Enterotoxigenic *Escherichia coli*: Pathogenesis, Natural Infection and the Current State of Vaccine Development

Stephen J. Savarino, MD, MPH
U.S. Naval Medical Research Center
Silver Spring, MD USA
Risk of travelers’ diarrhea by global region

Causes of under 5 mortality, 2000-2003


from Bryce J et al, Lancet 365:2005;1147
Outline

◆ Pathogenesis
◆ Natural Infection
◆ Models of ETEC disease and colonization
◆ Past and current state of ETEC vaccine development
  ○ Antitoxin vaccines
  ○ Live, attenuated vaccines
  ○ Whole-cell inactivated vaccines
  ○ Fimbrial and adhesin-based subunit vaccines
◆ Issues and Challenges Going Forward
ETEC Pathogenic Mechanisms

**Pathogenesis**

- Flagellum
- EtpA
- CFs
- LT
- GM1
- Guanylyl cyclase
- CFTR
- PKA
- AMP
- NAD
- Adenyl cyclase
- cAMP
- LT A subunit
- PKA
- Nucleus

**Knowledge gaps**

- Dearth of histopathologic correlation in human disease
- Orchestration of adhesion and colonization process
- Does LT play a role in colonization enhancement in vivo?
- Other undiscovered, critical virulence determinants?

---

## Other Proposed Virulence Factors

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Localization/Function</th>
<th>Relevant finding(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtpA</td>
<td>Extracellular adhesin</td>
<td>protective antigens in murine colonization model of ETEC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>recognized by human serum after ETEC infection</td>
</tr>
<tr>
<td>FliC</td>
<td>Flagellar monomer (i.e., H11 flagellum)</td>
<td>protective antigens in murine colonization model of ETEC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>recognized by human serum after ETEC infection</td>
</tr>
<tr>
<td>Ag₄₃, pAT, EatA, TibA</td>
<td>Autotransporter proteins; surface-exposed</td>
<td>recognized by ETEC convalescent serum (human)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Ag₄₃, pAT) protective in murine colonization model of ETEC</td>
</tr>
<tr>
<td>CexE</td>
<td>Secreted protein (unknown function)</td>
<td>expression under control of cfaD (virulence regulator)</td>
</tr>
</tbody>
</table>
(a few of the) Lessons Learned from Natural Disease and Volunteer Studies of ETEC

- Secretory, non-inflammatory diarrhea
  - ...but intestinal inflammatory markers in 15-30% of episodes (Boukenooghe 2000; Mercado 2011)

- Manifestations range from asymptomatic infection to severe cholera-like diarrhea

- Inverse relationship between age and incidence of ETEC diarrhea in endemic settings
  - ...but significant proportion of severe ETEC diarrhea observed in adults (e.g., Taneja 2006; Chowdhury 2011)

- In volunteers, prior ETEC challenge confers protection against homologous rechallenge (Levine 1979)
Models of ETEC Disease and Colonization

- Natural hosts of animal disease (e.g., young pigs, calves)
  - Species specificity of colonization factors
- Volunteer challenge model
  - Debate on suitability for screening vaccines for efficacy
  - Recent alterations achieve inoculum closer to natural infection
- Reversible intestinal tie adult rabbit diarrhea model
  - ‘RITARD’ used in early screening of whole-cell killed ETEC vaccines
- Mouse models
  - Colonization (streptomycin pre-treated CD-1)
  - Pneumonia model (intranasal instillation)
- Nonhuman primate models
  - Rhesus macaques
  - Aotus nancymaææ (new world owl monkeys)
ETEC Vaccine Approaches: Common Denominators

Live, attenuated vaccines

Killed whole-cell vaccine

Subunit vaccines

LT toxoid ± colonization factors [fimbriae]

Clear success has yet to be achieved for any ETEC vaccine
Colonization Factors of Human ETEC Pathogens

Archetypal Colonization Factor (CF)

Class 5 fimbriae alternate chaperone pathway

Class IV pili

FGL chaperone assisted ‘polyadhesins’

Class 1b pili
Prediction and Matching of ETEC Vaccine Coverage based on Global CF Distribution

adapted from Isidean SD et al, Vaccine 2011;29:6167.
# Investigational Human ETEC Vaccines: Current Landscape

