Supplementary materials

Supplementary methods

Human Study Design

research objectives

The objective of the human studies analysis was simply to determine whether blood group impacted the outcome of diarrheal illness. This hypothesis developed before initial analysis of any preliminary data.

research subjects

Samples were archived specimens from research subjects that were healthy adult human volunteers between 18 and 50 years of age without a prior history of exposure to enterotoxigenic *E. coli*. Each of the subjects in these studies served as untreated positive controls in one of four earlier clinical trials NCT01739231, NCT01060748, NCT00844493, and NCT01922856. For all these studies informed consent was obtained after the nature and possible consequences of the studies were explained. Pertinent details of the individual studies can be found at the ClinicalTrials.gov site.

experimental design

Blinded blood group determination on archived samples from prior CHIM studies with ETEC H10407.

sample size and selection criteria

A convenience sample of archived specimens of participants from the four, independent prior ETEC challenge studies was selected based exclusively on the following three criteria:

(1) Volunteers had been ETEC-naïve prior to challenge with the ETEC H10407 strain at an inoculum of ~10^7 colony forming units.

(2) Serum and/or salivary samples were available to determine blood type.

(3) The were available metadata on disease severity.

As the study subjects were from a convenience sample, predetermined power analysis was not performed. Variables were dichotomized to blood group A vs non-A due to the small sample size. After analysis of the first 63 subjects suggested that 103 participants were needed to achieve statistical significance with 80% power and a two-sided alpha of 0.05, additional samples were obtained from the NMRC-Ph2 study yielding 107 samples altogether.

data inclusion/exclusion criteria

All available samples for which there were accompanying metadata on diarrheal severity and outcome were included (n=107). A single sample was subsequently excluded from further analysis after we were unable to determine the blood type by PCR from an available salivary sample, leaving samples from a total of 106 subjects.

blinding

All blood group typing was done in a blinded fashion without access to the clinical metadata associated with the samples.

selection of endpoint criteria

Subjects in these studies were treated with antibiotics once they met previously defined CHIM endpoint criteria (summarized in table S1) or at 120 hours post inoculation to clear their infections.

glycan array production and screening

Glycan arrays containing 411 separate features were fabricated as previously reported (*1*) except for the addition of a washable fluorescent dye, the free acid of DyLight™649 (0.7 µg/mL, ThermoScientific), to the print buffer as an indicator of successful liquid deposition and spot morphology. The array format and assay have been described previously(*1*), along with analysis of reproducibility (*2*) and validation with numerous antibodies and lectins (*3, 4*).

Briefly, array components were printed onto epoxide-coated slides (SuperEpoxy 2; ArrayIt; Sunnyvale, CA) using a MicroGrid II arrayer (Genomic Solutions; Ann Arbor, MI) fitted with 946 Microspotting Pins (ArrayIt, Sunnyvale, CA). The printing spot size was ~80 μm. The humidity level in the arraying chamber was maintained at 40%~50% during the printing. All neoglycoproteins on the array have been characterized by MALDI-MS to determine the average number of glycans per molecule of BSA. The number after the glycan abbreviation indicates the average number of glycans per molecule of BSA. For example, BG-A1-04 indicates BG-A1 with an average of 4 copies of the glycan per molecule of BSA. Details for the preparation of the neoglycoproteins and characterization have been published previously (*5*). Printed slides were stored at −20 °C until used. Quality of each batch of slides is assessed by pre-scanning the slides to detect printing defects and testing a standard set of 3 lectins (ConA, WGA, and HPA) in quadruplicate on representative slides from the batch.

The array assay has been described in detail previously (*1*). Briefly, a 16-well module (Grace Bio-Lab) was affixed to the slide to create independent array wells. Slides were then blocked overnight at 4 °C with 3% BSA (w/v; 200 µL/well) in PBS and then washed 6 times

with PBST (PBS with 0.05% tween 20; 200 µL/well). Polyhistidine-tagged rEtpA-6His was diluted to final concentrations of 20 and 200 µg/ml in PBST buffer containing 3% bovine serum albumin (BSA, Sigma) and 1% human serum albumin (HSA, Sigma), and then 50 µL of each sample was added into two different wells on different slides. Incubation buffer alone served as the negative control for samples analyzed in duplicate. After incubation with rEtpA for 2 h at 37 °C (100 rpm), arrays were washed and then probed with mouse monoclonal IgG anti-His_5 antibody (Qiagen, 34660) diluted 1:500 to a final concentration of 0.4 µg/ml (1 h at 37 °C; 100 rpm), followed by Dylight 549-conjugated affinity purified Goat anti-mouse IgG (Jackson ImmunoResearch, 115-505-071; 1 h at 37 °C; 100 rpm). After washing 7 times with PBST (200 µL/well), slides were removed from modules, immersed in wash buffer for 5 min, and centrifuged at 1000 rpm for 5 min. Slides were scanned at 10 µm resolution with a Genepix 4000B microarray scanner (Molecular Devices Corporation) with excitation/emission wavelengths of 532/575nm and analyzed with Genepix Pro 6.0 software as previously reported(*1*). The spots were defined as circular features with a diameter of 90 µm. The background-corrected median was used for data analysis, and technical faults (e.g., missing spots, lint) were flagged and excluded from further analysis. Heatmaps were assembled in R version 3.2.2, The R Foundation for Statistical Computing, using the gplots and RColorBrewer packages made available through the Comprehensive R Archive Network (CRAN) at http://cran.wustl.edu/.

