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QseC inhibition as an antivirulence approach for colitis-associated bacteria

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Hosts and their microbes have established a sophisticated communication system over many millennia. Within mammalian hosts, this dynamic cross-talk is essential for maintaining intestinal homeostasis. In a genetically susceptible host, dysbiosis of the gut microbiome and dysregulated immune responses are central to the development of inflammatory bowel disease (IBD). Previous surveys of stool from the *T-bet$^{-/-}$Rag2$^{-/-}$* IBD mouse model revealed microbial features that discriminate between health and disease states. Enterobacteriaceae expansion and increased gene abundances for benzoate degradation, two-component systems, and bacterial motility proteins pointed to the potential involvement of a catecholamine-mediated bacterial signaling axis in colitis pathogenesis. Enterobacteriaceae sense and respond to microbiota-generated signals and host-derived catecholamines through the two-component quorum-sensing *Escherichia coli* regulators B and C (QseBC) system. On signal detection, QseC activates a cascade to induce virulence gene expression. Although a single pathogen has not been identified as a causative agent in IBD, adherent-invasive *Escherichia coli* (AIEC) have been implicated. Flagellar expression is necessary for the IBD-associated AIEC strain LF82 to establish colonization. Thus, we hypothesized that *qseC* inactivation could reduce LF82's virulence, and found that an absence of *qseC* leads to down-regulated flagellar expression and motility in vitro and reduced colonization in vivo. We extend these findings on the potential of QseC-based IBD therapeutics to three preclinical IBD models, wherein we observe that QseC blockade can effectively modulate colitogenic microbiotas to reduce intestinal inflammation. Collectively, our data support a role for QseC-mediated bacterial signaling in IBD pathogenesis and indicate that QseC inhibition may be a useful microbiota-targeted approach for disease management.

colitis | *Escherichia coli* | QseC | antivirulence | gut microbiome

Escherichia coli species, the predominant Gram-negative aerobes of the mammalian gut microbiota, contribute to the stability of the microbial community and maintenance of intestinal homeostasis (1). Compared with their symbiotic counterparts, pathogenic *E. coli* strains have acquired virulence factors involved in attachment, invasion, and toxin production (2). Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is a relapsing-remitting chronic inflammatory disorder influenced by genetic and environmental factors (3). IBD-associated gut microbiota alterations include a high abundance of *E. coli* in mucosal biopsy and stool specimens (4, 5). *E. coli* strains isolated from patients with active IBD frequently display pathogenic properties, such as adhesion to and invasion of host cells (6). A role for adherent-invasive *E. coli* (AIEC) in the pathogenesis of IBD, particularly in CD (7, 8), has been supported by whole metagenome sequencing data from human cohort samples (9).

The AIEC strain LF82 was isolated from a mucosal lesion of a patient with CD (10). LF82's virulence, including its ability to adhere to and invade intestinal epithelial cells (IECs) and to survive and replicate within macrophages, is well established (11, 12). A key mechanism driving this virulence, although undoubtedly not the sole virulence mechanism, is the production of flagella. Inactivating the flagellar gene *fliC* blocks LF82 invasion and reduces its adhesion to cultured IECs (13). In the setting of dextran sodium sulfate (DSS)-induced mucosal injury, LF82 can exacerbate colonic inflammation, an effect abrogated in the absence of *fliC* (14). This flagella-mediated mucosal immune response is elicited via direct signaling through its cognate pattern-recognition receptor, Toll-like receptor 5 (14, 15). Flagellin, a principal flagellar protein, continues to be implicated as a dominant antigen in CD and as a target of both innate and adaptive immune responses central to the immunopathogenesis of IBD (16, 17).

Beyond flagella and their proteins, the virulence of many pathogenic *E. coli* is dependent on quorum-sensing *E. coli* regulator C (QseC), a component of the quorum-sensing *E. coli* regulators B and C (QseBC) two-component system (TCS) (18, 19). QseC is

Significance

Bacteria use two-component quorum-sensing systems to communicate with each other and their hosts. Catecholamines are host stress signals that participate in this dialogue and drive bacterial growth and virulence. Evidence from a preclinical model of inflammatory bowel disease (IBD) revealed that Enterobacteriaceae and pathways linked to catecholamine-mediated bacterial virulence are enriched in active disease. Here we targeted the bacterial adrenergic sensor, quorum-sensing *Escherichia coli* regulator C (QseC) of the QseBC two-component system. Genetically inactivating *qseC* in a pathogenic, IBD-associated *E. coli* strain (LF82) reduced its virulence and ability to colonize a murine host. Furthermore, biochemically inhibiting QseC attenuated disease in multiple preclinical IBD models. This report demonstrates that QseC signaling influences IBD pathogenesis and identifies QseC blockade as a therapeutic strategy for colitis-associated bacteria.