<table>
<thead>
<tr>
<th>Preclinical</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Licensure</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtpA glycoprotein</td>
<td>Killed ETEC vaccine</td>
<td>Attenuated live vaccine, ACE527</td>
<td>LT skin patch</td>
<td>Killed cholera/CTB vaccine*</td>
</tr>
<tr>
<td>LT/ST-based toxins</td>
<td>ETEC adhesin based vaccine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>dmLT, modified LT adjuvant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Shigella-vectored ETEC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>S typhi-vectored ETEC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transgenic plant vaccines</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Licensed for ETEC indication in 29 countries, not including U.S.
### CT and LT Toxin-based Vaccines: ‘Then and Now’

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1968</td>
<td>Discovery of heat-labile enterotoxin (LT) (‘diarrhea factor’) from <em>E. coli</em> isolates of patients with cholera-like diarrhea (RB Sack <em>et al</em>)</td>
</tr>
<tr>
<td>1975</td>
<td>Large field trial of parenteral cholera toxoid (glutaraldehyde-treated CT); efficacy not seen against cholera in primary analysis, but short-term (3-month) protection in 5-14 yr-olds observed (G Curlin <em>et al</em>)</td>
</tr>
<tr>
<td>1985</td>
<td>CTB of killed <em>V cholerae</em> 01/CTB oral vaccine exerts positive short-term (6-month) impact on efficacy against endemic cholera (Clemens <em>et al</em>)</td>
</tr>
<tr>
<td>1990</td>
<td>WC/CTB oral cholera vaccine confers short-term cross-protection against LT-positive ETEC in endemic setting (JD Clemens <em>et al</em>)</td>
</tr>
<tr>
<td>2010</td>
<td>Native LT skin-patch vaccine showed significant efficacy against LT-ETEC but non-significant protection against all-ETEC in Phase 3 trial of travelers to Latin America (L Ellingsworth <em>et al</em>)</td>
</tr>
</tbody>
</table>
Efficacy of CTB and LT toxoid vaccines against ETEC diarrhea

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Location</th>
<th>Population</th>
<th>No. subjects (V:P)</th>
<th>Duration F/U</th>
<th>Vaccine Efficacy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTB oral¹</td>
<td>Bangladesh</td>
<td>Women/children (indigenous)</td>
<td>50K (1:1)</td>
<td>3 mos.</td>
<td>67</td>
</tr>
<tr>
<td>CTB oral¹</td>
<td>Morrocco</td>
<td>Finnish travelers</td>
<td>615 (1:1)</td>
<td>short-term</td>
<td>60</td>
</tr>
<tr>
<td>LT TCI</td>
<td>Latin America</td>
<td>U.S. travelers</td>
<td>170 (1:2)</td>
<td>1-3 wks</td>
<td>49 (66)³, 29 (75)³</td>
</tr>
<tr>
<td>LT TCI</td>
<td>Latin America</td>
<td>Europe travelers</td>
<td>1644 (1:1)</td>
<td>1-3 wks</td>
<td>58</td>
</tr>
</tbody>
</table>

¹Whole-cell killed cholera vaccine+CTB. ²Efficacy against LT and LTST ETEC. ³Efficacy estimates shown in parentheses are against moderate/severe diarrhea.

‘Testing of whether augmentation of the [WC/BS] vaccine with additional antigens…can improve efficacy against ETEC infections will be important….because the protective effect against LT-ETEC was short-lasting, consideration should be given to evaluating …methods of prolonging protection¹ (JD Clemens et al, JID 1988).

‘We suggest that immunity to LT blocks the conditioning of the gut wall for enhanced enteric pathogenicity, decreasing the attack rate of LT-containing and non-LT-containing ETEC as well as other pathogens‘ (Frech SA et al, Lancet 2008).

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>Discovery of heat-stable enterotoxin (ST) from porcine <em>E. coli</em> isolates</td>
<td>(HW Smith <em>et al</em>)</td>
</tr>
<tr>
<td>1976</td>
<td>Genetic determinant of STp (STIa) cloned</td>
<td>(M So <em>et al</em>)</td>
</tr>
<tr>
<td>1985</td>
<td>1st volunteer study of an ST-based oral vaccine</td>
<td>(synthetic ST peptide chemically conjugated to LTB); serum/intestinal neutralizing titers elicited (Klipstein <em>et al</em>)</td>
</tr>
<tr>
<td>1986</td>
<td>Volunteer study with an oral synthetic ST-LTB peptide vaccine</td>
<td>(STp 18 aa + LTB 26 aa), elicited serum/intestinal neutralizing titers (Klipstein <em>et al</em>)</td>
</tr>
<tr>
<td>2010-1</td>
<td>Panel of fusions produced comprising LTR192G genetically fused to series of modified STh (STIb) peptides; immunogenic in mice</td>
<td>(Zhang <em>et al</em>)</td>
</tr>
</tbody>
</table>
Live attenuated ETEC Vaccines: ‘Then and Now’