Propagation and Maintenance of human small intestinal enteroids

Enteroid based experiments were performed at both JHU and WUSTL using protocols developed and standardized in each institution. The protocols described below represent

the state-of-the-art at the time experiments were conducted for these studies. Each protocol is fully described and although methodologic variations exist, functional readouts were consistent at both institutions using the defined protocols. Furthermore, the enteroid field is rapidly evolving and investigators seeking the most recent protocols should contact the corresponding authors.

Human enteroid monolayer protocol (JHU)

Enteroids produced at Johns Hopkins University School of Medicine (JHU) were based on previously published methodology (*6*). A complete protocol is included here.

Transwell Supplies

24-well plate Transwells, PET membrane, 0.4 µm pore size (Corning 3470)

Collagen IV, from human placenta (Sigma C5533)

Media

(Media used in the preparation and maintenance of enteroids are based on methodology described by Sato, *et al* (*7*) with some modifications).

Prepared in Advanced DMEM/F12 (ThermoFisher 12634028) with the following supplementation:

Sato media		
reagent	final concentration	source
1 M HEPES	10 mM	ThermoFisher 15630080
Glutamax	1x	ThermoFisher 35050061
B-27	1x	ThermoFisher 17504044
N-acetylcysteine	1 mM	Sigma A9165
human EGF, recombinant	50 ng/ml	R&D Systems 236-EG
(Leu15)-gastrin-1	10 nM	AnaSpec AS-64149
A83-01	500 nM	Tocris 2939
SB 202190	10 µM	Tocris 1264
Primocin	100 µg/ml	Invivogen ant-pm
Wnt3A conditioned medium	50 % v/v	ATCC CRL-2647
R-spondin 1 conditioned medium	15 % v/v	HA-RSPo1-Fc; 3B cells; Kuo laboratory, Stanford University
Noggin conditioned medium	10% v/v	HEK293T/Noggin-Fc cells; GR van den Brink; Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Amsterdam, The Netherlands

Procedure

1. Prepare collagen IV solution from powder dissolved in 100 mM acetic acid (1 mg/mL). Store aliquots at 4 °C (short-term) or -20 °C (long-term).

    1.1.1. To coat Transwells, prepare a diluted collagen IV solution in sterile cell culture water at 34 µg/mL. This corresponds to 100 µL of collagen IV solution per 3 mL water.

    1.1.2. Add 100 µL of the diluted collagen IV solution to each Transwell surface to provide a plating density of 10 µg/cm^2. Transfer plate to 37 °C incubator for at least 2 h, or seal plate edges with parafilm and store at 4 °C overnight.

2. Prepare Sato medium as above with following additions:

Sato/+Y/+C medium		
reagent	final concentration	source
Y-27632	10 µM	Tocris 1254
CHIR99021	10 µM	Tocris 4423

3. Prepare enteroids from 3-D culture:

    3.1. Remove growth medium and replace with 1 mL Cell Dissociation Reagent (Stem Cell Techologies 07174).

    3.2. Break up Matrigel with a mini cell scraper and incubate the plate on a shaking platform at 250 rpm, 4 °C for ~30 min to aid in dissolution of Matrigel.

    3.3. Use a P200 pipet to triturate and fragment the enteroids.

    3.4. Transfer suspension to a 15 mL conical tube and wash with Advanced DMEM/F12. Spin at 1200 rpm, 10 min, 4 °C to pellet cells.

    3.5. Aspirate collagen IV solution and wash transwell surface with 100 µL Advanced DMEM/F12.

    3.6. Add 600 µL Sato/+Y/+C to the bottom chamber of each transwell.

    3.7. Resuspend enteroid fragments isolated from Matrigel in a volume of Sato/+Y/+C that will provide 100 µL of suspension/Transwell. Pipet 100 µL of suspension into the upper chamber of each Transwell.

> **n.b.** Small fragments are much more likely to attach and flatten successfully than large fragments/intact enteroids. The definition of a small fragment is something in the size range of a single, just-emerged bud.

3.8. Each Transwell should receive ~50 small enteroid fragments at seeding to yield efficient formation of a confluent monolayer within 4-7 d.

3.9. Feed every 2-3 days with Sato medium; 600 µL in bottom chamber and 100 µL in upper chamber until confluent.

3.10. Monitor Transwells daily for evidence of fragment flattening and spreading. Days to confluency can range from ~5 days to >2 weeks, depending on seeding density.

3.11. Examine by light microscopy and measure TER (Epithelial volt/Ohm meter, EVOM2, World Precision Instruments 300523) periodically to determine confluency.