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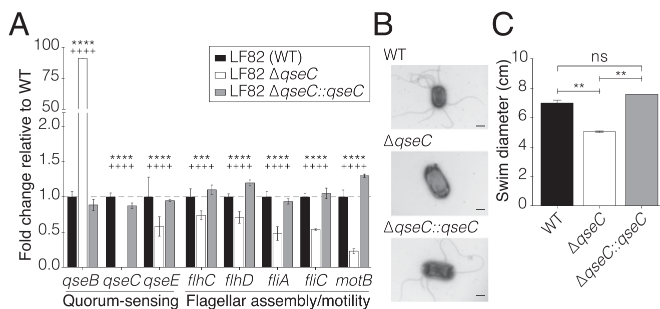

Fig. 1. *QseC* regulates LF82 flagellar expression and motility. (*A*) RT-qPCR of LF82 strains grown in LB. Genes normalized to *rpoA*. Fold change relative to WT strain. Data are mean ± fractional SD from two independent experiments. ****P* < 0.001; ****,++++*P* < 0.0001, two-way ANOVA with Tukey's post hoc test. * and + indicate differences relative to WT and complemented strains, respectively. (*B*) Representative TEM images. (Scale bar: 500 nm.) (*C*) Swim diameters on soft agar plates. Data are mean ± SEM from two independent experiments. ***P* < 0.01, one-way ANOVA with Tukey's test. ns, not significant.

a histidine sensor kinase activated on detection of microbiota-generated autoinducer-3 (AI-3) or host stress signals, specifically the catecholamines (CAs) norepinephrine (NE) and epinephrine (EPI) (20). Both NE and EPI induce QseC-mediated virulence in vitro and blocking QseBC signaling with the QseC inhibitor LED209 reduces the in vivo virulence of Gram-negative pathogens, including enterohemorrhagic *E. coli* and *Salmonella enterica* Typhimurium (21–25). Previous surveys of stool from the *T-bet*$^{-/-}$ *Rag2*$^{-/-}$ preclinical colitis model following various treatment interventions revealed microbial features that discriminate between active disease and remission. Gut microbiomes of mice with persistent colitis exhibited Enterobacteriaceae enrichment and, based on genomic functional analysis, an enhanced capacity for bacterial pathogenesis, including pathways involved in benzoate metabolism, TCSs, and cell motility (26). These pathways are linked to the QseBC signaling axis used by Enterobacteriaceae; catechols are intermediates of microbial benzoate degradation and CAs are host-derived catechols with a side-chain amine. Beyond their role as host stress hormones, CAs are also produced by the enteric nervous system and are important for regulating intestinal motility, electrolyte transport, and immune homeostasis (27). Moreover, evidence that levels of biologically active CAs in the intestinal lumen require specific bacterial-encoded enzymes suggests that luminal CA levels are gut microbiota-dependent (28). CAs continue to attract interest as communication molecules between host cells and microbes; these stress signals may influence microbial dysbiosis and increase the susceptibility to infection by altering the growth and virulence of human pathogens, including Enterobacteriaceae species (29, 30).

Given the essential function of flagella in LF82 virulence, we hypothesized that targeted inhibition of QseC would reduce its virulence potential. Thus, we generated isogenic LF82 *qseC* deletion and complemented strains to assess the effects of *qseC* inactivation on LF82 virulence. We found that the absence of *qseC* leads to down-regulated virulence gene expression and defects in flagellar assembly and motility in vitro and reduced colonization in vivo.

Furthermore, given that (*i*) microbial pathways involved in CA-related metabolism are enriched in experimental colitis (26) and human IBD (9) and (*ii*) QseC can mediate CA-induced virulence in pathogenic bacteria (31), we investigated whether QseC blockade with LED209 could be an effective microbiota-targeted approach for disease management. QseC inhibition attenuated disease in three preclinical models of colonic inflammation, including *T-bet*$^{-/-}$*Rag2*$^{-/-}$, *Il10*$^{-/-}$, and DSS-exposed mice. This study provides evidence that QseC may be an upstream virulence node used by colitogenic bacteria to survey their host and potentiate disease, and may be a useful target for microbiota-directed therapies for the treatment of IBD.

