Emerging Themes in the Molecular Pathogenesis of Enterotoxigenic Escherichia coli

James M. Fleckenstein *a, b, Alaullah Sheikh a.

a Department of Medicine, Division of Infectious Diseases, Washington University in Saint Louis, School of Medicine. Saint Louis, Missouri, USA. b Infectious Disease Section, Medicine Service, Saint Louis Veterans Affairs Health Care System, Saint Louis, Missouri, USA.

*corresponding author
James M. Fleckenstein
Division of Infectious Diseases
Department of Medicine
Washington University in Saint Louis, School of Medicine
660 South Euclid Avenue
Campus Box 8051
Saint Louis, Missouri 63110
jfleckenstein@wustl.edu

© The Author(s) 2021. Published by Oxford University Press for the Infectious Diseases Society of America.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
abstract

Enterotoxigenic *Escherichia coli* (ETEC) are ubiquitous diarrheal pathogens that thrive in areas lacking basic human needs of clean water and sanitation. These genetically plastic organisms cause tremendous morbidity among disadvantaged young children, both in the form of acute diarrheal illness and sequelae of malnutrition and growth impairment. The recent discovery of additional plasmid-encoded virulence factors and elucidation of their critical role in the molecular pathogenesis of ETEC may inform new approaches to the development of broadly protective vaccines. Although these pathogens have been closely linked epidemiologically with non-diarrheal sequelae, these conditions remain very poorly understood. Similarly, while canonical effects of ETEC toxins on cellular signaling promoting diarrhea are clear, emerging data suggest that these toxins may also drive changes in intestinal architecture and associated sequelae. Elucidation of molecular events underlying these changes could inform optimal approaches to vaccines that prevent acute diarrhea and ETEC-associated sequelae.

keywords

*Escherichia coli*, enterotoxigenic; malnutrition, child; brush border; microvilli; bacterial vaccines; enterotoxins; cyclic AMP; CEACAM6; Mucin 2.
epidemiology

The enterotoxigenic *E. coli* are common pathogens that comprise a genetically diverse diarrheagenic *E. coli* pathovar defined by the production of heat-labile (LT) and/or heat-stable (ST) toxins. It is estimated that worldwide hundreds of millions of symptomatic ETEC infections occur annually[1], a burden predominantly borne by young children of low-middle income countries (LMICs). Here, ETEC remain a major cause of deaths due to acute diarrheal illness. Abundant in LMIC regions where clean water and sanitation remain limited, ETEC are perennially the most common pathogen isolated from travelers with infectious diarrhea. Surprisingly however, they have also been associated with outbreaks[2, 3] and sporadic cases in more developed areas including the United States, where recently introduced culture-independent methods may accelerate their identification[4, 5].

canonical view of ETEC virulence

These pathogens were originally identified now more than five decades ago in patients presenting with severe cholera-like diarrheal syndromes[6]. Early investigation of these non-vibrio cholera patients in India, and Bangladesh soon revealed the presence of *E. coli* that produced a heat-labile toxin (LT) similar to cholera toxin, and subsequently the small peptide heat-stable toxins (ST), as well as the first of many plasmid-encoded fimbrial colonization factors (CFs). A canonical view of ETEC virulence in which these pathogens simply adhere to intestine via CFs to deliver their toxin payloads soon evolved, and much of the ensuing investigation of ETEC related to discovery and characterization of additional plasmid-encoded CFs or coli surface (CS) antigens. To date, more than 25 antigenically distinct CF/CS antigens have been defined. Elucidation of the structural biology and biogenesis of these antigens suggests development of a broadly protective vaccine would require a multivalent approach that incorporates multiple adhesins and toxoid molecules[7]. Approaches to vaccine development have focused primarily on these antigens and LT. Nevertheless, data generated over the past few years have highlighted major gaps in our understanding of these extraordinarily common pathogens to demonstrate that ETEC are far more complex than had been appreciated.