3.12. Upon reaching confluency, switch to Differentiation Media (remove all factors from the Advanced DMEM/F12 except B27, N-acetylcysteine, and EGF) for 5 days and follow TER each day to assess progress.

Human enteroid monolayer protocol (WUSTL)

Enteroids produced at Washington University School of Medicine (WUSTL) were based on previously published methodology(*8*). A complete protocol is included here.

Supplies

96 well plates (TPP 92096, TPP, Switzerland)
Transwells, PET membrane, 0.4 µm pore size (Corning 3470, Corning, NY, USA)

Matrigel (BD Cat. No. 354234)

media

L-WRN conditioned media was prepared based on methodology described by van Dussen, *et al(8)* with modifications as described below.

To culture and propagate enteroids , L-WRN conditioned media was diluted 1:1 with primary media (Advanced DMEM/F12 ThermoFisher, 12634-010) to prepare 50% L-WRN conditioned media which was supplemented with 10µM SB431542 (TGFβR inhibitor) and 10 µM Y27632 (Rock inhibitor) for use in the maintenance of enteroids.

50% L-WRN Media		
reagent	final concentration	source/(reference)
FBS	20%	Sigma F6178
Glutamax	1x	Sigma G7513
100x Penicillin/Streptomycin	1x	Sigma P4333
SB431542	10 µM	Selleckchem S1067
Y-27632	10 µM	Selleckchem S1049
L-WRN Media	50 % v/v	(*8*)

Wash Media: Prepared using DMEM/F12 with HEPES (SIGMA D6421-500ML) with the following supplementation:

Wash Media		
reagent	final concentration	source
FBS	10%	Sigma F6178
Glutamax	1x	ThermoFisher 35050061
100x Penicillin/Streptomycin	1x	Sigma P4333

Procedure

1. Dilute Matrigel 1:30 in PBS.
2. pre-coat wells of 96 well plate or Transwell at 37°C for 1 hour
3. Prepare enteroids from 3-D culture:
   - 3.1. Dislodge cells with a sterile 1 ml pipette tip
   - 3.2. re-suspend in 1 ml PBS-EDTA(0.5mM)
   - 3.3. pellet at 150 *g* for 5 min
   - 3.4. re-suspend in 300 ul 1x trypsin (Sigma t4549)
   - 3.5. Incubate at 37°C for 90 seconds.
   - 3.6. Add 1 ml wash media
   - 3.7. Use a P1000 pipet to triturate and fragment the enteroids (~300 times).
   - 3.8. Add another 4 ml wash media
   - 3.9. pellet at 150*g* for 5 min.
   - 3.10. Wash in 1 ml wash media
   - 3.11. pellet at 150*g* for 5 min.
   - 3.12. Re-suspend enteroids in 50% L-WRN media with inhibitors
   - 3.13. Aspirate the diluted Matrigel suspension from the 96 well plate or Transwell and add 200 ul of enteroids in 50% L-WRN media. If using transwells, add 500 µl of 50% L-WRN to the bottom of the transwell.
4. 24 hours later, remove the L-WRN media and replace with 200 µl of 5% L-WRN media with Y-27632 (replace media at bottom of Transwell with 500 µl of same 5% L-WRN media).
5. Monitoring of Transwells

5.1. Replace media every 24 hours*.

5.2. Monitor daily by microscopy for evidence of cell flattening and spreading. Days to confluency can range from ~5 days to >2 weeks, depending on seeding density.

5.3. Document TER using an epithelial Ohm/voltmeter upon confluency.

*5.4. *n.b.* for experiments involving bacteria penicillin and streptomycin are removed from media.