Results

Absence of *qseC* Suppresses LF82 Flagella Expression and Motility. To determine whether QseC plays an essential role in mediating LF82 virulence, we generated isogenic *qseC* deletion (*ΔqseC*) and in-genome complemented (*ΔqseC::qseC*) strains. To investigate whether *qseC* regulates LF82 virulence genes, we evaluated the expression of CA-mediated quorum-sensing genes (*qseB*, *qseC*, and *qseE*) and their transcriptional targets involved in flagellar activation, assembly, and motility (*flhC*, *flhD*, *fliA*, *fliC*, and *motB*) in the wild-type (WT) and mutant strains. *QseC* expression was not detected in *ΔqseC*, and *qseB* was up-regulated by >90-fold (Fig. 1*A*), consistent with previous findings indicating that QseC autoregulates QseBC TCS activation and that *qseC* deficiency leads to *qseB* up-regulation (32). Importantly, gene expression in the flagellar regulon (*flhDC*) and genes involved in flagellar assembly and motility (*fliA*, *fliC*, and *motB*) were all down-regulated in *ΔqseC* (Fig. 1*A*).

We used transmission electron microscopy (TEM) to visualize morphological differences in LF82 strains and to corroborate our flagellar expression data. There was marked reduction or complete loss of flagella on *ΔqseC* compared with the parental and complemented strains (Fig. 1*B*), supporting the idea that QseC regulates flagellar protein production. Given that flagella are required for LF82 virulence (13, 14) and that *qseC* inactivation suppresses the expression of flagellar genes and proteins (Fig. 1 *A* and *B*), we assessed flagella function via plate-based motility assays. The *ΔqseC* mutant exhibited motility defects based on a significantly smaller swim diameter compared with the parental strain (Fig. 1*C*). Collectively, these data substantiate that LF82 modulates its expression of flagellar genes and proteins through QseC.

QseC Mediates LF82 NE-Induced Flagella Expression and Motility. As a bacterial adrenergic receptor, QseC senses the host stress molecules NE and EPI and activates a signaling cascade that induces virulence gene expression (20). Thus, we evaluated the effects of *qseC* inactivation on CA-induced flagellar gene expression in the parental and *ΔqseC* mutant strains and selected NE rather than EPI because NE is more abundant in the intestinal lumen (28). NE up-regulated the expression of quorum-sensing and flagellar genes in the parental strain, but not in *ΔqseC* (Fig. 2*A*). Based on differential gene expression patterns with NE, we examined its effects on LF82 swimming motility. NE enhanced the flagella-mediated motility of WT LF82, but not of *ΔqseC*, in a dose-dependent manner (Fig. 2*B*). These observations confirm that QseC mediates NE's effects on LF82 flagellar expression and motility, with the caveat that NE-induced QseC-signaling has broad effects on bacterial physiology (19).

QseC Influences LF82's Effects in a Low-Complexity Microbiota. We next sought an in vivo system to assess whether QseC contributes

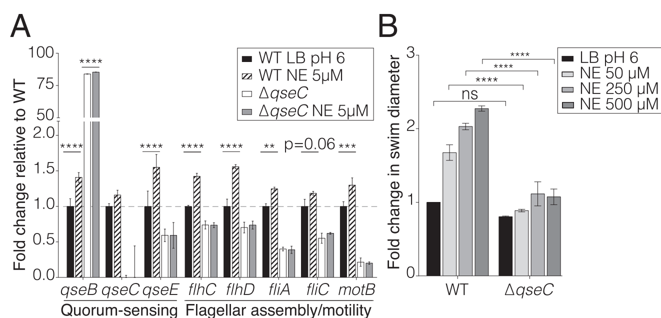

Fig. 2. *QseC* mediates NE-induced flagellar expression and motility in LF82. (*A*) RT-qPCR of LF82 strains grown in acidified or NE-supplemented LB. Genes normalized to *rpoA*. (*B*) Fold change in swim diameters on NE-supplemented soft agar plates. For *A* and *B*, fold change relative to WT grown in LB, pH 6. Data are mean ± fractional SD from two independent experiments. ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, two-way ANOVA with Sidak's multiple comparison test. ns, not significant.

