CRISPR screen reveals that EHEC?s T3SS and Shiga Toxin rely on shared host factors for infection

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Enterohemorrhagic Escherichia coli (EHEC) has two critical virulence factors?a type III secretion system (T3SS) and Shiga toxins (Stxs)?that are required for the pathogen to colonize the intestine and cause diarrheal disease. Here, we carried out a genome-wide CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats with Cas9) loss-of-function screen to identify host loci that facilitate EHEC infection of intestinal epithelial cells. Many of the guide RNAs identified targeted loci known to be associated with sphingolipid biosynthesis, particularly for production of globotriaosylceramide (Gb3), the Stx receptor. Two loci (TM9SF2 and LAPTM4A) with largely unknown functions were also targeted. Mutations in these loci not only rescued cells from Stx-mediated cell death, but also prevented cytotoxicity associated with the EHEC T3SS. These mutations interfered with early events associated with T3SS and Stx pathogenicity, markedly reducing entry of T3SS effectors into host cells and binding of Stx. The convergence of Stx and T3SS onto overlapping host targets provides guidance for design of new host-directed therapeutic agents to counter EHEC infection. IMPORTANCE Enterohemorrhagic Escherichia coli (EHEC) has two critical virulence factors?a type III secretion system (T3SS) and Shiga toxins (Stxs)?that are required for colonizing the intestine and causing diarrheal disease. We screened a genome-wide

collection of CRISPR mutants derived from intestinal epithelial cells and identified mutants with enhanced survival following EHEC infection. Many had mutations that disrupted synthesis of a subset of lipids (sphingolipids) that includes the Stx receptor globotriaosylceramide (Gb3) and hence protect against Stx intoxication. Unexpectedly, we found that sphingolipids also mediate early events associated with T3SS pathogenicity. Since antibiotics are contraindicated for the treatment of EHEC, therapeutics targeting sphingolipid biosynthesis are a promising alternative, as they could provide protection against both of the pathogen?s key virulence factors. © 2018 Pacheco et al.

CRISPR screen

EHEC

EPEC

Host susceptibility

LAPTM4A

Shiga toxin

Sphingolipid synthesis

T3SS

TM9SF2

globotriaosylceramide

Shiga toxin

sphingolipid

ceramide trihexoside

globotriaosylceramide

Shiga toxin

sphingolipid

virulence factor

Article

bacterial colonization

bacterial growth

bacterial strain

bacterial virulence

controlled study

CRISPR-CAS9 system

cytotoxicity

enterohemorrhagic Escherichia coli

host pathogen interaction

intestine epithelium cell

limit of quantitation

nonhuman

priority journal

biosynthesis

cell line

cell survival

clustered regularly interspaced short palindromic repeat

enterohemorrhagic Escherichia coli

epithelium cell

Escherichia coli infection

gene locus

gene targeting

genetics

genome-wide association study

host pathogen interaction

human

metabolism

microbiology

mutation

pathogenicity

physiology

type III secretion system

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Host-Pathogen Interactions

Humans

Mutation

Shiga Toxin

Sphingolipids

Trihexosylceramides

Type III Secretion Systems

Virulence Factors