Attenuated non-typhoidal *Salmonella* strains as live oral vaccines and as reagent strains for conjugate vaccine production

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Target age by disease burden

• Sub-Saharan Africa
  – Children < 36 months of age
  – Deliver through the Expanded Program on Immunization (EPI) along with pentavalent vaccine, OPV and rotavirus vaccine at ~ 6, 10 & 14 weeks of age

• North America, Europe
  – Elderly
  – Deliver along with future Clostridium difficile and norovirus vaccines, as well as influenza and future elderly pneumococcal vaccine
Roles for CVD attenuated NTS strains

• As *live oral vaccines* (*guaBA clpPX*)

• To make *conjugate vaccine* (COPS-FliC) production safer & more economical:

  \[\Delta guaBA\]- Primary attenuating mutation. Bacteria require exogenous guanine for growth in vitro. This deletion allows large-scale fermentation with enhanced occupational health safety

  \[\Delta clpPX\]- Secondary attenuating mutation results in enhanced flagella expression for *economical purification* of flagella

  \[\Delta fliD\]- Flagellin exported as monomers which enables *economical purification* of flagellin
Engineered NTS strains constructed

**Prototype strains (for proof of principle in mice)**

**S. Typhimurium I77 (ST19)**
- CVD 1921 -- ΔguaBA ΔclpP (hyperexpresses flagella)
- CVD 1925 -- ΔguaBA ΔclpP ΔfliD ΔfljB (hyperexpresses Phase 1, but not Phase 2, flagellin monomers)

**S. Enteritidis R11**
- CVD 1941 -- ΔguaBA ΔclpP
- CVD 1943 -- ΔguaBA ΔclpP ΔfliD

**Definitive oral vaccine strains (for clinical trials)**

**S. Typhimurium D65 (ST313)**
- CVD 1931 -- ΔguaBA ΔclpX

**S. Enteritidis R11**
- CVD 1944 -- ΔguaBA ΔclpX
Phenotypes of attenuated strains

- Flagella
- COPS

\[ \Delta \text{guaBA} \]
\[ \Delta \text{guaBA} \Delta \text{clpPX} \]
\[ \Delta \text{guaBA} \Delta \text{clpPX} \Delta \text{fliD} \]

- LIVE VACCINE
- REAGENT STRAIN

CVD 1925  wt 1943
NTS clpPX mutants are more motile than wild-type and fliD mutants are non-motile.
NTS strains harboring $\Delta guaBA$, $\Delta clpP$ and deletions are highly attenuated in mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>LD50$_{p.o.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>$2 \times 10^4$ CFU</td>
</tr>
<tr>
<td>$\Delta guaBA$</td>
<td>$&gt;10^9$ CFU</td>
</tr>
<tr>
<td>$\Delta guaBA, \Delta clpP$</td>
<td>$&gt;10^{10}$ CFU</td>
</tr>
</tbody>
</table>
Immunized mice produce high serum IgG anti-LPS and anti-FliC titers

Mice were immunized on days 0, 28 and 56 and challenged on day 84 with 100 LD50's.
Live attenuated S. Typhimurium vaccines mediate homologous but not heterologous protection

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Challenge</th>
<th>Challenge dose</th>
<th>Vaccine efficacy</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVD 1921 (ΔguaBA ΔclpP) ST19</td>
<td>S. Typhimurium I77 (B; i; 1,2)</td>
<td>100 X LD50s</td>
<td>86%</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>CVD 1931 (ΔguaBA ΔclpX) ST313</td>
<td>S. Typhimurium D65 (B; i; 1,2)</td>
<td>10,000 X LD50s</td>
<td>100%</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>CVD 1931 (ΔguaBA ΔclpX) ST313</td>
<td>S. Stanleyville J65* (B; z4,z23)</td>
<td>3 X LD50s</td>
<td>91%</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>CVD 1931 (ΔguaBA ΔclpX) ST313</td>
<td>S. Enteritidis R11 (D; gm)</td>
<td>50 X LD50s</td>
<td>51%</td>
<td>P=0.07</td>
</tr>
</tbody>
</table>

Mice were immunized orally on days 0, 28 and 56 and challenged orally on day 84

* i.p. challenge
# Live attenuated S. Enteritidis vaccines mediate homologous and heterologous protection

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<tr>
<td>CVD 1941 (ΔguaBA ΔclpP)</td>
<td>S. Enteritidis R11 (D; gm)</td>
<td>100 X LD50s</td>
<td>76%</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>CVD 1944 (ΔguaBA ΔclpX)</td>
<td>S. Enteritidis R11 (D; gm)</td>
<td>10,000 X LD50s</td>
<td>83%</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>CVD 1944 (ΔguaBA ΔclpX)</td>
<td>S. Dublin R17 (D; gp)</td>
<td>~800 X LD50s</td>
<td>85%</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>CVD 1944 (ΔguaBA ΔclpX)</td>
<td>S. Typhimurium D65 (B; i; 1,2)</td>
<td>~200X LD50s</td>
<td>81%</td>
<td>P=0.002</td>
</tr>
</tbody>
</table>

Mice were immunized orally on days 0, 28 and 56 and challenged orally on day 84
• Target populations for NTS vaccines include high risk groups for mortality in the USA (elderly) & Africa (infants & perhaps HIV-positive adults)

• Candidate live oral vaccines and reagent strains have been constructed

• Prototype \( \Delta guaBA \Delta clpPX \) attenuated NTS strains are:
  – Safe, immunogenic and protective in mice
  – Safe in SIV-infected Rhesus macaques
Live oral vaccine summary 2

• Live NTS vaccines elicit significant seroconversion (4-fold or > rise) of anti-LPS & anti-flagellin antibody titers

• Antibodies show functional antibody activity

• Definitive live oral NTS vaccines have been shown to be protective against a highly lethal challenge in mice

• A live attenuated S. Enteritidis vaccine was able to mediate cross-protection against S. Typhimurium but not vice versa
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