Enterotoxigenic *E. coli* (ETEC) vaccines for humans

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Importance of enterotoxigenic *E. coli* (ETEC)

- ETEC is the most common cause of bacterial diarrhea in children ≤ 3 years (10-20% of all cases)

- ETEC cause ca. 300 million episodes of diarrhea and ca. 300,000 deaths per year in children < 5 years in Latin America, Africa and Asia

- Repeated ETEC diarrheas may cause malnutrition

ETEC is also the most common cause of diarrhea in travelers (20-45% of all TD)
Why is no ETEC vaccine available yet?
Large heterogeneity of diarrheaogenetic ETEC makes vaccine development complicated

Enterotoxins:
LT ~33%; ST ~33%, LT+ST ~33%

Colonization factors (CFs):
> 25 CFs; CFA/I and CS1-CS6 on 50-75% of all ETEC

O-antigens: > 70 in ETEC

Putative protective surface antigens:
e.g. EtpA, flagellin, TibA
Most common CFs and serotypes of human ETEC strains

Colonization factors:

<table>
<thead>
<tr>
<th>Common (50-75%)</th>
<th>Less common</th>
<th>Rare</th>
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<tbody>
<tr>
<td>CFA/I</td>
<td></td>
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<tr>
<td>CFA/II CS1+CS3</td>
<td>CS7</td>
<td>CS13</td>
</tr>
<tr>
<td>CS2+CS3</td>
<td>CS8</td>
<td>CS15</td>
</tr>
<tr>
<td>CFA/IV CS4+CS6</td>
<td>CS12</td>
<td>CS18</td>
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<tr>
<td>CS5+CS6</td>
<td>CS14</td>
<td>CS20</td>
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<tr>
<td>CS6</td>
<td>CS17</td>
<td>CS22</td>
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<tr>
<td>CS21</td>
<td>CS19</td>
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</tbody>
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Common serotypes:

O (LPS): O6, O25, O78, O115, O128
H (flagellae): H12, H16, H21, H45 and H9
Potential ETEC vaccine

In spite of this heterogeneity it has been suggested that a vaccine containing LT antigen (e.g. LTB or the immunologically cross-reactive CTB) and CFA/I, CS3, CS5 and CS6 may have 80-85% protective coverage.
Can genome sequence analyses guide further ETEC vaccine development?

- Should additional components/antigens be added to current candidate vaccines (new CFs, conserved protein antigens etc)?

- Can the same vaccine be used in different geographic areas and populations?

- Is there a risk of antigen changes/antigen drift over time?
Protective immunity against ETEC

Antibodies against CFs and LT (LTB) are protective and cooperate synergistically in animal models

Antibodies against homologous O-antigen can also protect

Mainly secretory IgA (SIgA) antibodies produced locally in the small intestine
Protection by anti-CFA immunity

- **Rabbits:**
  - Intestinal loops and RITARD

- **Human volunteers:**
  - Protection against reinfection with ETEC expressing homologous CFAs in challenge studies

- **Birth cohort studies:**
  - Initial infection with CF positive ETEC “protects” against reinfection with ETEC expressing homologous CFs
Protection by antitoxic immunity; CTB cross-reacts immunologically with LTB

Protective effect by oral CTB-WC cholera vaccine (Dukoral®) against LT+ ETEC diarrhea

- Field study in Bangladesh 67%
- Finnish travelers to Morocco 60%
- U.S. students to Mexico 50%

The CTB component provides significant short-term protection (up to 6 months)
Candidate ETEC vaccines tested in clinical trials

Oral

- LT like antigens (e.g. CTB, LTB, LCTBA, dmLT)
- Purified CFs
- Inactivated whole bacteria expressing CFs + CTB or LCTBA
- Live bacteria expressing CFs and producing LTB

Parenteral / patches on the skin

- LT patch, CS6 patch
- CF-tip (adhesion-based) proteins
- Purified CFs (e.g. CS6)

tested in clinical trials 2012-2013
Immunization with double-mutated LT (dmLT) adjuvant/antigen in adult US volunteers
(Chen W et al, VED 2013)

A single oral immunization with 5, 25, 50 or 100 µg of dmLT

- All doses well tolerated – no significant adverse events
- Strongest anti-LT IgA immune responses to 50µg dose (less after 100µg)

May be used as adjuvant and immunogen in different enteric vaccines
LT patch - Transcutaneous application of E. coli LT: Phase III trial in travelers

- No significant protection against ETEC and other enteric pathogens (Steffen et al, 2013)
- Reduction in duration of travelers diarrhea and total number of stools

Further development of the LT patch terminated by the producer
Colonization factor (CF) antigens

- Purified CFs
  - Recombinantly produced
    - oral or transcutaneous

- CF tip proteins, conserved among several different CFs)
  - transcutaneous or intradermal (id)