1986 Spontaneous ETEC tox⁻ plasmid derivative (E1392/75 2A) protects against wild type ETEC challenge in small volunteer study (MM Levine et al)

2006 Genetically defined oral, live-attenuated ETEC strain (PTL-003, aroC, ompC, ompF) safe and immunogenic in volunteers (R McKenzie et al)

2008 PTL-003 oral ETEC oral vaccination-challenge volunteers study; two-dose series not protective against mod-severe diarrhea (R McKenzie et al)

2009 ACE527, genetically attenuated trivalent live ETEC vaccine; no significant reactogenicity, robust immunogenicity in volunteers (C Harro et al)

2012 ACE527 volunteer vaccination-challenge study does not show significant protection against moderate-severe CFA/I-ETEC diarrhea, but results in diminution in diarrheal output and challenge strain shedding (M Darsley et al)
Findings from Phase 1, 2 Clinical Trials of ACE527 (multivalent live, attenuated vaccine)

Vaccine Composition

<table>
<thead>
<tr>
<th>Vaccine Strain</th>
<th>Expressed CFs</th>
<th>Expressed Toxoid</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACAM 2022</td>
<td>CS5, CS6</td>
<td>LTB</td>
<td>O141:H5</td>
</tr>
<tr>
<td>ACAM 2025</td>
<td>CFA/I</td>
<td>LTB</td>
<td>O71:H-</td>
</tr>
<tr>
<td>ACAM 2027</td>
<td>CS1, CS2, CS3</td>
<td>LTB</td>
<td>O39:H12</td>
</tr>
</tbody>
</table>

* wild type ETEC derivatives with deletion of *aroC* and *ompF* as well as all native enterotoxin genes.

Pertinent GI Adverse Events (with 2-dose regimen ACE527)

<table>
<thead>
<tr>
<th></th>
<th>Phase 1 Trial</th>
<th>Phase 2 Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>10^10 cfu</em></td>
<td><em>10^11 cfu</em></td>
</tr>
<tr>
<td></td>
<td>n=12</td>
<td>n=12</td>
</tr>
<tr>
<td></td>
<td>buffer</td>
<td>buffer</td>
</tr>
<tr>
<td>n=12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>2 (16.7)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
<td>1 (8.3)</td>
</tr>
</tbody>
</table>

* *p* < 0.05

**Additional Findings from Phase 2 Clinical Trials of ACE527 (live, attenuated vaccine)**

- Optimized volunteer challenge with CFA/I ETEC strain H10407 with **overnight fast**, and dose of \(2 \times 10^7\) cfu
- Immune response rates (serum IgG, IgA, and/or ALS) against LTB (94%) and CFA/I (81%) were high

**Phase 2 Efficacy Results** (*primary end point*)

<table>
<thead>
<tr>
<th></th>
<th>ACE527 n=29</th>
<th>Placebo n=27</th>
<th>PE (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moderate-severe diarrhea</strong>*</td>
<td>15 (51.7)</td>
<td>19 (70.4)</td>
<td>26.</td>
<td>0.12</td>
</tr>
<tr>
<td>Severe diarrhea</td>
<td>14 (48.3)</td>
<td>16 (59.3)</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>All diarrhea</td>
<td>16 (55.2)</td>
<td>20 (74.1)</td>
<td>25.5</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Total weight grade 3-5 stools per subject (median, gms)</strong></td>
<td>390</td>
<td>1128</td>
<td>65.4</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>No. grade 3-5 stools per subject (median)</strong></td>
<td>5</td>
<td>10</td>
<td>50.0</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>H10407 shedding, day 4 (cfu/gm stool)</strong></td>
<td>(7.5 \times 10^5)</td>
<td>(2.9 \times 10^6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Darsley et al, Clin Vacc Immunol 2012*
Whole-cell (WC) Killed ETEC Vaccines: ‘Then and Now’

1988, 91  WC/CTB oral cholera vaccine confers short-term cross-protection against LT-positive ETEC in endemic & travel settings (Clemens ‘88; Peltola ‘91)

