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EDITORIAL COMMENTARY

# Providing Structure to Enterotoxigenic *Escherichia coli* Vaccine Development

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**(See the major article by Savarino et al, on pages 7–13.)**

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Enterotoxigenic *Escherichia coli* (ETEC) are ubiquitous contributors to the estimated 1.7 billion annual episodes of diarrheal illness in low-income countries lacking access to clean water and fundamental sanitation. In the developing world, these pathogens are among the most frequent causes of moderate-to-severe diarrheal illness and, consequently, deaths after diarrhea among young children [1]. Although the overall death rate from acute diarrheal illness has declined dramatically in the past few decades [2], ETEC appear to contribute substantially to postdiarrheal sequelae that follow repeated enteric infections. More than 100 million children are currently thought to suffer from gut dysfunction and associated morbidities of stunted growth, malnutrition, poor response to oral vaccines, and impaired cognitive development [3].

Consequently, ETEC and other diarrheal pathogens remain high priority targets for vaccine development [4]. Enterotoxigenic *E. coli* vaccines have been in development since shortly after the identification of these pathogens as a cause of severe cholera-like diarrheal illness >4 decades ago [5]. Canonical vaccine approaches to date have targeted a collection of antigenically distinct, plasmid-encoded antigens known as colonization factors [6], which are thought to be critical for bacterial adhesion to small intestinal surfaces where ETEC deliver heat-stable and heat-labile toxin payloads [7]. Many colonization factors are fimbrial structures composed of a major structural protein subunit that makes up the shaft and minor subunits, which present the actual adhesin molecule at the tip.

The seminal studies reported by Savarino et al in this issue of the *Journal of Infectious Diseases* represent the culmination of years of outstanding fundamental efforts to define the biology of CFA/I, the first fimbrial ETEC colonization factor to be identified. CFA/I was initially found to be encoded on a large virulence plasmid of ETEC H10407, a strain isolated in 1971 from a case of severe cholera-like diarrheal illness in Bangladesh [8], and early controlled human infection model (CHIM) studies showed that H10407-P, a strain of H10407 cured of the plasmid encoding CFA/I, was avirulent [9]. Investigators subsequently demonstrated protection against H01407 challenge following passive immunization with hyperimmune bovine milk immunoglobulin (bIgG) directed at whole CFA/I fimbriae [10], engendering enthusiasm for colonization factor–based approaches to ETEC vaccines. Important developments preceding the current vaccine studies included the definition of the molecular biogenesis, structure, and function of CFA/I [11–14]. A critical step was the elucidation of the CFA/I tip structure and the recognition that the major fimbrial subunit, CfaB, nonconvalently donates an N-terminal extension to the CfaE tip adhesin moiety during CFA/I biogenesis. Inclusion of this extension at the C-terminus of recombinant "donor-strand complemented" CfaE (dscCfaE), permitted the production of soluble, properly folded, functionally active adhesin molecules [15]. This stable recombinant version of the CfaE tip adhesin of CFA/I was used in the studies by Savarino et al to generate bIgG and passively immunize human volunteers.

The robust protective efficacy (approximately 84%) against moderate-to-severe diarrhea in CfaE-vaccinated participants challenged with $10^9$ colony-forming units of H10407 is particularly impressive in light of prior studies that showed that $10^7$ colony-forming units are sufficient to cause significant diarrhea in the majority of naive subjects [16] in the ETEC controlled human infection model. The strong passive protection achieved with the anti-CfaE adhesin bIgG provides important clinical confirmation of the importance of CfaE and critical validation of the tip adhesin approach. Likewise, those in the positive control group, who were passively vaccinated with bIgG against whole CFA/I fimbriae, were equally protected. Although it is not possible from the study design to discern whether antibodies directed against the tip adhesin, the major fimbrial structural

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subunit (CfaB), or both were responsible for protection, this arm of the study reaffirms the importance of colonization factors as protective antigens [10].

Because the incidence of ETEC infections declines with age among young children in developing countries, it is presumed that they are being naturally immunized with repeated infections and that anti-colonization factor immunity contributes to this apparent protection [17]. Nevertheless, it has been difficult to definitively establish mechanistic correlates of protection in field studies [18, 19]. The data reported here by Savarino et al highlight the distinct advantage of conducting well-designed CHIM studies in immunologically naive volunteers to inform ETEC translational vaccinology.

These studies have significant implications for the rational design of vaccines against ETEC and other pathogens that adhere via fimbriae (also referred to as pili) that assemble through similar chaperone-usher-pilus pathways and that terminate in tip adhesins that bind directly to mucosal receptors with stereochemical specificity. Importantly, although the clinical studies reported here provide an important precedent for ETEC structural vaccinology, they also validate the concept of targeting tip adhesins in vaccines for therapeutic strategies to combat infections by ETEC and other pathogens, including uropathogenic *E. coli* [20, 21].

