Stimulation of mucosal immunity by pulmonary delivery of live vaccines.
A safe and effective strategy against TB

Nacho Aguilo
(naguilo@unizar.es)
Pulmonary *Mycobacterium bovis* BCG Vaccination Confers Dose-Dependent Superior Protection Compared to That of Subcutaneous Vaccination

Nacho Aguiló, Ana María Toledo, Eva Maria Lopez-Roman, Esther Perez-Herran, Eamonn Gормley, Joaquin Rullas-Trincado, Inigo Angulo-Barturen, Carlos Martin

Pulmonary but Not Subcutaneous Delivery of BCG Vaccine Confers Protection to Tuberculosis-Susceptible Mice by an Interleukin 17-Dependent Mechanism

Nacho Aguiló, Samuel Alvarez-Arquedas, Santiago Uranga, Dessislava Marinova, Marta Monzón, Juan Badiola, and Carlos Martin
ANIMAL MODELS TESTED WITH PULMONARY LIVE VACCINES

Goonetilleke et al. 2003
Tree et al. 2004
Chen et al. 2004
Giri et al. 2006
Aguilo et al. 2015

Lagranderie et al. 1993

Kaushal et al. 2015
Pulmonary vaccination with live vaccines is safe in preclinical models

Tree et al. 2004

Lagranderie et al. 1993
AEROSOL BCG TREATMENT OF CARCINOMA METASTATIC TO THE LUNG: A PHASE I STUDY

Fredric B. Garner, MD, Carol A. Meyer, RN, Deborah S. White, RN, and Allan Lipton, MD

BCG (TICE) was safely administered to 15 patients with metastatic cancer to the lungs in weekly doses of up to $3 \times 10^7$ organisms by the aerosol route. The aerosol route of administration is associated in approximately 33% of the doses with a toxicity syndrome of malaise, fever, and chills beginning 4 to 8 hours after treatment and ending within 24–36 hours. This syndrome is experienced by all patients and symptoms gradually subside with continuation of therapy. No hepatic or pulmonary toxicity was documented during the 221 treatment doses.


- Weekly BCG doses of $3 \times 10^7$ by the aerosol route
- No hepatic or pulmonary toxicity was documented during the study
### EFFICACY DATA IN ANIMAL MODELS

**Guinea Pigs**

<table>
<thead>
<tr>
<th>Level</th>
<th>n</th>
<th>Mean LogCFUs</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>aerosol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>4</td>
<td>1.7350</td>
<td>0.9618</td>
</tr>
<tr>
<td>Group 2</td>
<td>4</td>
<td>1.0100</td>
<td>0.0000</td>
</tr>
<tr>
<td>Group 3</td>
<td>4</td>
<td>2.9750</td>
<td>0.6504</td>
</tr>
<tr>
<td><strong>subcutaneous</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>4</td>
<td>3.1575</td>
<td>0.1889</td>
</tr>
<tr>
<td>Group 5</td>
<td>4</td>
<td>2.7000</td>
<td>0.8943</td>
</tr>
<tr>
<td>Group 6</td>
<td>4</td>
<td>2.9375</td>
<td>0.8514</td>
</tr>
<tr>
<td>Group 7</td>
<td>4</td>
<td>2.9050</td>
<td>1.5018</td>
</tr>
<tr>
<td>Group 8</td>
<td>4</td>
<td>2.3100</td>
<td>0.9918</td>
</tr>
<tr>
<td>Group 9</td>
<td>4</td>
<td>2.3200</td>
<td>0.6845</td>
</tr>
<tr>
<td>Group 10</td>
<td>4</td>
<td>2.3050</td>
<td>0.6396</td>
</tr>
<tr>
<td><strong>Placebo</strong></td>
<td>4</td>
<td>4.9425</td>
<td>0.2371</td>
</tr>
</tbody>
</table>

Lagranderie et al. 1993. Tubercle and Lung Disease
Efficacy data in animal models

Balb/c mice

A. CFU/lung (log10) 4 weeks post-i.n. M.tb challenge

- PBS: p=0.000019
- BCGsub.c: p=0.00015
- BCGi.n.

