Pulmonary mucosal BCG vaccination shows protection of infection in a novel repeated ultra-low dose challenge model in rhesus macaques.

Frank Verreck
5th Global Forum on TB Vaccines [OA-04]
21 February 2018, New Delhi
Using Macaque Species in TB Vaccine Research

- naturally susceptible to Mtb
- great face validity of TB in many aspects

Macaca mulatta  
* rhesus

Macaca fascicularis  
* cynomolgus

to support preclinical vaccine development by efficacy testing

to investigate mechanisms of disease and protective infection

exploratory research

applied research

translation

vaccine R&D

biomarker R&D

back-translation & verification
Today's Presentation

• on optimizing model conditions for vaccine efficacy evaluation in rhesus macaques
ultimately demonstrating the feasibility of Repeated Ultra-Low Dose (RULD) infection as useful modelling condition

• on investigating mechanisms of disease and of protective immunity, towards correlates/biomarkers
finding local polyfunctional IL17+ CD4 cells and specific immunoglobulins as distinctive markers of protection
Standardising NHP TB Studies for Vaccine Research

host

Rhesus (*Macaca mulatta*)

*M. tuberculosis* challenge

strain Erdman K01 (harmonisation strain, from BEI Resources, USA)

by endobronchial instillation

*BCG vaccine*

standard human dose of

strain Moscow (a.k.a. Sophia), while in earlier studies using Danish 1331
Single High Dose (500 CFU) Mtb Challenge

12 months follow-up, group size N=12
Verreck et al (unpublished)

Modus of Infection: high dose

Modus of readout: time-to-humane-endpoint (survival)
Single High Dose (500 CFU) Mtb Challenge

- **Modus of Infection**: high dose
- **Modus of readout**: time-to-humane-endpoint (survival)

12 weeks follow-up, group size N=6
Verreck et al (PLoS One) 2009

![Graph showing lung PA levels](chart.png)
Is High Dose Challenge in Rhesus Too Stringent for TB Vaccine Evaluation?

<table>
<thead>
<tr>
<th>Modus of Infection</th>
<th>Modus of readout</th>
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<tbody>
<tr>
<td>✓ high dose</td>
<td>time-to-humane-endpoint (survival)</td>
</tr>
<tr>
<td>✓ high dose</td>
<td>advanced disease</td>
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</tbody>
</table>

- 100% infection take in nv.ctrls
- rapid onset to progressive TB (≤ 12 wks)
- power to demonstrate reduction of TB (relative to nv.ctrls) with group size N=6 only

**BUT**

- unable to demonstrate statistically significant improvement over BCG; finding positive indicators only
Pulmonary BCG protects where standard BCG fails

Corroborating and extending beyond earlier studies showing the superiority of i.v. > pulm > i.d BCG administration in NHP.
Barclay et al (1973) IAI;
Verreck et al (2017) Tuberculosis
## Working towards "low" dose infection modelling

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</tr>
<tr>
<td>✓ low dose</td>
<td>mild disease</td>
</tr>
<tr>
<td>? repeated low dose</td>
<td>infection (and/or mild disease)</td>
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<td></td>
<td>natural transmission</td>
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12 months follow-up, group size N=12

12 weeks follow-up, group size N=6

- 10 to 25 CFU
- NHP TB Research Community of CTVD
Finding the lowest amenable dose for RLD infection
a comparative infection dose-escalation study in rhesus versus cynomolgus

Karin Dijkman, Ph.D student

Study Weeks (post-primary infection)

K. Dijkman et al. (in preparation)
Visit poster PD-10, on disease susceptibility and immune profiles of rhesus versus cyno
Repeated Ultra-Low Dose (RULD) Infectious Challenge
an alternative study design in rhesus (N=8 per group) toward readout of Pol

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Vaccination Phase</th>
<th>Infection Phase</th>
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<tbody>
<tr>
<td></td>
<td>non-vaccinated</td>
<td></td>
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<tr>
<td></td>
<td>standard BCG</td>
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<tr>
<td></td>
<td>pulmonary MUCosal BCG</td>
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<tr>
<td></td>
<td>endobronchial Mtb challenge</td>
<td>8x, targeting 1 CFU/dose/week</td>
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<td>euthanasia &amp; PME</td>
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K. Dijkman et al. (in preparation)
IGRA response dynamics over time
by specific IFNγ ELISPOT

PPD stimulated

ESAT6-CFP10 stimulated

K. Dijkman et al. (in preparation)
Mucosal BCG shows significantly delayed IGRA conversion, a Pol signal

K. Dijkman et al. (in preparation)
Mucosal BCG significantly reduces pathology

K. Dijkman et al. (in preparation)
Mucosal BCG significantly reduces bacterial load

K. Dijkman et al. (in preparation)
CD4 T cells in BAL by multi-label flow-cytometry, 8 weeks post-vaccination
mucosal BCG induces highest cytokine levels and an exclusive IL17 signal

K. Dijkman et al. (in preparation)
mucosal BCG excl. induces a local poly-functional Th17 response

K. Dijkman et al. (in preparation)
mucosal BCG induces specific local IgG and IgA

K. Dijkman et al.  
(in preparation)
Summarizing Conclusions

RULD challenge is a feasible strategy for enhanced vaccine efficacy readout in rhesus toward PoI and PoD signals showing more subtle TB infection and disease phenotypes.

Pulmonary mucosal BCG vaccination in NHP (again) is superior over standard intradermal BCG administration by delayed IGRA conversion and significant reduction of pathology and bacterial load (no CFU detectable in 2 out of 8).

Local, Ag-specific, multi-functional IL17+ CD4+ T cells and IgG & IgA appear as correlates of protective immunity.

Local mucosal vaccination per se and perhaps pulmonary BCG administration in particular, provides a great perspective for improved BCG vaccination or BCG re-vaccination to fight TB.
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AnimalCARE Team
VetCARE Team

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