Supplementary figures

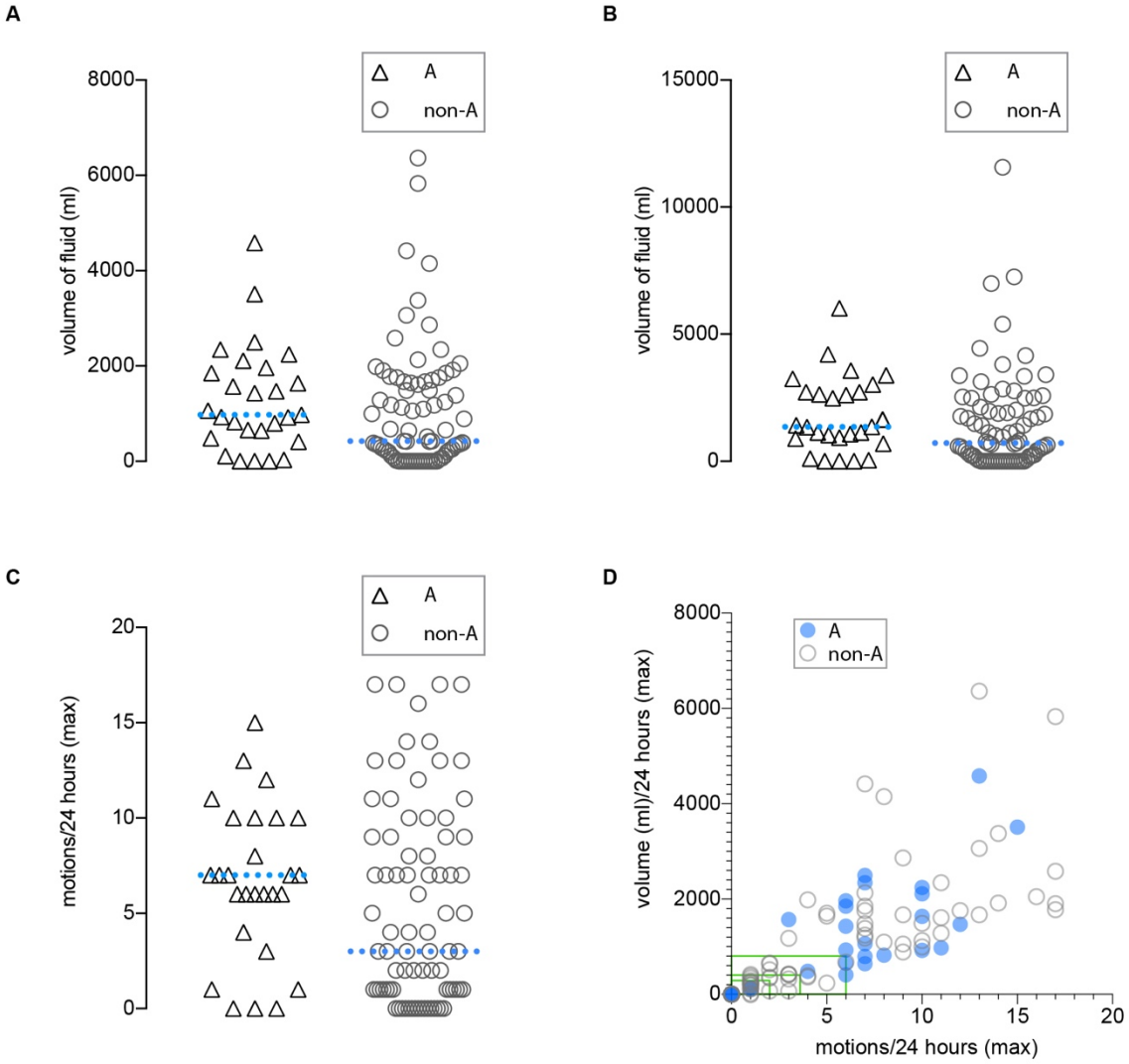

Fig. S1. characteristics of diarrheal illness in volunteers by blood group

A. maximal volume of diarrheal stool in a 24 hour period; blood group A median=979; non-A=424 ml). **B.** total volume of diarrheal stool prior to treatment (median blood group A: 1352 ml; median non-A: 719). **C.** maximal number of diarrheal stools in a 24 hour period media blood group A: 7; median non-A: 3). Dashed lines in **A-C** for each group represent median values. **D.** Comparison of maximal stool volume and maximal number of diarrheal episodes in a 24 hour period following infection of volunteers of blood group A (n=27, blue circles) and non-A (n=79, open circles) with ETEC H10407. Green rectangles at lower left of the figure represent threshold values for mild, moderate and severe diarrhea(9).

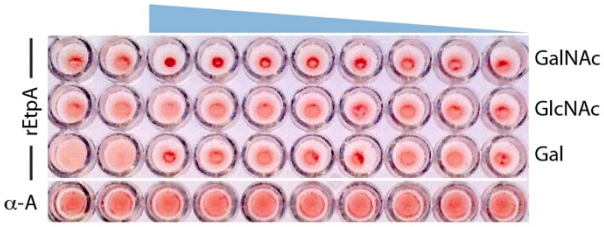

Fig. S2. GalNAc, the terminal sugar of A blood group inhibits EtpA-mediated hemagglutination.

N-Acetylgalactosamine (GalNAc), the terminal saccharide residue on blood group A, but not N-Acetylglucosamine (GlcNAc), or Galactose (Gal) the terminal saccharide residue on blood group B, inhibits EtpA-mediated hemagglutination of A1 RBCs. Positive control wells containing rEtpA and A1 RBCs are shown in first 2 columns at left. Anti-A blood group antibody positive controls (α–A) are shown in the bottom row. Wells at right contain 2-fold decreasing concentrations of the indicated sugars, beginning at a starting concentration of 50 mM.

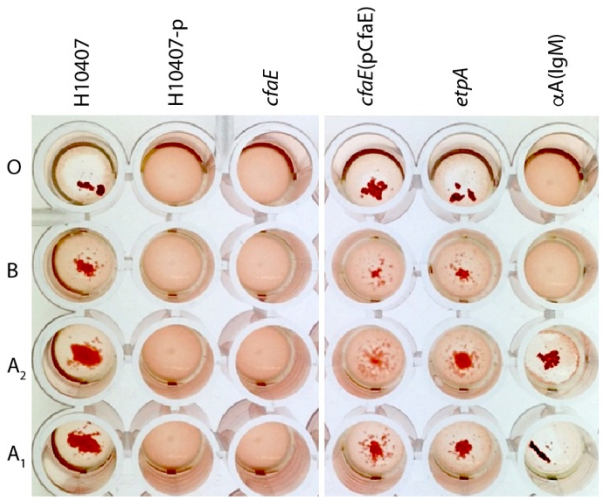

Fig. S3. CFA/I promotes non-selective hemagglutination of erythrocytes.