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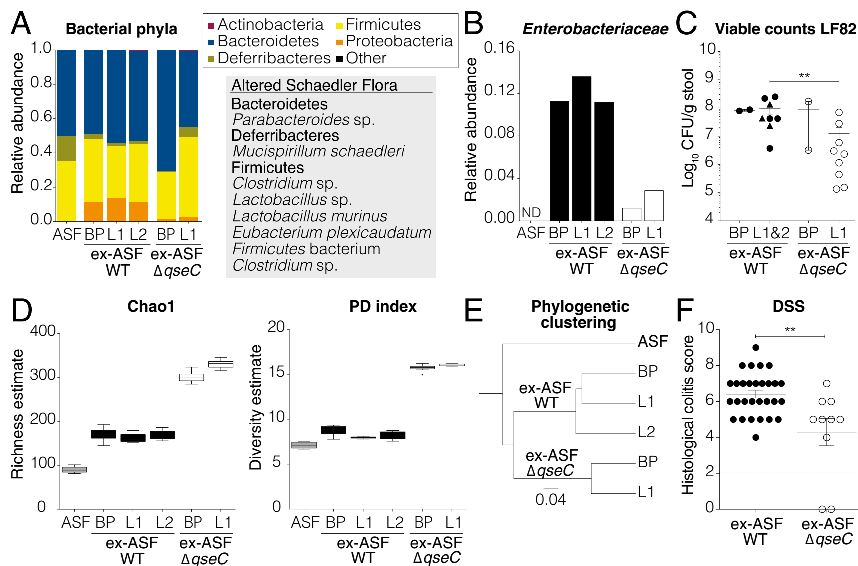

Fig. 3. *QseC* influences LF82's effects in a low-complexity microbiota. 16S rRNA gene surveys of cecal content from a gnotobiotic-ASF mouse and stool from LF82-inoculated ex-ASF breeding pairs (BP) and litters (L). Number of pooled samples for sequencing: WT LF82-associated mice: BP, $n = 2$; L1, $n = 3$; L2, $n = 5$; Δ*qseC*-associated mice: BP, $n = 2$; L1, $n = 9$. (*A*) Phylum-level relative abundances from QIIME-classified sequences. ASF strain list. (*B*) Enterobacteriaceae relative abundances. ND, not detected. (*C*) LF82 CFU in stool. Filled triangles represent L1 progeny; filled circles, L2 progeny. Data are mean ± SEM. $^{**}P < 0.01$, Kruskal–Wallis test with Dunn's post hoc test. (*D*) Alpha diversity using Chao1 and phylogenetic diversity (PD) indices. Boxplots are top, median, and bottom quartiles. Whiskers and outliers are plotted with Tukey's method. (*E*) Beta diversity using unweighted UniFrac distances. (*F*) Histological colitis scores of DSS-treated WT LF82-associated ($n = 29$) and Δ*qseC*-associated ($n = 10$) ex-ASF mice. Data are mean ± SEM from two to three independent experiments. A colitis score >2 indicates active disease; a score ≤2, remission. $^{**}P < 0.01$, Mann–Whitney *U* test.

to LF82 colonization. In specified pathogen-free (SPF) WT mice, LF82 colonization does not alter bacterial load, microbiota composition, or induce intestinal inflammation (33). This suggests that LF82 colonization is transient and does not activate innate immune responses in a healthy host. Thus, we hypothesized that gnotobiotic mice harboring the altered Schaedler flora (ASF) (34), an eight-bacterial strain consortium devoid of Proteobacteria (including *E. coli*), could provide a permissive niche for LF82 and furnish an in vivo system to assess whether *qseC* plays a role in LF82 colonization and persistence. This system has been successfully used to study other Gram-negative pathogens that are unable to stably colonize mice under SPF conditions (35). Using gnotobiotic-ASF mice also provides an opportunity to mirror in vivo features of a low-complexity microbiota commonly seen in IBD (36).

We transferred gnotobiotic-ASF mice from gnotobiotic isolators to our conventional animal facility, hereinafter referred to as ex-gnotobiotic-ASF (ex-ASF) mice. To maintain a low-complexity microbiota, ex-ASF mice were handled separately using animal husbandry practices that minimize microbial transfer. On transfer to conventional housing conditions, ex-ASF mice were orally inoculated with 10^8 colony-forming units (CFUs) of the WT or Δ*qseC* mutant LF82 strains. Experimental schema details are provided in Fig. S1.