It is important to relate these studies to the current landscape for ETEC vaccine development and what is currently known regarding the target antigens. CFA/I is estimated to be present in <20% of ETEC strains worldwide [22] and is part of a large antigenically heterogeneous family of colonization factors. More than 25 distinct colonization factors have been reported, and novel pili are likely to emerge as DNA sequencing uncovers similar chaperone-usher-pilus operons in the roughly one half of all ETEC strains that currently lack recognizable pathovar-specific pili [23]. The tip adhesin molecules may be more highly conserved than the corresponding major pilin subunits, and there is intriguing in vitro evidence that antibodies raised against dscCfaE can neutralize the activity of similar tip adhesins from other colonization factors [15]. The degree to which 1 colonization factor tip adhesin will afford heterologous cross-protection against ETEC expressing a panoply of pilus antigens, however, is not presently clear, and not all colonization factors involve pili.

There are several practical challenges facing ETEC vaccine development. Future ETEC vaccine approaches will need to establish the appropriate antigenic valency that will be required for broad coverage based on a global assessment of antigen conservation, something that to date has confounded canonical approaches to vaccine development [22]. It seems likely that a combination of antigens will ultimately be needed to achieve broad protection against these organisms of remarkable genetic plasticity [24–26]. Incorporation of emerging recombinant toxoids [27], including mutant forms of heat-labile toxin, which can also serve as a potent mucosal adjuvant [28], and more recently discovered antigens [29] from the ETEC pathovar could potentially complement the tip adhesin approach. It is possible that a subunit vaccine could combine multiple classes of antigens, modeling the present acellular pertussis vaccines [30, 31]. Moreover, from a practical perspective, future ETEC vaccines may need to be delivered with antigens from *Shigella* [32] and perhaps other *E. coli* diarrheal pathovars to yield a combined multivalent vaccine that would achieve protection against the most common bacterial pathogens that afflict young children in developing countries [1]. Finally, a vaccination strategy that provides sustained mucosal protection will be needed for developing countries where oral vaccine performance in target populations is frequently suboptimal.

These challenges are not insurmountable. Recent microbial pathogenesis studies, the availability of multiple whole-genome sequences from a diverse global collection of ETEC isolates [29, 33], and emerging immunoproteomic platforms can further inform and complement colonization factor–centered vaccine development. The studies reported by Savarino et al offer an unprecedented clinical proof of principle for vaccination approaches that target tip adhesins, providing an essential solid foundation on which to build rationally engineered, broadly protective vaccines for ETEC and other pathogens that rely on similar structures to effectively engage the host.

## Notes

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## References

1. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet **2013**; 382:209–22.
2. Walker CL, Rudan I, Liu L, et al. Global burden of childhood pneumonia and diarrhoea. Lancet **2013**; 381:1405–16.
3. MAL-ED Network Investigators. The MAL-ED study: a multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and children up to 2 years of age in resource-poor environments. Clin Infect Dis **2014**; 59(suppl 4):S193–206.
4. Bourgeois AL, Wierzba TF, Walker RI. Status of vaccine research and development for enterotoxigenic *Escherichia coli*. Vaccine **2016**; 34:2880–6.
5. Sack RB, Gorbach SL, Banwell JG, Jacobs B, Chatterjee BD, Mitra RC. Enterotoxigenic *Escherichia coli* isolated from patients with severe cholera-like disease. J Infect Dis **1971**; 123:378–85.
6. Lundgren A, Bourgeois L, Carlin N, et al. Safety and immunogenicity of an improved oral inactivated multivalent enterotoxigenic *Escherichia coli* (ETEC) vaccine administered alone and together with dmLT adjuvant in a double-blind, randomized, placebo-controlled phase I study. Vaccine **2014**; 32:7077–84.
7. Fleckenstein JM, Hardwidge PR, Munson GP, Rasko DA, Sommerfelt H, Steinsland H. Molecular mechanisms of enterotoxigenic *Escherichia coli* infection. Microbes Infect **2010**; 12:89–98.
8. Evans DG, Silver RP, Evans DJ Jr, Chase DG, Gorbach SL. Plasmid-controlled colonization