Whole, wild type H10407 bacteria expressing CFA/I agglutinate erythrocytes from all major blood types (column 1). Loss of either the full plasmid carrying CFA/I (H10407-p, column 2) or the CFA/I tip adhesin (*cfaE*, column 3) abrogates agglutination. Agglutination is restored by complementation of *cfaE* (column 4). Loss of EtpA has no effect on CfaE-dependent non-specific agglutination (column 5). Control wells containing anti-A specific antibodies (αA IgM) are shown in column 6. (Note that experiments were performed with whole bacteria under conditions that favor CFA/I expression, but minimize the impact of EtpA. Assays in this experiment also used flat-bottom plates in which agglutinated erythrocytes clump and settle to the bottom while non-agglutinated cells are dispersed).

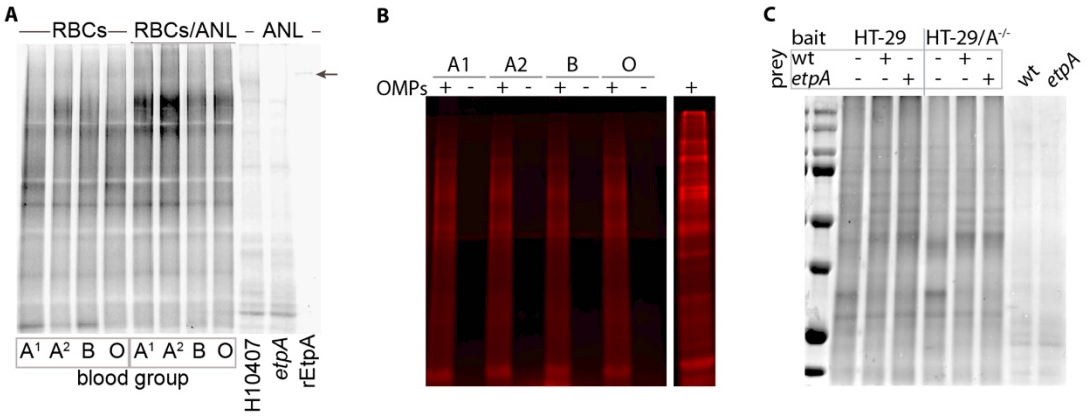

Fig. S4. EtpA is the major blood group A lectin in ETEC

A. loading controls for the data shown in figure 4E. Shown in the image of a Coomassie stained gel are RBCs from different blood groups at left and the RBC mixed with ANL labeled peptides at right. **B**. ANL-labeled outer membrane proteins (OMPs) (+ lanes) were added to erythrocytes ghosts from each of the blood groups. Negative lanes contain only the corresponding RBC ghosts. Lane at right shows the input ANL-tagged OMPs. **C.** Coomassie-stained gel image of samples from experiment shown in 5B.