B. CFU/spleen (log10) 4 weeks post-i.n. M.tb challenge

- PBS: p=0.00655
- BCGsub.c: p=0.11
- BCGi.n.
C57BL/6 mice

Aguilo et al. 2012. Clinical Vaccine Immunology
Efficacy data in animal models

C57BL/6 mice Dose response profile in BCG pulmonary vaccination

Aguilo et al. 2012. Clinical Vaccine Immunology
BCG IN boosts BCG SC vaccination

Unpublished results
Does pulmonary BCG work where parenteral immunization fails?
Parenteral BCG immunization confers good protection in preclinical models used to test TB vaccines
TB susceptible and resistant mouse strains

Medina et al. 1998 Immunology
BCG subcutaneous poorly protects DBA/2 mice against TB challenge

Medina et al. 1999 Immunology
Gruppo et al. 2002 Microbiology
PROTECTIVE EFFICACY (CFU in lungs)

s.c. BCG Danish $10^6$ CFUS

i.n. Challenge
H37Rv $\approx 150$ CFUS

Sacrifice

8 weeks

4 weeks

BCG s.c does not confer protection to DBA/2 mice

Aguilo et al. Journal of Infectious Diseases. IN PRESS
PROTECTIVE EFFICACY (CFU in lungs and spleen)

8 weeks

s.c. or i.n BCG Danish $10^6$ CFUS

4 weeks

i.n. Challenge H37Rv $\approx 150$ CFUS

Sacrifice

---

BCG i.n confers protection to DBA/2 mice in lungs and spleen

---

Aguilo et al. Journal of Infectious Diseases. IN PRESS
PROTECTIVE EFFICACY (Survival)

s.c. or i.n BCG Danish $10^6$ CFUS

i.n. Challenge
H37Rv $\approx 1000$ CFUS

Aguilo et al. Journal of Infectious Diseases. IN PRESS
BCG intranasal induces both local and systemic higher Th1 and Th17
s.c. or i.n BCG Danish $10^6$ CFUS

8 weeks

i.n. Challenge H37Rv $\approx 150$ CFUS

4 weeks

Sacrifice: CFUs and PPD-specific immune response

PPD-specific immunity

CFUs

Aguilo et al. Journal of Infectious Diseases. IN PRESS
Th17 lung response post-challenge correlates with better protection.
ROLE OF IL17A IN PROTECTIVE EFFICACY

Aguilo et al. 2016. Journal of Infectious Diseases. IN PRESS
MUCOSAL IMMUNOGLOBULINS (IgA)

Johansen et al. 2011. Mucosal Immunology
pIgR FUNCTION

WT  pIgR -/-

Johansen et al. 1999. Journal of Experimental Medicine
# ROLE OF IgA IN PROTECTIVE EFFICACY

