Salmonella Typhi bactericidal antibody activity is a correlate of disease severity, but not protection against typhoid fever

After live oral vaccine in the human challenge model

Andrew J Pollard

Helene B Juel*, Helena B Thomaides-Brears*, Thomas C Darton, Claire Jones, Elizabeth Jones, Sonu Shrestha, Rebecca Sie, Ushma Galal, Stephen Baker, Christoph J Blohmke
Using a human challenge model of typhoid infection to measure vaccine efficacy

Attenuated S. Typhi strain
2 independent, (ssaV and aroC) which limit replication and growth

Vaccination

Placebo
M01ZH09
Ty21a
D-28 Pre-vaccination
28 days

Challenge

1-5x10⁴ CFU S. Typhi Quailes
D0 Challenge

No Diagnosis

Acute disease

nTD
TD
Using a Human Challenge Model of Infection to Measure Vaccine Efficacy: A Randomised, Controlled Trial Comparing the Typhoid Vaccines M01ZH09 with Placebo and Ty21a

Thomas C. Darton, Claire Jones, Christoph J. Blohmke, Claire S. Waddington, Lin Zhan, Anna Peters, Kathryn Haworth, Rebecca Sin, Christopher A. Green

Cumulative proportion with infection

- Placebo
- M01ZH09
- Ty21a

Time to infection, hours

Number at risk

<table>
<thead>
<tr>
<th></th>
<th>M01ZH09</th>
<th>Placebo</th>
<th>Ty21a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-96</td>
<td>31</td>
<td>30</td>
<td>30</td>
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<tr>
<td>97-144</td>
<td>31</td>
<td>30</td>
<td>30</td>
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<tr>
<td>145-192</td>
<td>28</td>
<td>20</td>
<td>21</td>
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<tr>
<td>193-236</td>
<td>25</td>
<td>17</td>
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<td>237-280</td>
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<td>15</td>
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<td>281-324</td>
<td>18</td>
<td>13</td>
<td>18</td>
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<tr>
<td>325-360</td>
<td>17</td>
<td>11</td>
<td>18</td>
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</tbody>
</table>

Placebo 67% TD
M01ZH09 58% TD; VE 13%
Ty21a 39% TD; VE 35%
Serum bactericidal antibody assay and typhoid

The SBA titre defined as the lowest dilution of test serum to achieve ≤50% bacterial killing relative to complement alone

modified from M. Sadarangani

A correlate of protection with encapsulated bacteria like Neisseria meningitidis Goldschneider, I, Gotschlich, El B Artenstein MS, 1969
M01ZH09 vaccination induces LPS IgG

- Darton et al 2016: Showed M01ZH09 induced high antibody titres against key antigens

**C**

Log_{10} anti-LPS IgG ELISA units/mL

- **LPS IgG**
  - Day -28
  - Day 0
  - M01ZH09
  - Placebo
  - Ty21a
  - p < 0.0001

- **H IgG**
  - Day -28
  - Day 0
  - M01ZH09
  - Placebo
  - Ty21a
  - p < 0.0001

- Significant p value: Wilcoxon Signed Rank test between D-28 and D0
M01ZH09 vaccination induces bactericidal antibodies

- Pre-vaccination
- Challenge

### Placebo
- D-28
- D0

### Ty21a
- D-28
- D0

### M01ZH09
- D-28
- D0

- Significance: *p* = 0.001

- Significant *p* value: Wilcoxon Signed Rank test between D-28 and D0

![Box plots showing log10 SBA titre for Placebo, Ty21a, and M01ZH09 groups before and after challenge.](image-url)

**Note:** The image contains box plots that illustrate the distribution of log10 SBA titre for the Placebo, Ty21a, and M01ZH09 groups pre- and post-challenge. The plots highlight the significant difference (*p* = 0.001) in bactericidal antibody levels induced by M01ZH09 vaccination compared to the placebo or Ty21a groups.
Typhoid challenge induces bactericidal antibodies in placebo and Ty21a

Mainly in TD groups

- Day 60 post-challenge shown
- Significant p values in colour: Wilcoxon Signed Rank test against 0 FC from D0
- Significant p value in black: Mann-Whitney between TD and nTD for that study arm
M01ZH09 bactericidal antibody activity persists after infection

- Only TD participants shown
- Significant p values in colour: Wilcoxon Signed Rank test to D0 within study arm
- Significant p value in black: Mann-Whitney test to D0 of Placebo
Bactericidal antibody activity does not protect against typhoid infection

No significant p values following Mann-Whitney test between TD and nTD in combined or individual study arms
High bactericidal antibody activity reduces disease severity and cytokine response of vaccinated participants.