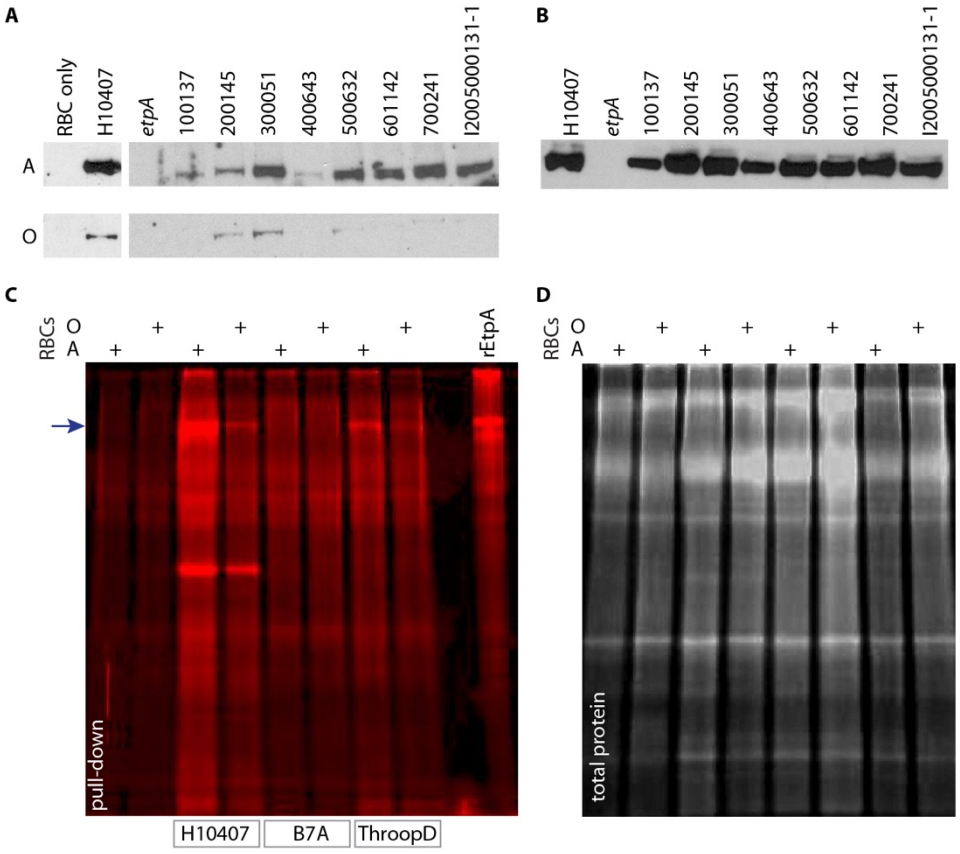

Fig. S5. preferential binding of blood group A is shared by EtpA molecules from a diverse collection of isolates.

A. Anti-EtpA-immunoblots of RBC ghost pulldowns following incubation of concentrated culture supernatants from a geographically disparate collection of EtpA producing strains with erythrocytes of blood group A (top) and blood group O (bottom). RBC only control and H10407+ control are shown in blot images on left of each panel. The *etpA* deletion mutant negative control and additional strains at shown on right. Geographic origin of the isolates is as follows: H10407 (Bangladesh); 100137 (Gambia); 200145 (Mali); 300051 (Mozambique); 400643 (Kenya); 500632 (India); 601142 (Bangladesh); 700241 (Pakistan); I2005000131-1 (Mexico). **B.** relative abundance of EtpA in supernatants from indicated strains. **C.** pull-down of EtpA from supernatants of ANL-labelled strains with erythrocytes from blood group O and A. Shown from left to right are RBC-pull downs with H10407; B7A, an *etpA*-negative isolate; and ThroopD. Arrow at far left indicates migration of EtpA. **D.** total protein loading controls (Oriole-stained gel) for samples in C.

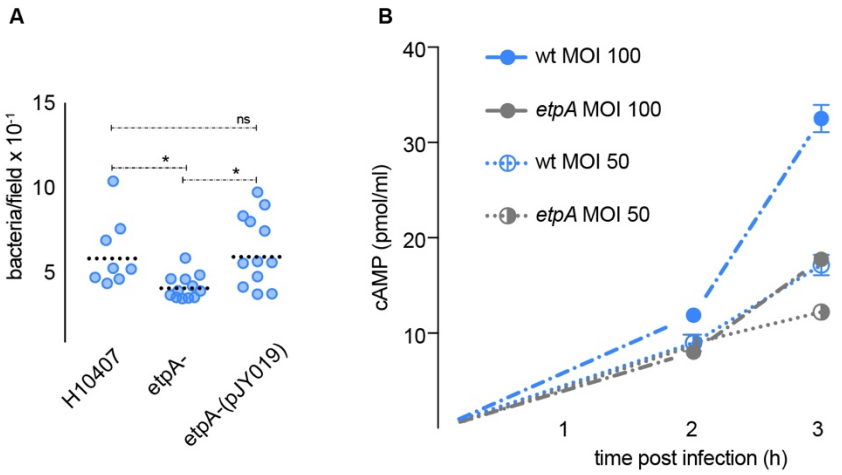

Fig. S6. EtpA is required for optimal adhesion and toxin delivery

A. EtpA is required for optimal interaction of bacteria with blood group A expressing enterocytes (shown are the wild type H10407 (n=8), the *etpA* mutant (n=12) and the complemented mutant *etpA*(pJY019) (n=12). Data represent combined results of two separate experiments. Statistical significance determinations were made Kruskal-Wallis testing for multiple comparisons of non-parametric data *p≤0.05; ns non-significant. **B.** EtpA accelerates toxin delivery to A blood group positive HT-29 cells. Shown are the H10407 wild type (wt), and the *etpA* mutant at two differerent mulitplicities of infection (MOI), 100 and 50 bacteria per cell. Points represent mean ± SEM of n=3 replicates.

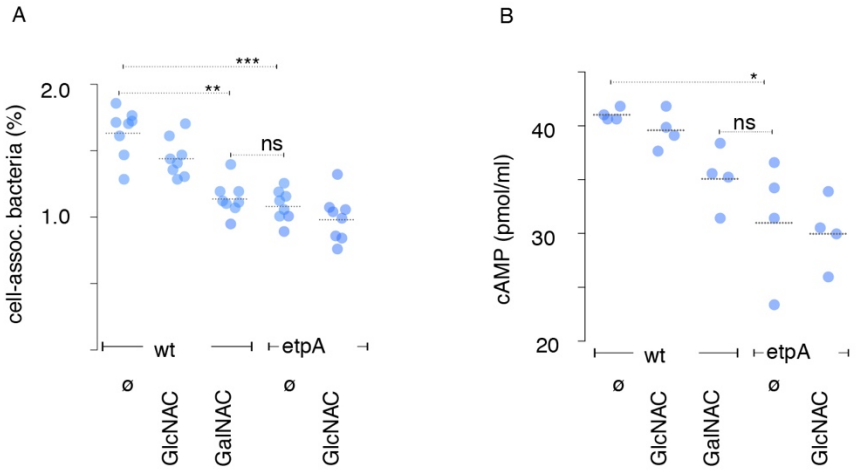

Fig. S7. exogenous GalNAC inhibits bacterial adhesion and toxin delivery.