To determine whether QseC promotes LF82 persistence within a low-complexity microbiota, we used 16S ribosomal RNA (rRNA) gene surveys to compare gut microbial communities of gnotobiotic-ASF and ex-ASF mice inoculated with the parental or Δ*qseC* mutant strains. DNA from the cecum of a gnotobiotic-ASF mouse and stool pooled from LF82-associated ex-ASF breeding pairs and corresponding litters were sequenced. Reads were binned into approximately species-level operational taxonomic units (OTUs) and analyzed using QIIME, a bioinformatics pipeline for microbial community analysis (37).

When phylum-level relative abundances were examined, only reads aligning to the microbial clades of the ASF were present in the gnotobiotic-ASF cecal sample, which is representative of the initial microbial community before LF82 inoculation (Fig. 3*A*). Higher levels of Proteobacteria were observed in mice exposed to the LF82 WT vs. the Δ*qseC* mutant strain (Fig. 3*A*). Of sequences that mapped to Proteobacteria, >98% belonged to the family Enterobacteriaceae, with WT LF82-associated mice having an average sixfold greater Enterobacteriaceae abundance than Δ*qseC*-associated mice (Fig. 3*B*).

To examine whether Enterobacteriaceae abundances correspond with LF82 viable counts, we plated stool samples from individual mice of each breeding pair and litter. LF82 CFUs were higher in WT LF82-associated progeny compared with Δ*qseC*-associated progeny (Fig. 3*C*). Thus, *qseC* loss may hinder LF82's ability to colonize and persist in a host even when there is minimal niche competition.

To characterize differences in community structure in the presence of WT and mutant LF82 strains, we analyzed microbial community diversity. Within-sample diversity of gut microbiomes of WT LF82-associated mice deviated less from the original ASF input community compared with Δ*qseC*-associated mice (Fig. 3*D*), suggesting that the parental strain is more adept at colonizing and persisting in a low-complexity microbiota and may even facilitate a state of reduced diversity. Between-sample diversity and hierarchical clustering by similarity revealed that microbial communities segregated according to ASF input or presence of LF82 WT or Δ*qseC*, indicating that these strains induce distinct microbiota responses (Fig. 3*E*). Moreover, these data show that LF82-associated microbiota phenotypes may be transmissible to offspring, given the similar community composition, structure, and diversity in breeding pairs and their corresponding litters. These findings support LF82's ability to sustain and thrive in a microbiota with reduced diversity.

Although ex-ASF mice harboring LF82 WT or Δ*qseC* do not display histological signs of intestinal inflammation, their reduced microbial community diversity and the fact that LF82 is an IBD-associated AIEC prompted us to ask whether LF82-associated ex-ASF mice would display exacerbated colitis in response to the mucosal disruptant and colitogenic agent DSS. Treating LF82 WT- and Δ*qseC*-associated ex-ASF mice with DSS could reveal whether the presence of functional *qseC* is sufficient to worsen colitis and whether QseC is a worthy microbial target in IBD pathogenesis. Indeed, after DSS treatment, WT LF82-associated mice had higher colitis scores than Δ*qseC*-associated mice (mean colitis score, 6.41 ± 0.22 vs. 4.3 ± 0.76) (Fig. 3*F*), and both strains were detectable in stool at similar levels (mean CFU/g stool, $7.62 \pm 3.23\mathrm{e}10^7$ for LF82 WT vs. $4.54 \pm 2.27\mathrm{e}10^7$ for LF82 Δ*qseC*).

As a virulence master regulator, QseC broadly affects bacterial physiology. Previous gene expression analysis in WT vs. Δ*qseC* uropathogenic *E. coli* strains revealed that *qseC* deletion results in altered nucleotide, amino acid, and carbon metabolism (19). Observing that LF82 is more adept at persisting in a low-complexity microbiota (Fig. 3), we queried whether *qseC* loss affects growth kinetics in vitro and survival in vivo. LF82 strains cultivated in aerobic or anaerobic conditions showed comparable growth and survival (Fig. S2). Thus, whereas the absence of *qseC* does not result in growth or survival defects, *qseC* has an appreciable influence on

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LF82's persistence over time and, importantly, may affect the susceptibility to mucosal injury, as observed with DSS treatment.