essential elements of ETEC as a successful pathogen

In effect, ETEC molecular pathogenesis can be viewed as the summary of events that enable these bacteria to reach the small intestine, traffic to the mucosal surface, engage epithelial cell receptors and ultimately deliver their LT and/or ST toxin payloads. This process ultimately requires a coordinated division of labor between highly conserved core elements encoded the *E. coli* chromosome and the ETEC pathovar-specific features encoded on virulence plasmids[8].
importance of chromosomally-encoded virulence traits

With time, it has become clear that ETEC rely on a number of chromosomally-encoded features. Virtually all ETEC are motile, can be serotyped according to their flagellar H antigens, and flagellar motility is essential to successful delivery of both LT and ST. The flagellar assembly apparatus is encoded on the chromosome as are genes encoding type 1 pili common to all E. coli[9], and those encoding additional outer membrane surface adhesins[10]. Likewise, although the toxins that define ETEC are plasmid-encoded, the type II secretion system (T2SS) for LT[11], and the type I TolC secretion system responsible for export of ST[12] are both encoded on the chromosome. Remarkably, prior to the discovery of its cognate export system, ETEC were thought to simply release LT by lysis, as expression of the structurally similar cholera toxin in a laboratory strain of E. coli (which happened to lack the T2SS) failed to export the toxin. Fortunately, we now know that these chromosomally-encoded features work in a highly orchestrated and cooperative fashion[8] with plasmid-borne pathovar-specific virulence traits to facilitate toxin delivery (table1).

identification and characterization of additional plasmid-encoded ETEC virulence factors

The identification of additional surface-expressed antigens has expanded the repertoire of ETEC virulence factors and contributed to our understanding of ETEC molecular pathogenesis. Two plasmid-encoded loci, discovered in a search for novel secreted molecules, have emerged as important virulence factors. These include EatA, a member of the serine protease autotransporter of the Enterobactericiae (SPATE) family[13], and the etpBAC two-partner secretion locus responsible for export of EtpA, an extracellular adhesin[14]. (figure 1)

Although the layer of secreted mucin in the small intestine is relatively thin compared to that in the colon, it is likely that this effectively prevents effective engagement by pathogenic bacteria including ETEC, and that LT, like cholera toxin, is capable of inducing the production and release of mucin from goblet cells. Recent studies have shown that the secreted passenger domain of EatA, which contains both mucin-binding activity as well as the serine protease catalytic triad, degrades MUC2, the major mucin secreted by intestinal goblet cells. MUC2 is a very large glycoprotein that has been dimerizes at its C-terminal end, and is thought to form complex trimeric structures at the N-terminus of the molecule. This results in hexameric arrays of MUC2 arranged in sheets[15] that in effect trap the bacteria and prevent pathogen-host engagement. Data emerging from EatA-mediated degradation of MUC2 suggests that this conserved protease, as well as SepA, a homologous protein from Shigella flexneri, work by cleaving MUC2 to effectively dissolve the protective sheets of mucin. ETEC equipped with EatA can therefore overcome the mucin barrier allowing these pathogens to engage in productive pathogen-host interactions essential for toxin delivery[16].

EtpA promotes these interactions by acting as a molecular bridge[17] between the bacteria and host cell glycans present on mucins [18] and intestinal epithelial cells. Like a host of
bacterial adhesins, including another two-partner secretion protein, filamentous hemagglutinin of *Bordetella pertussis*, part of acellular pertussis vaccines, EtpA also possesses hemagglutinating activity. In the case of EtpA, this activity is very specific for human A blood group erythrocytes. As blood group A glycans are also present on intestinal epithelia, the preferential binding of EtpA may explain observations of more severe illness observed among blood group A + naturally infected young children[19], and human volunteers challenged with ETEC[20].

Several findings attest to the importance of these more recently discovered “non-canonical” virulence proteins in the molecular pathogenesis of ETEC. First, both antigens discovered in ETEC H10407[13, 14], an early isolate from a patient with severe cholera-like diarrhea, have been identified in multiple isolates from severe diarrheal illness, and they are conserved and have been retained over time among a large and geographically dispersed population of ETEC[21-24]. Both antigens are highly immunogenic in human volunteers challenged with H10407 [25, 26] and naturally infected hosts[24] suggesting that they are expressed *in vivo*. Notably, recent analysis of strains obtained from a birth cohort of young children in Bangladesh indicate that both molecules are significantly associated with symptomatic infection[27]. Conversely, antibodies against either antigen were associated with asymptomatic colonization. Collectively, the data emerging from molecular pathogenesis as well as clinical studies would appear to support a role for these recently discovered “non-canonical” antigens in virulence.