A. Adhesion is significantly impaired by exogenous GalNAc the terminal A blood group sugar residue. Dashed horizontal lines indicate geometric mean values (n=8 technical replicates). **B.** Toxin delivery is impaired by exogenous GalNAc. Dashed horizontal lines indicate geometric mean values (n=4 technical replicates). Adjusted p values: ***p<0.001, **p<0.01, and *p<0.05 obtained by Kruskal-Wallis testing for multiple comparisons of non-parametric data.

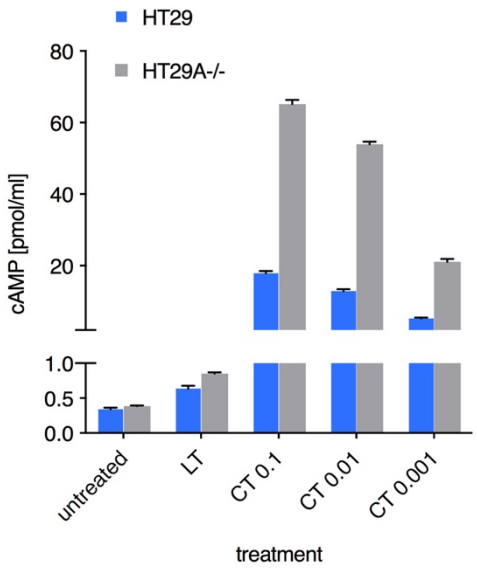

Fig. S8. Differential activation of HT-29 and HT-29A$^{-/-}$ cells by heat-labile toxin (LT) and cholera toxin (CT).

Results are the summary of n=6 technical replicates following stimulation of HT29 cells with either LT or CT at the indicated dose.

Supplementary tables

table s1

Endpoint criteria for human challenge studies					
	None	Mild		Moderate	Severe
Number of stools Grade 3-5*	0	1	2-3	4-5	≥ 6
		AND	AND	OR	OR
Stool Weight of Grade 3-5 stools (g)	Not Applicable	≥ 300	≥ 200 in 24 hours	401-800	> 800

Treatment was initiated for severe diarrhea, moderate diarrhea ≥48 hours, *Stool was graded as follows: grade 1, firm formed; grade 2, soft formed; grade 3, viscous opaque liquid or semiliquid which assumed the shape of the container; grade 4, watery opaque liquid; and grade 5, clear watery or mucoid liquid.

table s2

Summary of H10407 controlled human infection studies

		blood group			
		O	B	A or AB†	total (%)
study (NCT identifier)	Illness				
NCT01739231	None	7 (54)	1 (20)	1 (11)	9 (33)
	Moderate-severe	6 (46)	4 (80)	8 (89)	18 (67)
	Total	13	5	9	27
NCT01060748	None	4 (27)	3 (60)	1 (14)	8 (30)
	Moderate-severe	11 (73)	2 (40)	6 (86)	19 (70)
	Total	15	5	7	27
NCT00844493*	None	2 (29)	0 (0)	1 (50)	3 (33)
	Moderate-severe	5 (71)	0 (0)	1 (50)	6 (67)
	Total	7	0	2	9
NCT01922856	None	15 (52)	3 (60)	2 (22)	20 (47)
	Moderate-severe	14 (48)	2 (40)	7 (78)	23 (53)
	Total	29	5	9	43

Study numbers at left refer to ClinicalTrials.gov identifiers. * Johns Hopkins University Center for Immunization Research human challenge model refinement study***(10)***. †Only 3 patients had a blood type of AB, 2 in the NCT01739231 study (1 in each illness category), and one with severe diarrhea in the NCT01922856 study.

table s3

Table S3. diarrheal severity is not race dependent

	Diarrheal Severity (N, (%))		
Race	None or Mild	Moderate or Severe	Total (N)
Black	33 (36)	59 (64)	92
White	4 (40)	6 (60)	10
Other	3 (60)	2 (40)	5
Total	40 (37)	67 (63)	107*

($\chi 2$ (df = 2, n=107), = 1.212, p = 0.546). *One additional patient is included in this analysis for which blood typing was not available.

table s4

Table S4. Diarrheal severity does not correlate with age

Diarrheal Severity	Age	
	Median	Range
None or Mild	35.4	21.8-49.0
Moderate or Severe	34.0	18.0-49.0

(Mann Whitney U p=0.784). (n=107; One additional patient is included in this analysis for which blood typing is unavailable).