LED209 Inhibition of QseC Attenuates Colitis in SPF Mice. Based on (*i*) our observation that QseC functions in AIEC persistence and exacerbated colitis in ex-ASF mice and (*ii*) work demonstrating that the QseC inhibitor LED209 reduces Enterobacteriaceae pathogen virulence in vivo (21, 22), we examined the effects of QseC blockade on host disease status in three experimental colitis models maintained under SPF conditions. This approach would allow us to explore the translational applicability of a QseC-targeted antivirulence strategy for IBD. We hypothesized that LED209 may reduce disease activity, given that the Enterobacteriaceae family and Proteobacteria phylum have been implicated in instigating and/or perpetuating intestinal inflammation in preclinical colitis models (38). Thus, LED209 was administered daily to SPF *T-bet*$^{-/-}$*Rag2*$^{-/-}$, *Il10*$^{-/-}$, and DSS-treated WT mice. Study design, histological colitis scores, and other parameters of disease are provided in Fig. 4 and Fig. S3.

Treatment with LED209 conferred almost complete protection from colitis in *T-bet*$^{-/-}$*Rag2*$^{-/-}$ mice and reduced disease severity in DSS-exposed mice (Fig. 4 *A* and *C*). In *Il10*$^{-/-}$ mice, a greater proportion of LED209-treated mice (9 of 13; 69.2%) showed no signs of mucosal inflammation or injury compared with sham (7 of 11; 63.6%) and vehicle control (6 of 11; 54.5%) mice (Fig. 4*B*). *Il10*$^{-/-}$ mice often show gender differences in disease activity, with males tending to have more severe disease than females, as was also observed with LED209 treatment (Fig. S4). Our histopathology data indicate that LED209 can attenuate disease severity in genetic and chemically induced models of colonic inflammation, presumably by inhibiting QseC virulence pathways.

Enterobacteriaceae can increase their colitogenic potential under inflammatory conditions and may participate in initiating and potentiating inflammation in IBD (1, 38–40). LED209 studies measuring pathogen growth and survival in vitro and in infection models suggest that this antivirulence approach can reduce pathogenicity without affecting bacterial growth (21). Thus, we measured shifts in Enterobacteriaceae levels in stool from baseline using qPCR to assess whether we could relate abundance to treatment response in our preclinical colitis models. In *Il10*$^{-/-}$ and DSS-treated mice, LED209 blocked the expansion of Enterobacteriaceae compared with vehicle controls, which exhibited a >5.5-fold increase from baseline (Fig. S5 *B* and *C*). *T-bet*$^{-/-}$*Rag2*$^{-/-}$ mice displayed an inverse trend, with levels remaining stable in vehicle controls and increasing by 2.4-fold in LED209-treated mice (Fig. S5*A*).

Segregating Enterobacteriaceae relative abundances of LED209-treated mice based on active disease vs. remission for each model revealed comparable levels between disease states in *T-bet*$^{-/-}$*Rag2*$^{-/-}$ and DSS-exposed mice (Fig. S5*D*). However, *Il10*$^{-/-}$ mice with active disease had marked variations in Enterobacteriaceae, with higher levels observed in several mice with active disease compared with mice in remission (Fig. S5*D*). Thus, LED209 has differential effects on Enterobacteriaceae levels that are distinct between models and between disease states of treated mice.

Given that QseC is important for Enterobacteriaceae virulence and that QseC senses host NE and EPI, we evaluated the effects of QseC inhibition on the luminal CA pool of the cecum and colon. Cecal CA levels were comparable between treatment groups and across models (Fig. S6*A*). Significant differences in cecal NE and EPI were observed only in DSS-treated mice, but this effect was driven by a single outlier (Fig. S6*A*). There were no significant differences or trends between treatment groups or across models in stool CA level changes from baseline (Fig. S6*B*). Despite differences in disease severity and Enterobacteriaceae abundances between treatment groups in these models, we did not observe major perturbations to the CA economy of the intestinal lumen.