The potential importance of non-canonical antigens as vaccine targets

These recent studies of ETEC molecular pathogenesis may afford new approaches to the development of a broadly protective vaccine. The conservation of these more recently discovered highly immunogenic molecules suggests that they may provide alternative targets for vaccine development. Recently, available ETEC genomes were mined to identify potential surface antigens that were conserved in at least 40% of isolates, and these were then spotted onto protein microarrays to identify those recognized during the course of infection[27]. Interestingly, and somewhat surprisingly, these “open-aperture” immunoproteome studies demonstrated relatively few immunogenic pathovar-specific proteins, among them EtpA and EatA. Altogether, these data suggest that targeting these two molecules could help to overcome some of the hurdles inherent in targeting the less highly conserved CF antigens. Molecular epidemiology of more than one thousand strains from disparate sources indicate that a vaccine targeting just two antigens CS6, and EtpA would likely encompass more than three quarters of all ETEC[21].

Studies are also underway to elucidate the molecular structure of EtpA by cryo-electron microscopy (cryo-EM), map the antibody responses onto the antigen, and determine the functional activity of monoclonal antibodies which recognize specific epitopes. These collaborative “structural vaccinology” efforts should facilitate the identification of critical regions of this large (~170 kD) molecule to target in a polyvalent vaccine.
the utility of intestinal organoids as a novel model in vitro system for ETEC pathogenesis

Seminal studies by Hans Clevers and his laboratory in the Netherlands defined the signals required to maintain and propagate intestinal epithelial cells from stem cells[28-30]. Stem cells obtained from biopsies performed during routine endoscopic procedures can now be preserved, expanded, and differentiated to recapitulate many of the features of normal human intestinal epithelia normally encountered by the bacteria. Unlike transformed cells derived from gastrointestinal malignancies that have been widely used in the past to study ETEC pathogen-host interactions, intestinal organoids reflect the population of cells normally present in the intestinal epithelium including Paneth cells, goblet cells, enteroendocrine cells, and importantly enterocytes with a well-defined brush border, and glycocalyx (figure 2a). Importantly, with access to large biobanks of cells from multiple individuals, rigorous data can now be generated and the importance of features unique to the original host can be examined in detail.

These small intestinal “enteroid” systems have already proven to be extraordinarily useful in exploring pathogen-host interactions. Their ability to recapitulate important features of human biology is illustrated in experiments showing that ETEC deliver toxins more efficiently to blood group A enterocytes, reflecting increased disease severity in human volunteers and naturally infected children who express A blood group antigens[20].

In addition, goblet cells present in the enteroids produce significant amounts of MUC2 mucin, which normally serves as a protective barrier to mitigate interaction of ETEC with enterocytes goblet cells (figure 2b). Data emerging from experiments with small intestinal enteroids as well as with surgical explants of human small intestine demonstrate that EatA plays a critical role by eliminating this protective barrier to permit access of the bacteria to the surface of enterocytes where they can adhere and deliver their toxin payloads.

enigmatic sequelae associated with ETEC infection

While the mortality from acute diarrheal diseases has declined appreciably since the introduction of oral rehydration therapy and other measures, even less severe episodes of ETEC diarrhea can exert lasting effects on children, including growth faltering, and malnutrition[31]. Although ETEC, as well as other pathogens, have been repeatedly linked to these sequelae in children, the molecular events underlying these non-diarrheal conditions collectively referred to as environmental enteric dysfunction (EED) or environmental enteropathy remain very poorly understood.