table s5

Table S5. bacterial strains and plasmids used in these studies

Strain	Description	Reference(s)
H10407-P	plasmid cured strain of H10407	(*11*)
H10407kan	*cfaE* deletion mutant; Km^R	(*12*)
H10407kan(p*cfaE*)	plasmid complemented deletion strain Km^R, Gm^R	(*12*)
jf576	LT^- (*eltAB*::Km^R) mutant of H10407	(*13*)
jf1668	etpA::Cm^R mutant of H10407	(*14*)
jf1696	Top10(pJL017, pJL030), Amp^R, Cm^R	(*15*)
jf1697	jf1668 transformed with pJY019, Amp^R	(*15*)
jf1700	jf1668 tranformed with pBAD/Myc-His A, Amp^R	(*15*)
jf3422	H10407 transformed with pBAD-MetRS, Amp^R	this study
jf3904	jf1668 transformed with pBAD-MetRS, Amp^R	this study
Top10	F- mcrA Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lacZ*ΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*araleu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G	Invitrogen
	wild type ETEC strains	
strain	**description, (origin, year of isolation)**	**references**
H10407	O78:H11; CFA/1;LT+/STh/STp+;EtpA+ (Bangladesh, 1970s)	(*11, 16*)
ThroopD	063:Nm; LT+/ST+; EtpA+ (Texas, 1976)	(*17*)
B7A	O148:H28; LT+/ST+;EtpA- (Vietnam, 1970)	(*18*)
100137	O64; CS6;LT+;EtpA+ (Gambia, 2013)	(*19*)
200145	LT+/STh+;CS1,CS3,CS21; EtpA+ (Mali, 2013);	(*19*)
300051	O71:H44;CFA/I,CS21;STh;EtpA+ (Mozambique, 2013)	(*19*)
400643	CS5, CS14;STh; EtpA+ (Kenya, 2013);	(*19*)
500632	O78:NT; CS14; STh; EtpA+ (India, 2013);	(*19*)
601142	CF-negative;LT; EtpA+ (Bangladesh, 2013)	(*19*)
700241	CS12; LT; EtpA+ (Pakistan, 2013);	(*19*)
I2005000131-1	LT+STh+; EtpA+ (Mexico, 2005).	(*20*)
	Plasmids	
Plasmid		
pJL017	*etpBA* cloned into pBAD/*Myc*-His A, with *etpA* in-frame with myc and 6His coding regions, Amp^R	(*21*)
pJL030	*etpC* gene cloned into pACYC*184,* Cm^R	(*14*)
pJY019	*etpBAC locus cloned into* pBAD/*Myc*-His A	(*15*)
pAM1	*expresses NLL-MetRS from endogenous MetG promotor, Km^R, Cm^R*	(*22*)
pBAD/Myc-His B	arabinose inducible expression plasmid, Amp^R	Invitrogen
pBAD-MetRS	1647 bp MetRS fragment from pAM1 cloned into *NcoI-EcoRI* sites of pBAD/Myc-His B by In-Fusion; Amp^R	this study

Km^R =kanamycin resistant; Gm^R=gentamicin resistant; Cm^R = chloramphenicol resistant; Amp^R = ampicillin resistant

table s6

Table S6. primers used in these studies

primer designation	sequence (5′-3′)	description	reference(s)
jf091715.1	gaggaattaa ccatgATGAC TCAAGTCGCG AAGA	5′ primer to amplify MetRS ORF. vector sequence for In-Fusion cloning into pBAD/Myc-His B at NcoI site is underlined	this study
jf091715.2	agaaagcttcgaattTCATTTAGAGGCTTCCACC	3′ primer to amplify MetRS ORF. vector sequence for In-Fusion cloning into pBAD/Myc-His B at *EcoRI* site is underlined	this study
jf062014.1	CAGAAGCTGAGTGGAGTTTCCAGG	ABO glycosyltransferase Exon 6 5′ primer	(*23*)
jf062014.2	CTGAACTGCTCGTTGAGGATGTCG	ABO glycosyltransferase Exon 6 3′ primer	(*23*)
jf062014.3	AAGGACGAGGGCGATTTCTACTAC	ABO glycosyltransferase Exon 7 5′ primer	(*23*)
jf062014.4	CGTTGGCCTGGTCGACCATCATG	ABO glycosyltransferase Exon 7 3′ primer	(*23*)
jf062014.7	GTGCAGTAGGAAGGATGTCCTCGTGACG	ABO glycosyltransferase Exon 6 5′ primer AB mismatch	(*23*)
jf062014.8	GTGCAGTAGGAAGGATGTCCTCGTGACA	ABO glycosyltransferase Exon 6 5′ primer AO mismatch	(*23*)
jf062014.11	AAGGACGAGGGCGATTTCTACTCTC	ABO glycosyltransferase Exon 7 5′ primer AO mismatch	(*23*)
jf062014.12	AAGGACGAGGGCGATTTCTACTCTA	ABO glycosyltransferase Exon 6 5′ primer B mismatch	(*23*)

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