1990  Formalinized WC ETEC vaccine (E1392/75 2A; spontaneous ETEC tox-p-lasmid derivative) shows no indication of protection against wild type ETEC challenge in small volunteer study (MM Levine et al)

1990s  Extensive evaluation of killed, oral WC/rCTB ETEC vaccine indicates safety and immunogenicity (Svennerholm AM and others)

2003  Phase 3 field trial of WC/rCTB ETEC vaccine in young Egyptian children indicates lack of significant protective efficacy (Savarino SJ et al)

2007  Two Phase 3 trials of WC/rCTB ETEC vaccine in U.S. adults traveling to Latin America show some indication of protection against moderate-severe ETEC diarrhea (Sack DA et al, 2007; Bourgeois AL et al)

2011  Phase 1 clinical trials initiated with 2nd generation WC ETEC vaccine with ‘over’-expression of CFs and new toxoid (LCTBA)
Phase 1 Clinical Trials of 2nd Generation WC Inactivated ETEC Vaccines (A-M Svennerholm et al)

- Tetravalent (TV) oral inactivated ETEC vaccine
  - Recombinant *E. coli* strains produce **4-20 times more** CF (i.e., CFA/I, CS3, CS5, CS6) than original ETEC vaccine strains
  - LCTBA - LT-like toxoid with 8 LT residue replacements in CTB
  - Add mucosal adjuvant, double mutant LT (dmLT), LTR192G/L211A

- Phase 1 clinical trial of prototype, monovalent CFA/I WC strain plus LCTBA (OEV-120) in Swedish adults

<table>
<thead>
<tr>
<th>Strain</th>
<th>cfu ($\times10^{10}$)</th>
<th>CFA/I content (μg)</th>
<th>Toxoid, mg</th>
<th>No. subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st gen</td>
<td>3</td>
<td>200</td>
<td>rCTB, 1</td>
<td>20</td>
</tr>
<tr>
<td>2nd gen</td>
<td>3</td>
<td>600</td>
<td>LCTBA, 1</td>
<td>19</td>
</tr>
<tr>
<td>2nd gen</td>
<td>12</td>
<td>2400</td>
<td>LCTBA, 4</td>
<td>19</td>
</tr>
</tbody>
</table>

- Phase 1 trial of TV WC ETEC vaccine begun Mar 2012
  - Groups (n=30) given (1) TVWC/LCTBA (1 mg); (2) TVWC/LCTBA (1 mg) + dmLT (10 μg); (3) TVWC/LCTBA (1 μg) + dmLT (25 μg); (4) buffer
# ETEC Fimbrial Vaccines for Humans: ‘Then and Now’

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>First human-specific CF (CFA/I fimbriae) of ETEC identified</td>
<td>Evans et al</td>
</tr>
<tr>
<td>1984</td>
<td>Purified CFA/I given as parenteral (sc) prime and oral booster vaccine suggested protective intestinal immunity achievable against CFA/I-ETEC challenge in volunteers</td>
<td>Evans et al</td>
</tr>
<tr>
<td>1994</td>
<td>Purified CFA/II vaccine (given by intestinal instillation) not protective against CF-homologous ETEC challenge in volunteers</td>
<td>CO Tacket et al</td>
</tr>
<tr>
<td>1998</td>
<td>Passive oral vaccination with anti-CFA/I bovine colostral IgG protects against CF-homologous challenge in volunteers</td>
<td>D Freedman et al</td>
</tr>
<tr>
<td>2002</td>
<td>CS6+LT vaccine given by skin-patch elicited very modest anti-CS6 responses</td>
<td>F Guerena et al</td>
</tr>
<tr>
<td>2004</td>
<td>Conserved tip-localized fimbrial adhesins of CFA/I (Class 5 fimbriae) elucidated</td>
<td>ST Poole et al</td>
</tr>
<tr>
<td>2011-2</td>
<td>Phase 1 clinical trials begun with prototype ETEC adhesin vaccine</td>
<td>Riddle</td>
</tr>
</tbody>
</table>
Adhesin-Based ETEC Vaccine

ETEC Bacterium

CFA/I Fimbria


Prototype CfaE Adhesin: Lessons learned from
development and evaluation of several variants

- Excellent stability profile, adhesion-competent, homogeneous monomer
- Oral anti-dscCfaE bovine colostral IgG protective in volunteers
- dscCfaE ± LTB intranasal (IN) vaccination protective in *Aotus nancymaeae*
- In Phase 1 clinical trials (transcutaneous [TCI] vaccine trial Aug 2011-Sep 2012; Intradermal vaccine trial started Aug 2012)

