2.3 ± 0.05</td>
<td>1.4 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Drug+DNA-hsp65</td>
<td>1.6 ± 0.03</td>
<td>0.1 ± 0.01*</td>
</tr>
</tbody>
</table>
Therapeutic Protocol in “Cornell” Model of Latent TB

- Balb/c + H37Rv
DNA vaccination reduces the number of bacteria remaining after drug therapy.
DNA Vaccination Improves Treatment of MDR-TB


Immunotherapy with plasmid DNA encoding mycobacterial hsp65 in association with chemotherapy is a more rapid and efficient form of treatment for tuberculosis in mice.
Improves Treatment with the Latest Anti-TB Drug in BCG immunized, TB challenged mice

Data of Jacques Grosset, Washington DC
CFU TB Lung

Days Post-infection

Controls
Octagam (3+5d)
Octagam (6+24hrs)

CFU M. tuberculosis per tissue

Days Post-infection

0 2 0 4 0 6 0 8 0 1 0 0

10^3
10^4
10^5
10^6

Lung
The effect of IVIg and albumin on the growth of M. tuberculosis in mice

![Graph showing CFU counts of M. tuberculosis in spleen over days post-infection for controls, IVIg, and albumin treatments.](image)
CFU TB Lung

Days Post-infection

Lung

CFU M. tuberculosis per tissue

- Controls
- Octagam Late treatment
- Octagam (3+5)

Octagam
Dose response curve of the effect of IVIg on M. tuberculosis in mice

A Lung

CFU/tissue

Day

Control
IVIg (normal)
IVIg (0.1g/kg)
IVIg (0.5g/kg)
IVIg (1g/kg)
Therapeutic Effect of anti-IL-4

Log$_{10}$ CFU

Days

untreated
gammaglob 0.1
gammaglob 0.5
anti-IL-4 high
anti-IL-4 low

(0.7 and 0.07 mg/Kg)
Therapeutic Effect of anti-IL-4

Spleen

Log 10 CFU

untreated
- gammaglob 0.1
- gammaglob 0.5
- anti-IL-4 high
- anti-IL-4 low

(0.7 and 0.07 mg/Kg)
Prolonged Effect of anti-IL-4
Where are we Now?

- Around half a dozen new vaccines are in or entering Phase 1 clinical trials.
- Prime-boost is the favoured approach, but there is no evidence yet that any provide improved protection in people.
- Animal vaccines and immunotherapeutic vaccines may be the first to yield clear results.