Interestingly, another enigmatic condition linked to toxin-producing E. coli, tropical sprue, remains the most common cause of chronic small intestinal malabsorption and diarrhea in areas of high ETEC prevalence including India[32]. Notably, multiple studies in the 1970’s identified “toxin producing E. coli” in luminal aspirates from patients with tropical sprue[33]. Unfortunately, these studies relied on rabbit ileal loops to demonstrate enterotoxin production, and we lack definitive molecular characterization of isolates from these patients.
Both EED and tropical sprue are characterized by shortening of the small intestinal villi, lengthened crypts, mucosal inflammation, and nutrient malabsorption[34]. Despite these common features and epidemiologic associations connecting these non-diarrheal sequelae to ETEC, we lack a molecular understanding of how these pathogens might promote the development of intestinal changes characteristic of enteropathy, or whether ETEC alone are sufficient to elicit them in addition to causing diarrhea. Essentially, we have yet to fulfill “molecular Koch’s postulates”[35] that firmly establish ETEC as the causative agents.

**an expanded role for toxins**

Notably, both ETEC enterotoxins target intestinal epithelia to activate the production of cyclic nucleotides which serve as major cellular “second messengers” involved in the modulation of multiple cellular pathways. Soon after the discovery of heat-labile toxin, it was recognized that it stimulates the production of cAMP by adenylate cyclase[36]. Intracellular accumulation of cyclic AMP leads to activation of the protein kinase A heterotetramer by liberating its two catalytic subunits from regulatory subunits. The catalytic PKA subunits are then free to phosphorylate cellular targets including chloride and sodium ion channels to modulate their activity resulting in the net release of salt and water into the lumen of the small intestine. In addition, the free catalytic subunits also move into the nucleus where they phosphorylate and activate the cyclic AMP response element binding protein (CREB) transcription factor. Binding of CREB to cyclic AMP response element (CRE) sites in promoter regions can potentially modulate the activity of thousands of genes[37]. Similarly, ST, binds to guanylate cyclase C on the surface of intestinal epithelial cells where it activates production of cGMP, to activate protein kinase G, which in turn can phosphorylate multiple cellular proteins in addition to those directly related to modulation of ion channels and development of diarrhea. Remarkably, however despite the capacity for these toxins to induce pleiotropic effects on the host cell via their impact on critical “second messenger”[38], pathways, we presently have very little information regarding other potential impacts that LT and ST induced signaling may have on the host other than diarrhea.

Interestingly, LT was previously shown to play an important role in promoting ETEC adhesion to intestinal epithelial cells *in vitro*[39] and small intestinal colonization in animal models[40, 41]. Recent investigations demonstrate that intracellular increases in cAMP lead to enhanced production of a number of glycoproteins known as CEACAMs, expressed on the surface of small intestinal epithelial cells[42]. These proteins, in particular CEACAM6 (figure 2c), then serve as receptors for FimH, the tip adhesin molecule expressed at the ends of chromosomally-encoded type 1 pili[9]. In effect, ETEC use the plasmid-encoded heat labile toxin to change the surface architecture of target host enterocytes enhancing production of their own receptor.

While these events *transiently* benefit the pathogen, it is possible that these changes come at the expense of the host as these architectural changes are occurring on the surface of enterocytes, the major site of nutrient absorption in the human small intestine. Enterocytes are propagated from stem cells in the small intestinal crypts, and migrate to the distal end of small intestinal villi as they mature, eventually being released from the extracellular matrix.
and shed from the villus tip to undergo programmed cell death termed “anoikis”[43], a process inhibited by deregulated CEACAM6 expression[44]. Alteration of this normal homeostatic mechanism of intestinal villi could ultimately prove to be detrimental to the host. Conversely, enhanced expression of CEACAMs by the epithelia could also prove to be important as a host defense strategy as large amounts of CEACAMs shed in stool[45] could serve as molecular decoys that mitigate binding of ETEC to intestinal epithelia.

**Do ETEC toxins drive enteropathy?**

At a microscopic level, both tropical sprue and EED are characterized morphologically by substantial alterations in the small intestinal villous architecture characterized by blunting of the villi. The epithelial surface of intestinal villi is comprised of enterocytes, goblet cells, tuft cells and enteroendocrine cells that differentiate they propagate up the villus surface from progenitor cells in the crypts of Lieberkuhn where intestinal stem cells and Paneth cells reside. Enterocytes are the most abundant cells in intestinal epithelia, and the major site of nutrient absorption. At an ultrastructural level, the intestinal “brush border” is normally formed by densely packed regular arrays of microvilli at the surface of enterocytes. In essence, the microvilli are extensions of the enterocyte apical plasma membrane surrounding a central core of actin microfilaments. These protrusions greatly increase the available surface area at luminal surface of the intestine, allowing for maximal nutrient absorption. As such, insults which interfere with the development of microvilli can potentially result in malnutrition.