- Heterobifunctional, heterohexameric LT-like adhesin-toxoid chimera
- Instability at ≥ 4°C (over 4-6 mos) may be overcome with lyophilization
- Chimera+LTR192G ID vaccination protective against CFA/I-ETEC in NHP
- Phase 1 clinical trial started Aug 2012 (ID vaccination ± LTR192G)

- Conceptual shift to adhesin-pilin fusion (1:1 ratio vs. 1:1100 in fimbria)
- Down selected CfaE variant in sp-NMRC CRADA mouse evaluation
- dscCfaEB + adjuvant by ID vaccination protective against CFA/I-ETEC challenge in NHP
ETEC Prototype Adhesin-Based Vaccine Development Schedule

- **cGMP dscCfaE released**
- **cGMP CfaE/LTB chimera released**

2010

- **dscCfaE TCI**
- **Phase 1 FIH**

2011

- **dscCfaE/chimera ID**
- **Phase 1**

2012

- **vaccination/challenge**
- **Phase 2b**

2013

- **Multivalent adhesin-based vaccine**
  - **GMP mfg**
  - **GLP tox**

2014

2015

2016

2017

Expanding R&D on co-adhesins
Specific Issues and Challenges

◆ LT-based vaccine component
  ○ Different delivery routes have achieved similar results
  ○ Short-term protective effect is a consistent finding
  ○ Protection limited to LT-producing ETEC

◆ ST-based vaccine component
  ○ Protective capacity remains to be demonstrated
  ○ Will durability be similar to that of LT-based vaccines?

◆ Live, attenuated ETEC vaccines
  ○ Will safety, immunogenicity be similar in ETEC endemic populations?
  ○ What is the utility of other carrier host strains (e.g., *Shigella*, *S. typhi*)?

◆ Fimbrial adhesin vaccines
  ○ Can these be produced in a cost-efficient way?
  ○ What is the most suitable route of delivery and adjuvant to achieve induction of protective mucosal immunity?

◆ Other candidate vaccines and approaches
General Issues and Challenges

◆ How best to achieve sufficiently broad coverage against ETEC
  ○ LT toxoid + ST toxoid?
  ○ Colonization factors? CFA/I + CFA/II + CFA/IV; more?
  ○ Combination of CFs + toxoids
  ○ Other common antigens (e.g., CS21, EtpA, undiscovered antigens)

◆ What are the effectors of natural and vaccine induced immunity?
  ○ Specific sIgA
  ○ Systemic IgG (via transudation to mucosal surface)
  ○ Is antibody functional activity important (antitoxic, anti-adhesion, bactericidal effects?)

◆ What are the most suitable, available adjuvants for stimulating mucosal immune responses against ETEC
  ○ CT-, LT-like adjuvants (mLT, dMLT in evaluation)
  ○ Retinoic acid
  ○ Others?

◆ Can one vaccine be developed for two distinct populations
  ○ Young children in resource-limited settings (repetitive endemic exposure)
  ○ Adult travelers from non-endemic areas (transient exposure)?
Acknowledgments

Contributors

◆ Naval Medical Research Center
  ○ Aisling O’Dowd
  ○ Steven Poole
  ○ Milton Maciel
  ○ Annette McVeigh
  ○ Chad Porter
  ○ Mark Riddle

◆ U.S. NAMRU-6, Lima, PERU
  ○ Michael Gregory

◆ PATH, Washington, D.C.
  ○ A. Louis Bourgeois

◆ University of Colorado
  ○ Randall Holmes
  ○ Michael Jobling

◆ sanofi pasteur vaccines
  ○ Genevieve Renauld-Mongenie
  ○ Pascal Chaux
  ○ Prem Dinadayala
  ○ Marie Garinot

Funding & Sponsorship

◆ U.S. Army: Military Infectious Diseases Research Program (MIDRP)

◆ U.S. Navy: Medical Advanced Development Program

◆ sanofi pasteur vaccines

◆ PATH Vaccine Solutions: Enteric Vaccine Initiative (EVI)

◆ U.S. National Institute of Allergy and Infectious Diseases

◆ Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF)
Disclaimers

◆ The views expressed in this presentation are those of the speaker and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government

◆ Human research reported herein was approved by the Naval Medical Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects

◆ Animal experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with the principles set forth in the ‘Guide for the Care and Use of Laboratory Animals,’ Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 1996.