factor associated with virulence in *Esherichia coli* enterotoxigenic for humans. Infect Immun **1975**; 12:656–67.
9. Satterwhite TK, Evans DG, DuPont HL, Evans DJ Jr. Role of *Escherichia coli* colonisation factor antigen in acute diarrhoea. Lancet **1978**; 2:181–4.
10. Freedman DJ, Tacket CO, Delehanty A, Maneval DR, Nataro J, Crabb JH. Milk immunoglobulin with specific activity against purified colonization factor antigens can protect against oral challenge with enterotoxigenic *Escherichia coli*. J Infect Dis **1998**; 177:662–7.
11. Li YF, Poole S, Nishio K, et al. Structure of CFA/I fimbriae from enterotoxigenic *Escherichia coli*. Proc Natl Acad Sci U S A **2009;** 106:10793–8.
12. Mu XQ, Savarino SJ, Bullitt E. The three-dimensional structure of CFA/I adhesion pili: traveler's diarrhea bacteria hang on by a spring. J Mol Biol **2008**; 376:614–20.
13. Li YF, Poole S, Rasulova F, McVeigh AL, Savarino SJ, Xia D. A receptor-binding site as revealed by the crystal structure of CfaE, the colonization factor antigen I fimbrial adhesin of enterotoxigenic *Escherichia coli*. J Biol Chem **2007**; 282:23970–80.
14. Sakellaris H, Munson GP, Scott JR. A conserved residue in the tip proteins of CS1 and CFA/I pili of enterotoxigenic *Escherichia coli* that is essential for adherence. Proc Natl Acad Sci U S A **1999**; 96:12828–32.
15. Poole ST, McVeigh AL, Anantha RP, et al. Donor strand complementation governs intersubunit interaction of fimbriae of the alternate chaperone pathway. Mol Microbiol **2007**; 63:1372–84.
16. Harro C, Chakraborty S, Feller A, et al. Refinement of a human challenge model for evaluation of enterotoxigenic *Escherichia coli* vaccines. Clin Vaccine Immunol **2011**; 18:1719–27.
17. Qadri F, Saha A, Ahmed T, Al Tarique A, Begum YA, Svennerholm AM. Disease burden due to enterotoxigenic *Escherichia coli* in the first 2 years of life in an urban community in Bangladesh. Infect Immun **2007**; 75:3961–8.
18. Steinsland H, Valentiner-Branth P, Gjessing HK, Aaby P, Mølbak K, Sommerfelt H. Protection from natural infections with enterotoxigenic *Escherichia coli*: longitudinal study. Lancet **2003**; 362:286–91.
19. Rao MR, Wierzba TF, Savarino SJ, et al. Serologic correlates of protection against enterotoxigenic *Escherichia coli* diarrhea. J Infect Dis **2005**; 191:562–70.
20. Langermann S, Möllby R, Burlein JE, et al. Vaccination with FimH adhesin protects cynomolgus monkeys from colonization and infection by uropathogenic *Escherichia coli*. J Infect Dis **2000**; 181:774–8.
21. Langermann S, Palaszynski S, Barnhart M, et al. Prevention of mucosal *Escherichia coli* infection by FimH-adhesin-based systemic vaccination. Science **1997**; 276:607–11.
22. Isidean SD, Riddle MS, Savarino SJ, Porter CK. A systematic review of ETEC epidemiology focusing on colonization factor and toxin expression. Vaccine **2011**; 29:6167–78.
23. von Mentzer A, Sjoling A, Dougan G, Svennerholm A. Whole genome sequencing of enterotoxigenic *Escherichia coli* (ETEC)—search for novel colonization factors. In: Program and abstracts of the 50th US-Japan Cooperative Medical Sciences Program Joint Panel Conference on Cholera and Other Bacterial Enteric Infections. Bethesda, MD, **2016**:115–8.
24. Begum YA, Baby NI, Faruque AS, et al. Shift in phenotypic characteristics of enterotoxigenic *Escherichia coli* (ETEC) isolated from diarrheal patients in Bangladesh. PLoS Negl Trop Dis **2014**; 8:e3031.
25. Rasko DA, Rosovitz MJ, Myers GS, et al. The pangenome structure of *Escherichia coli*: comparative genomic analysis of *E. coli* commensal and pathogenic isolates. J Bacteriol **2008**; 190:6881–93.
26. Crossman LC, Chaudhuri RR, Beatson SA, et al. A commensal gone bad: complete genome sequence of the prototypical enterotoxigenic *Escherichia coli* strain H10407. J Bacteriol **2010**; 192:5822–31.
27. Taxt AM, Diaz Y, Aasland R, et al. Towards rational design of a toxoid vaccine against the heat-stable toxin of *Escherichia coli*. Infect Immun **2016**; 84:1239–49.
28. Norton EB, Lawson LB, Freytag LC, Clements JD. Characterization of a mutant *Escherichia coli* heat-labile toxin, LT(R192G/L211A), as a safe and effective oral adjuvant. Clin Vaccine Immunol **2011**; 18:546–51.
29. Luo Q, Qadri F, Kansal R, Rasko DA, Sheikh A, Fleckenstein JM. Conservation and immunogenicity of novel antigens in diverse isolates of enterotoxigenic *Escherichia coli*. PLoS Negl Trop Dis **2015**; 9:e0003446.
30. Robbins JB, Schneerson R, Trollfors B, et al. The diphtheria and pertussis components of diphtheria-tetanus toxoids-pertussis vaccine should be genetically inactivated mutant toxins. J Infect Dis **2005**; 191:81–8.
31. Cherry JD. The protein content of diphtheria-tetanus toxoids-acellular pertussis vaccines and an emerging clinical problem. J Infect Dis **2005**; 191:1386–8.
32. Martinez-Becerra FJ, Chen X, Dickenson NE, et al. Characterization of a novel fusion protein from IpaB and IpaD of *Shigella* spp. and its potential as a pan-*Shigella* vaccine. Infect Immun **2013**; 81:4470–7.
33. von Mentzer A, Connor TR, Wieler LH, et al. Identification of enterotoxigenic *Escherichia coli* (ETEC) clades with long-term global distribution. Nat Genet **2014**; 46:1321–6.