Intriguingly, transmission electron microscopy of small intestinal biopsies of patients suffering from tropical sprue obtained by Mathan, et al, in the 1970’s demonstrated marked derangement of the enterocyte brush border with irregular, short and sparse microvilli[46]. Notably, she found that in patients with cholera[47], the ultrastructural derangements of the small intestinal epithelia were remarkably similar to those with tropical sprue, raising the possibility that these changes are toxin mediated.

Not surprisingly, given the importance of cAMP as a second messenger in the cell, we found by RNA sequencing that treatment of small intestinal enteroids with heat-labile toxin (LT), a close homologue of cholera toxin, results in the modulation of many cellular genes. Among the hundreds of genes altered by LT were those encoding proteins needed for the biogenesis of microvilli[48], and transmission electron micrographs of enterocytes look astoundingly like those seen in tropical sprue and cholera. Moreover, many of the transporters for vitamins and nutrients were also impacted by the toxin. Collectively, these findings suggest that impairment of the major site of nutrient absorption in the small intestine may be part of the “collateral damage” that occurs following ETEC infection. While it is certainly premature to equate these changes with the development EED and malnutrition, at the very least they suggest that we still have much to learn about the molecular pathogenesis of these pathogens that are virtually ubiquitous in regions lacking the basic human necessities of clean water and sanitation.
future directions

Unfortunately, in the absence of a broadly protective vaccine, young children in LMICs will likely continue to bear the burden of acute diarrheal illness imposed on them by enterotoxigenic \textit{E. coli}. Resolving the role of more recently discovered virulence proteins in the molecular pathogenesis of ETEC may focus vaccine development on antigens that encompass the breadth of ETEC.

While ETEC have been epidemiology linked to development of sequelae including malnutrition, additional study is needed at the molecular level to understand how these pathogens might promote changes in the small intestinal architecture that underlie the sequelae. It is becoming clear however that one or both toxins secreted by ETEC can affect a variety of cellular pathways that govern normal homeostasis and ultimately absorptive capacity of the small intestine in addition to causing diarrhea. An ideal ETEC vaccine would mitigate or prevent both the acute diarrheal illness caused by ETEC as well as the substantial burden of non-diarrheal morbidity linked to these pathogens.

Funding

These studies were supported in part by NIH grants R01AI89894, R01AI126887 and Grant I01BX004825 from the Department of Veterans Affairs. Additional support was received from NIH Clinical and Translational Sciences Award UL1 TR002345; NIH Washington University Digestive Diseases Research Core Center (DDRCC) Grant No. NIDDK P30 DK052574.

conflicts of interest

Dr. Fleckenstein is listed as inventor on U.S. patent 8,323,668 “Prevention and treatment of Gram-negative, flagellated bacterial infections” that relates to use of EtpA in vaccines.
figure legends

figure 1

figure 1. summary of steps in the molecular pathogenesis of ETEC

(1) ETEC are propelled to the small intestinal lumen via peritrichous flagella. (2) ETEC engage the mucin overlying small intestinal epithelia cells, in part mediated by EtpA-bridging of the bacterial flagella with glycans present in mucin. (3) degradation of MUC2 by the proteolytically active EatA passenger domain (EatA_p) permits bacterial access to the epithelial surface. (4) ETEC engage enterocytes via plasmid-encoded CF molecules in addition to EtpA, where delivery of LT activates production of cellular cAMP (5), which in turn activates protein kinase A (PKA). PKA catalytic subunits phosphorylates sodium and chloride channels that result in the net export of salt and water into the intestinal lumen and diarrhea. PKA also phosphorylates other cellular target proteins, and enters the nucleus to alter transcription via CREB. ST binds to guanylate cyclase C to elicit the production of cGMP (6) activating protein kinase G (PKG) which phosphorylates ion channel proteins and other targets. (7) LT modulates the transcription of multiple genes including those encoding (8) CEACAMs that then serve as receptors for ETEC expressing type 1 fimbriae, and (9) MUC2 enhancing the mucin barrier. (Figure created with BioRender.com).
figure 2.