**Incubation period**
- Day of diagnosis vs. log$_{10}$ (SBA titre) D0, p=0.013, Rho=0.58

**Bacteraemia**
- Bacteraemia CFU/ml vs. log$_{10}$ (SBA titre) D0, p=0.006, Rho=-0.69
- Symptom severity score vs. log$_{10}$ (SBA titre) D0, p=0.021, Rho=-0.52

No such correlations seen with placebo.

**Cytokines**
- TNFα pg/ml vs. log$_{10}$ (SBA titre) D0, p=0.019, Rho=-0.60
- IL-8 pg/ml vs. log$_{10}$ (SBA titre) D0, p=0.005, Rho=-0.71
- IL-8 pg/ml vs. log$_{10}$ (SBA titre) D0, p=0.004, Rho=-0.69
- IL-1RA pg/ml vs. log$_{10}$ (SBA titre) D0, p=0.051, Rho=0.51

M01ZH09 & Ty21a TD
Anti-O9: LPS antibodies are the key mediators of bactericidal antibody activity. Shown are fits to a mixed effects model that takes into account the multiple time points from each participant.

HlyE, CdtB and PiiL IgG and IgA titres also tested for fit in mixed effects model.
Anti-O9:LPS antibodies are the key mediators of bactericidal antibody activity

Significant p values: Wilcoxon Signed Rank test against UD
Conclusions

In the challenge model:

• Bactericidal antibodies do not protect against typhoid infection

• ......But do reduce disease severity

• Most detectable bactericidal antibodies after live oral vaccines are directed at LPS

• Field?
• Paratyphoid?
Acknowledgements

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*Joint 1st authors

SBA assays ELISA assays SBA assays

- Prathiba Kurupati (Weatherall Institute of Molecular Medicine, University of Oxford) purified the HlyE antigen.
- Matthew K Siggins, Peter Hart, and Calman A MacLennan (School of Immunity and Infection, College of Medicine and Dental Sciences, University of Birmingham) and Leanne Marsay (OVG) advised on SBA assay optimisation.
- Elizabeth Bateman (Dept. of Clinical Immunology, Churchill Hospital, Oxford) performed the sheep erythrocyte lysis assay on sera for complement in the SBA.
Supplementary

Estimate and p values in mixed effects models that correlated SBA data from all arms and time points to ELISA titres against specific antigens, taking into account random effects from grouping multiple time points from the same participants.

<table>
<thead>
<tr>
<th>Correlation with</th>
<th>Estimate (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>O9:LPS IgG</td>
<td>0.5957 (0.3, 0.9)</td>
<td>7.3e-5</td>
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<tr>
<td>O9:LPS IgA</td>
<td>0.9531 (0.7, 1.2)</td>
<td>1.6e-9</td>
</tr>
<tr>
<td>O9:LPS IgM</td>
<td>0.8876 (0.7, 1.1)</td>
<td>1.1e-13</td>
</tr>
<tr>
<td>H IgG</td>
<td>0.3703 (-0.1-0.8)</td>
<td>0.123</td>
</tr>
<tr>
<td>H IgA</td>
<td>0.5031 (0.1-1.0)</td>
<td>0.036</td>
</tr>
<tr>
<td>H IgM</td>
<td>0.3078 (0.2, 0.4)</td>
<td>1.4e-6</td>
</tr>
<tr>
<td>Vi IgG</td>
<td>0.2538 (-0.1, 0.6)</td>
<td>0.109</td>
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<tr>
<td>HlyE IgG</td>
<td>0.6144 (0.1, 1.2)</td>
<td>0.037</td>
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<tr>
<td>HlyE IgA</td>
<td>0.3073 (-0.2, 0.8)</td>
<td>0.223</td>
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<tr>
<td>CdtB IgG</td>
<td>0.0978 (-0.4, 0.6)</td>
<td>0.718</td>
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<tr>
<td>CdtB IgA</td>
<td>-0.0678 (-0.8, 0.6)</td>
<td>0.855</td>
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<tr>
<td>PilL IgG</td>
<td>-0.0433 (-0.5, 0.4)</td>
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<tr>
<td>PilL IgA</td>
<td>-0.1738 (-0.8, 0.5)</td>
<td>0.619</td>
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