<table>
<thead>
<tr>
<th>mAb (isotype)</th>
<th>Target Antigen (Type)</th>
<th>Model</th>
<th>Organism/Antigen Challenge (Route)</th>
<th>mAb Administration (Timing to Infection)</th>
<th>Change in CFU</th>
<th>Biological Effect</th>
<th>Quantitative Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBA51 (IgA) and TBA84 (IgA)</td>
<td>16 kDa α-crystallin (intracellular and cell-wall protein) and 38 kDa PstS-1 (CF protein)</td>
<td>Mouse (BALB/c)</td>
<td>M. tuberculosis (i.t.)</td>
<td>i.t. (mAb given 30 min prior to infection)</td>
<td>↓ Lungs (only for TBA61)</td>
<td>Reduced lung pathology (only for TBA61)</td>
<td>Reduced lung colonization by $\sim 200 \times 10^5$ CFUs 21 days postinfection in mAb TBA61-treated mice compared to controls and TBA84-treated mice ($p &lt; 0.05$) and reduced peribronchial inflammation in TBA61-treated mice compared to controls 21 days postinfection ($p &lt; 0.05$)</td>
<td>López et al., 2009</td>
</tr>
<tr>
<td>TBA61 (IgA)</td>
<td>16 kDa α-crystallin (intracellular and cell-wall protein)</td>
<td>Mouse (BALB/c, C57BL/6, and C3H/HeJ)</td>
<td>M. tuberculosis (i.n. and i.v.)</td>
<td>i.n. and i.v. given at 3, 5, or 7 weeks as CIT with INF-γ, polyclonal Ab against IL-4, and mAbTBA61 in mice treated for 4 weeks with INH/R</td>
<td>↓ Lungs in CIT-treated mice</td>
<td>Prevention of TB relapse in mice treated with CIT together with ↑ granuloma formation and ↑ cyto- and chemokine levels</td>
<td>Reduced lung colonization by $\sim 3-4 \log(10)$ CFUs 8 weeks postinfection in CIT-treated mice compared to controls; strongest protection when CIT given 5 weeks postinfection ($p = 0.001$)</td>
<td>Buccheri et al., 2009</td>
</tr>
<tr>
<td>2E9 (IgA1)</td>
<td>16 kDa α-crystallin (intracellular and cell-wall protein)</td>
<td>Mouse (CD89tg)</td>
<td>M. tuberculosis (i.n.)</td>
<td>i.n. with and without INF-γ (2 hr prior and 1 or 21 days post)</td>
<td>↓ Lungs</td>
<td>Reduced lung pathology</td>
<td>Reduced lung colonization by $&lt; 1 \log(10)$ CFUs 4 weeks postinfection in mAb plus INF-γ-treated mice compared to controls ($p &lt; 0.05$) and significantly reduced lung granuloma formation in mAb-treated mice ($&lt; 10%$) compared to controls ($\sim 45%$; $p &lt; 0.001$)</td>
<td>Balu et al., 2011</td>
</tr>
</tbody>
</table>

Achkar et al. 2013
Induction of MTB-specific IgA by BCG intranasal in an IL17-dependent fashion

**MTB-SPECIFIC IgA**

<table>
<thead>
<tr>
<th>Group</th>
<th>IgA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG sc</td>
<td>1.75 ± 0.25</td>
</tr>
<tr>
<td>BCG in+isotype</td>
<td>1.50 ± 0.30</td>
</tr>
<tr>
<td>BCG in+αIL17A</td>
<td>0.50 ± 0.10</td>
</tr>
<tr>
<td>NV</td>
<td>0.25 ± 0.05</td>
</tr>
</tbody>
</table>

**plgR band intensity**

<table>
<thead>
<tr>
<th>Group</th>
<th>plgR band intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NV</td>
<td>5000 ± 500</td>
</tr>
<tr>
<td>BCG sc</td>
<td>1000 ± 100</td>
</tr>
<tr>
<td>BCG in+isotype</td>
<td>2000 ± 200</td>
</tr>
<tr>
<td>BCG in+αIL17A</td>
<td>20000 ± 2000</td>
</tr>
</tbody>
</table>

Aguilo et al. Journal of Infectious Diseases. IN PRESS
PROTECTION AND IgA INDUCTION BY HEAT-KILLED MTB

BAL SAMPLES

PPD-SPECIFIC IgA

Unpublished results
SUMMARY

- Good safety profile of live pulmonary vaccines in different preclinical models.
- Pulmonary route of administration is more effective than parenteral vaccination in animal models.
- Pulmonary vaccination can work where parenteral immunization fails.
- Possible role of IL17 in BCG pulmonary-induced protection.
- Good correlation of specific IgA in BAL with protection.
Nacho Aguiló
José Antonio Aínsa*
Henar Alonso
Esther Broset
Alberto Cebollada
Ana Belén Gómez
Jesús Gonzalo
Begoña Gracia
Mª José Iglesias Gozalo*
Carmen Lafoz
Elena Mata
Raquel Tarancón

Carlos Lampreave
Dessi Marinova
Isabel Otal*
Ana Pico
Sofía Samper*
Luis Solans
Santiago Uranga
Samuel Álvarez
Liliana Rodrigues
Irene Perez

Microbiología Clínica
Mª José Revillo Pinilla
Asunción Vitoria

Dirección General de Salud Pública

Grupo de Apoptosis, Inmunidad y Cancer
Alberto Anel
Julian Pardo

Centro de Encefalopatías y Enfermedades Emergentes
Marta Monzón
Juan J Badiola

* IP coordinadores de línea
thank you