The image shows a transmission electron microscopy (TEM) image of ETEC (Enterotoxigenic E. coli) adhering to the apical surface of small intestinal enteroids derived from human ileum. ETEC is shown in cross section approaching an enterocyte flanked by two goblet cells which have released mucin (arrows). Enterotoxigenic E. coli adhering to the apical surface of small intestinal enteroids in regions of CEACAM6 expression. Enterotoxigenic E. coli (H10407 strain, isolated from a case of cholera-like diarrhea in Bangladesh) adhering to the brush border formed by microvilli on the surface of small intestinal enteroids propagated from human (blood group A+) small intestine (Scanning Electron Microscopy, 27,700 x).
table 1. summary of ETEC virulence factors

| virulence factor/locus | gene(s) location | description and known dominant function(s) | ETEC-specific |
|-----------------------|-----------------|---------------------------------------------|---------------|
| **canonical ETEC virulence traits** | | | |
| colonization factors (CF)/coli surface (CS) antigens | plasmid | fimbrial and afimbrial structures that promote adhesion and small intestinal colonization | yes |
| heat-labile toxin (LT) | plasmid | ADP ribosylating toxin, activates cAMP production to alter ion channels | yes |
| heat-stable toxins (STh, STp) | plasmid | binds guanylate cyclase C to activate cGMP production to alter ion channels | yes |
| **chromosomally-encoded *E. coli* virulence traits** | | | |
| flagella | chromosome | motility; essential for toxin delivery | no |
| type 1 pili | chromosome | adhesion to mannosylated glycoproteins | no |
| EaeH | chromosome | outer membrane protein/adhesion | no |
| YghJ | chromosome | metalloprotease | no |
| T2SS | chromosome | responsible for secretion of LT, YghH | no |
| TolC T1SS | chromosome | responsible for secretion of STh, STp | no |
| **non-canonical ETEC virulence factors** | | | |
| EatA | plasmid | mucin-degrading serine protease autotransporter protein | yesa |
| EtpBAC | plasmid | two-partner secretion system responsible for export of EtpA, an extracellular adhesin which binds to GalNAc and A blood group glycans | yes |