To determine whether LED209 may exert direct anti-inflammatory effects on the host, we tested its effects on cytokine gene expression in immune cell populations in vitro. LED209 exposure did not alter the expression of *Il10* or *Tnfα* in mesenteric lymph node (MLN)-derived CD11c^{+} dendritic cells (Fig. S7*A*) and had negligible

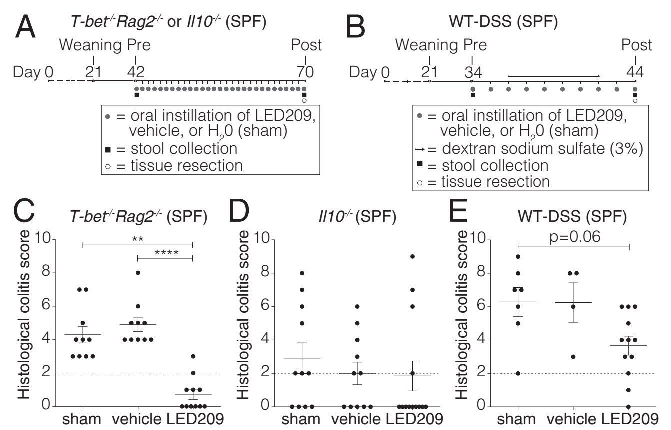

Fig. 4. LED209 inhibition of QseC attenuates colitis. (*A* and *B*) Study design: *T-bet*$^{-/-}$*Rag2*$^{-/-}$ (n = 31), *Il10*$^{-/-}$ (n = 35), and WT DSS-treated (n = 23) mice under SPF conditions. Mice were orally administered LED209, vehicle, or water (sham) daily. (*C*–*E*) Histological colitis scores. Symbols represent individual mice. Data are mean ± SEM from two to three independent experiments. A colitis score >2 indicates active disease; a score ≤2, remission. $^{**}P$ < 0.01, $^{****}P$ < 0.0001, Kruskal–Wallis test with Dunn's post hoc test.

effects on *Il10*, *Tnfα*, and *Ifnγ* in splenic-derived CD4^{+} cells (Fig. S7*B*). These observations suggest that LED209 may disrupt the ability of bacteria to communicate with each other and their host and in turn attenuate bacterial virulence and intestinal inflammation without directly influencing the luminal CA pool or host cytokine gene expression.

Discussion

Enterobacteriaceae, particularly *E. coli*, have been implicated in IBD pathogenesis given their abundance in mucosal lesions of patients with CD and pathogenic properties in vitro and in vivo (4, 5, 7). The prototype AIEC strain LF82 requires flagella expression to adhere to and invade host cells (13). As a virulence master regulator, QseC plays a crucial role in promoting the virulence of pathogenic *E. coli*, including enterohemorrhagic and enteropathogenic *E. coli* strains (41), but little is known about its regulatory function in AIEC. To examine whether QseC regulates LF82 virulence, we genetically manipulated the *qseC* locus of the parental strain to generate an isogenic *qseC* deletion mutant and in-genome complemented strain. Our characterization revealed that the *ΔqseC* mutant had down-regulated expression of flagellar assembly and motility genes, loss of flagellar surface proteins, and reduced swimming motility, which were rescued with *qseC* complementation (Fig. 1). These findings support the idea that QseC regulates LF82 virulence, at least in part, by activating flagellar expression and motility.

Host–microbiota communication is increasingly recognized as an important aspect of both symbiosis and pathogenesis. Integral to the microbiota's surveillance and collective decision making process are quorum-sensing TCSs. For QseBC, the presence of microbiota-generated hormone-like compounds (AI-3) or host stress signals (NE or EPI) can initiate a virulence program with detrimental consequences for the host (42). This property motivated us to examine whether QseC is necessary for NE-induced flagellar gene expression in LF82. We found that the parental strain, and not *ΔqseC*, had enhanced expression of NE-regulated TCS and flagellar genes and exhibited a dose-dependent increase in swimming motility (Fig. 2), indicating that QseC functions as an adrenergic receptor in LF82. We also observed that QseEF, an alternative NE-sensing TCS, was also regulated by QseC activation, given that expression of the *qseE* sensor kinase was reduced in the *ΔqseC* mutant and its levels did not change in the presence of NE (Figs. 1*A* and 2*A*). These findings are in agreement with other reports of QseC acting upstream of QseEF (43) and establish that NE-induced virulence in LF82 is regulated primarily by QseC.

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Because LF82 is unable to colonize the gut of a healthy host (33), we established an in vivo system for examining the effects of *qseC* inactivation on LF82 colonization, persistence, and response to the mucosal disruptant and colitogenic agent DSS using ex-ASF mice. The absence of Proteobacteria in the ASF community enabled colonization of LF82. The 16S rRNA gene surveys revealed that total Enterobacteriaceae were more enriched in ex-ASF mice associated with LF82 WT vs. *ΔqseC*, which were consistent with culturable counts of LF82 (Fig. 3*B*). These results suggest that despite similar growth kinetics in vitro, LF82 may have a fitness advantage compared with the *ΔqseC* mutant within the gastrointestinal tract of a host over time.