- CFs expressed on the surface of recombinant bacteria
  - Inactivated or live vaccines - oral
Fimbrial tip adhesin, CfaE
(Porter et al, US Naval NMRC, VED 2013)

Recent Phase I trial:
3 doses of CfaE +/- dmLT adjuvant transcutaneously or
CfaE or a CfaE chimera (Cfa-sCT2a/LTB) intradermally

• Local skin reactions common after both administration routes
• Id vaccination induced robust serum antibody responses against
  LTB and CfaE and some mucosal responses
• Challenge studies in progress
Live attenuated ETEC vaccine ACE527; three genetically modified strains expressing CFA/I, CS1, CS2, CS3 CS5, CS6 and LTB (Harro et al, 2012, Darsley et al 2012, Harro et al, VED 2013)

Initial Phase I/IIb trials with 2 doses (\(10^{11}\) cfus):
- Mucosal (ALS) responses to CFA/I, CS3, CS6 and LTB
- No significant protection, but reduced severity and colonization
- Side effects: vomiting in 19% and diarrhea in 17%

Phase I trial with 3 doses lyophilized ETEC (\(10^{10}\) cfus) +/-dmLT:
- Mucosal (ALS) responses to LTB, CFA/I and CS3, not CS6
- Mostly mild and a few moderate/severe side-effects
- Results from CFA/I challenge study soon
1st generation oral inactivated rCTB-CF whole cell ETEC vaccine
(developed together with SBL Vaccin, Sweden)

- Formalin-inactivated *E. coli* (1-2 x10^{11} bacteria) expressing CFA/I, CS1, CS2+CS3, CS4+CS5 (4 strains) + rCTB (1 mg); 2 oral doses given in buffer

- Well tolerated, few mild side-effects - in children <12 months after reduced vaccine dose

- Robust intestinal IgA and ASC responses in all age groups in endemic areas and in adult travelers etc
Oral inactivated rCTB-CF ETEC vaccine - rationale for reformulation

- 70 - 80% protection against more severe ETEC diarrhea, i.e. interfering with daily activity, in travellers

but

- No significant protection in 6-18 months old children in Egypt

- Full dose of vaccine in infants have resulted in side-effects; ¼ dose not significant side-effects
Strategies to improve the ETEC vaccine for use in young children

**Over-expression** of CFs - reduced dose of bacteria required?

Replace CTB with **LT like toxoid** - better neutralization of LT?
CTB with LCTBA
7 amino acids changed compared to CTB

**Addition of an adjuvant (dmLT)** to the vaccine - enhanced immunity?
CFA/I expression by "old vaccine" (reference) and new recombinant over-expressing ETEC strains in dot blot test (using specific anti-CFA/I MAb).
2nd generation Multivalent oral ETEC vaccine

Multivalent ETEC vaccine + Adjuvant

CFA/I
CS3
CS5
CS6

Total: $10^{11}$ bacteria
(ca $2 \times 10^{10}$ bacteria/strain)

DMLT

>4 fold higher expression of CFs than in the first generation ETEC vaccine, CS6 added
Testing of the Multivalent oral ETEC vaccine in a phase I, double blind study in adult Swedes

Four groups with 129 volunteers have received:

A. Placebo (bicarbonate buffer only); n=34
B. Multivalent ETEC vaccine alone; n=35
C. Multivalent CF ETEC vaccine + 10 ug of dmLT; n=30
D. Multivalent CF ETEC vaccine + 25 ug of dmLT; n=30

Objective:
To test safety and immunogenicity of the vaccine
The Multivalent ETEC vaccine is safe and tolerable

- No differences in frequencies or intensities of adverse events between the different study groups A-D, i.e., vaccine and placebo recipients.

- 85% of the adverse events were mild, 15% moderate and none severe; same distribution in vaccine and placebo groups.

Lundgren et al, VED 2013
Mucosal immune responses against the oral Multivalent ETEC vaccine

Frequencies of responders against the primary vaccine antigens in mucosal specimens:

~90% of the vaccinated subjects mounted mucosal immune responses to at least 4 of the 5 primary vaccine antigens, i.e. CFA/I, CS3, CS5, CS6 and LTB
Continued studies with Multivalent ETEC vaccine planned

- Will the vaccine induce an immunological memory? Booster vaccination 1-2 years after initial vaccination (initiated at UG)
- Phase I/II studies in descending age groups (adults, toddlers, infants (Bangladesh))
- Phase III study (of protection) in infants (Bangladesh)
- Phase II/III studies in European travelers to ETEC endemic countries
Studies to evaluate possible reasons for poor vaccine protection and immunogenicity of oral vaccines in infants in developing countries

- Is breast milk preventing “take” of oral vaccines? Yes
- Are nutritional deficiencies, e.g. zinc, affecting vaccine immunogenicity? Yes
- Are concomitant infections (helminths and other parasites) interfering with vaccine immune responses in the gut? Not in our studies
- Does arsenic contamination of drinking water affect immune responses to oral vaccines? No
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