*a*type 2 secretion system

*b*type 1 secretion system

*c*also found in some *Shigella* spp, including *Shigella sonnei*
1. Khalil IA, Troeger C, Blacker BF, et al. Morbidity and mortality due to shigella and enterotoxigenic Escherichia coli diarrhoea: the Global Burden of Disease Study 1990-2016. Lancet Infect Dis 2018; 18:1229-40.
2. Jain S, Chen L, Dechet A, et al. An outbreak of enterotoxigenic Escherichia coli associated with sushi restaurants in Nevada, 2004. Clin Infect Dis 2008; 47:1-7.
3. Roels TH, Proctor ME, Robinson LC, Hulbert K, Bopp CA, Davis JP. Clinical features of infections due to Escherichia coli producing heat-stable toxin during an outbreak in Wisconsin: a rarely suspected cause of diarhrea in the United States. Clin Infect Dis 1998; 26:898-902.
4. Medus C, Besser JM, Juni BA, et al. Long-Term Sentinel Surveillance for Enterotoxigenic Escherichia coli and Non-O157 Shiga Toxin-Producing E. coli in Minnesota. Open Forum Infect Dis 2016; 3:ofw003.
5. Buuck S, Smith K, Fowler RC, et al. Epidemiology of Enterotoxigenic Escherichia coli Infection in Minnesota, 2016-2017. Epidemiol Infect 2020; 1-26.
6. 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.
7. Liu Y, Shahabudin S, Farid S, et al. Cross-Reactivity, Epitope Mapping, and Potency of Monoclonal Antibodies to Class 5 Fimbrial Tip Adhesins of Enterotoxigenic Escherichia coli. Infect Immun 2020; 88.
8. Kansal R, Rasko DA, Sahl JW, et al. Transcriptional modulation of enterotoxigenic Escherichia coli virulence genes in response to epithelial cell interactions. Infect Immun 2013; 81:259-70.
9. Sheikh A, Rashu R, Begum YA, et al. Highly conserved type 1 pili promote enterotoxigenic E. coli pathogen-host interactions. PLoS Negl Trop Dis 2017; 11:e0005586.
10. Sheikh A, Luo Q, Roy K, et al. Contribution of the highly conserved EaeH surface protein to enterotoxigenic Escherichia coli pathogenesis. Infect Immun 2014; 82:3657-66.
11. Tauschek M, Gorrell RJ, Strugnell RA, Robins-Browne RM. Identification of a protein secretory pathway for the secretion of heat-labile enterotoxin by an enterotoxigenic strain of Escherichia coli. Proc Natl Acad Sci U S A 2002; 99:7066-71.
12. Zhu Y, Luo Q, Davis SM, Westra C, Vickers TJ, Fleckenstein JM. Molecular Determinants of Enterotoxigenic Escherichia coli Heat-Stable Toxin Secretion and Delivery. Infect Immun 2018; 86.
13. Patel SK, Dotson J, Allen KP, Fleckenstein JM. Identification and molecular characterization of EatA, an autotransporter protein of enterotoxigenic Escherichia coli. Infect Immun 2004; 72:1786-94.
14. Fleckenstein JM, Roy K, Fischer JF, Burkitt M. Identification of a two-partner secretion locus of enterotoxigenic Escherichia coli. Infect Immun 2006; 74:2245-58.
15. Johansson ME, Sjovall H, Hansson GC. The gastrointestinal mucus system in health and disease. Nat Rev Gastroenterol Hepatol 2013; 10:352-61.
16. Kumar P, Luo Q, Vickers TJ, Sheikh A, Lewis WG, Fleckenstein JM. EatA, an Immunogenic Protective Antigen of Enterotoxigenic Escherichia coli, Degradates Intestinal Mucin. Infect Immun 2014; 82:500-8.
17. Roy K, Hilliard GM, Hamilton DJ, Luo J, Ostmann MM, Fleckenstein JM. Enterotoxigenic Escherichia coli EtpA mediates adhesion between flagella and host cells. Nature 2009; 457:594-8.
18. Kumar P, Kuhlmann FM, Bhullar K, et al. Dynamic Interactions of a Conserved Enterotoxigenic Escherichia coli Adhesin with Intestinal Mucins Govern Epithelium Engagement and Toxin Delivery. Infect Immun 2016; 84:3608-17.
19. 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.
20. Kumar P, Kuhlmann FM, Chakraborty S, et al. Enterotoxigenic Escherichia coli-blood group A interactions intensify diarrheal severity. J Clin Invest 2018; 128:3298-311.
21. Kuhlmann FM, Martin J, Hazen TH, et al. Conservation and global distribution of non-canonical antigens in Enterotoxigenic Escherichia coli. PLoS Negl Trop Dis 2019; 13:e0007825.
22. Sahl JW, Sistrunk JR, Baby NI, et al. Insights into enterotoxigenic Escherichia coli diversity in Bangladesh utilizing genomic epidemiology. Sci Rep 2017; 7:3402.
23. Rasko DA, Del Canto F, Luo Q, Fleckenstein JM, Vidal R, Hazen TH. Comparative genomic analysis and molecular examination of the diversity of enterotoxigenic Escherichia coli isolates from Chile. PLoS Negl Trop Dis 2019; 13:e0007828.
24. 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.
25. Chakraborty S, Randall A, Vickers TJ, et al. Interrogation of a live-attenuated enterotoxigenic Escherichia coli vaccine highlights features unique to wild-type infection. NPJ Vaccines 2019; 4:37.
26. Chakraborty S, Randall A, Vickers TJ, et al. Human Experimental Challenge With Enterotoxigenic Escherichia coli Elicits Immune Responses to Canonical and Novel Antigens Relevant to Vaccine Development. J Infect Dis 2018; 218:1436-46.
27. Kuhlmann FM, Laine RO, Afrin S, et al. Contribution of noncanonical antigens to virulence and adaptive immunity in human infection with enterotoxigenic E. coli. Infect Immun 2021.
28. Jung P, Sato T, Merlos-Suarez A, et al. Isolation and in vitro expansion of human colonic stem cells. Nat Med 2011; 17:1225-7.
29. Sato T, van Es JH, Snippert HJ, et al. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature 2011; 469:415-8.
30. Sato T, Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. Science 2013; 340:1190-4.
31. Kotloff KL, Nasrin D, Blackwelder WC, et al. The incidence, aetiology, and adverse clinical consequences of less severe diarrhoeal episodes among infants and children residing in low-income and middle-income countries: a 12-month case-control study as a follow-on to the Global Enteric Multicenter Study (GEMS). Lancet Glob Health 2019; 7:e568-e84.
32. Ghoshal UC, Srivastava D, Verma A, Ghoshal U. Tropical sprue in 2014: the new face of an old disease. Curr Gastroenterol Rep 2014; 16:391.
33. Klipstein FA, Engert RF, Short HB. Enterotoxigenicity of colonising coliform bacteria in tropical sprue and blind-loop syndrome. Lancet 1978; 2:342-4.
34. Korpe PS, Petri WA, Jr. Environmental enteropathy: critical implications of a poorly understood condition. Trends Mol Med 2012; 18:328-36.
35. Falkow S. Molecular Koch’s postulates applied to bacterial pathogenicity--a personal recollection 15 years later. Nat Rev Microbiol 2004; 2:67-72.
36. Evans DJ, Jr., Chen LC, Curlin GT, Evans DG. Stimulation of adenyl cyclase by Escherichia coli enterotoxin. Nat New Biol 1972; 236:137-8.
37. Zhang X, Odom DT, Koo SH, et al. Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc Natl Acad Sci U S A 2005; 102:4459-64.
38. Antoni FA. New paradigms in cAMP signalling. Mol Cell Endocrinol 2012; 353:3-9.
39. Johnson AM, Kaushik RS, Francis DH, Fleckenstein JM, Hardwidge PR. Heat-labile enterotoxin promotes Escherichia coli adherence to intestinal epithelial cells. J Bacteriol 2009; 191:178-86.
40. Allen KP, Randolph MM, Fleckenstein JM. Importance of heat-labile enterotoxin in colonization of the adult mouse small intestine by human enterotoxigenic Escherichia coli strains. Infect Immun 2006; 74:869-75.
41. Roy K, Hamilton DJ, Fleckenstein JM. Cooperative role of antibodies against heat-labile toxin and the EtpA Adhesin in preventing toxin delivery and intestinal colonization by enterotoxigenic Escherichia coli. Clin Vaccine Immunol 2012; 19:1603-8.
42. Sheikh A, Tumala B, Vickers TJ, et al. CEACAMs serve as toxin-stimulated receptors for enterotoxigenic Escherichia coli. Proc Natl Acad Sci U S A 2020; 117:29055-62.
43. Beumer J, Clevers H. Cell fate specification and differentiation in the adult mammalian intestine. Nat Rev Mol Cell Biol 2021; 22:39-53.
44. Ilantzis C, DeMarte L, Screaton RA, Stanners CP. Deregulated expression of the human tumor marker CEA and CEA family member CEACAM6 disrupts tissue architecture and blocks colonocyte differentiation. Neoplasia 2002; 4:151-63.
45. Matsuoka Y, Matsuo Y, Sugano K, Ohkura H, Kuroki M, Kuroki M. Characterization of carcinoembryonic antigen-related antigens in normal adult feces. Jpn J Cancer Res 1990; 81:514-9.
46. Mathan M, Mathan VI, Baker SJ. An electron-microscopic study of jejunal mucosal morphology in control subjects and in patients with tropical sprue in southern India. Gastroenterology 1975; 68:17-32.
47. Mathan MM, Chandy G, Mathan VI. Ultrastructural changes in the upper small intestinal mucosa in patients with cholera. Gastroenterology 1995; 109:422-30.
48. Crawley SW, Mooseker MS, Tyska MJ. Shaping the intestinal brush border. J Cell Biol 2014; 207:441-51.