Additional insights relevant to the underlying microbial dysbiosis associated with IBD were gleaned from our 16S rRNA gene sequence analysis. We observed that microbiotas of WT LF82-associated mice had less within-sample diversity and were more analogous to the ASF input community compared with microbiotas of *ΔqseC*-associated mice. Because LF82 is unable to stably colonize conventional mice under SPF conditions (33), these data show that LF82 can successfully colonize a host harboring a low-complexity microbiota and may even promote the maintenance of a less diverse state to ensure its survival. Whether the loss in microbial community diversity associated with dysbiosis is a cause or consequence of chronic intestinal inflammation remains elusive. Perhaps certain bacteria, like AIEC, are involved throughout the disease continuum, contributing to both the initiation and progression of disease by promoting and sustaining dysbiosis and altered host–microbiota interactions. Evidence that LF82 can preserve a specific microbiota phenotype was observed, with breeding pairs of LF82-associated ex-ASF mice exhibiting similar microbial community structures as their litters (Fig. 3), demonstrating that features of an LF82-associated microbiota may be transmissible. With the genetic foundation of most complex immune-mediated disorders, such as IBD, the increased risk of disease between family members may be augmented by the transmissibility of the microbiome.

Given that LF82 is more adapted to colonize the intestines of humans rather than mice, human CEACAM6 transgenic mice may be a useful model for understanding LF82–host interactions (44). However, intestinal inflammation in this model requires both antibiotic-mediated microbiome alterations and mucosal injury induced by DSS. Although this system affords opportunities for studying LF82 in vivo, its applicability as a robust preclinical model has major caveats. Our results support the idea that QseC is an important regulator of LF82 virulence and modulator of the microbiome and host–microbiota homeostasis, all of which strengthen the case for further investigation into perturbing QseC and other key virulence mediators as an approach to IBD therapeutics.

Quorum-sensing through TCSs is considered an adaptive and auxiliary function of bacteria—one that is critical for virulence, infection, and enhanced fitness but not essential for growth and survival (45). This concept has been strengthened by studies demonstrating that quorum-sensing inhibitors, like LED209, can reduce bacterial virulence without affecting growth (21). Thus, we investigated whether inhibition of QseC could reduce disease severity in three preclinical models of colonic inflammation under SPF conditions. We performed LED209 interventions in the (*i*) *T-bet*$^{-/-}$*Rag2*$^{-/-}$ model, which has defects in both innate and adaptive immunity; (*ii*) *Il10*$^{-/-}$ model, in which mice develop spontaneous colitis from loss of regulatory immune function; and (*iii*) DSS-induced acute colonic injury model, which recapitulates histological features of human UC. Using these distinct models of experimental colitis allowed us to assess the applicability of QseC inhibition under different genetic contexts. We observed an attenuation of disease in *T-bet*$^{-/-}$*Rag2*$^{-/-}$ and DSS-exposed mice, and a modest benefit in *Il10*$^{-/-}$ mice (Fig. 4). Intriguingly, LED209's ability to ameliorate disease in *Il10*$^{-/-}$ and DSS-treated mice appeared to be dependent on blocking the expansion of Enterobacteriaceae, given mice with active colitis had a greater Enterobacteriaceae abundance on average compared with mice in remission (Fig. S5). Taken together, the data from LED209 interventions in genetically distinct mouse models of colonic inflammation suggest an underlying role for QseC-mediated virulence in disease pathogenesis.

Several mechanisms for how antivirulence drugs inhibit exogenous pathogens have been suggested, including preventing the colonization of pathogens during passage through the gastrointestinal tract and facilitating the elimination of pathogens by the host immune system (46). Whether these mechanisms hold true for colitogenic bacteria, which are already a part of the endogenous microbiota, requires further study.

In summary, we have demonstrated that perturbing the Enterobacteriaceae adrenergic receptor QseC with the biochemical inhibitor LED209 can attenuate experimental colitis and that genetically inactivating *qseC* in a pathogenic IBD-associated *E. coli* strain can reduce its virulence in vitro and abrogate its ability to persist in a low-complexity microbiota in vivo. These results provide insight into the use of an antivirulence approach for targeting not only pathogens, but also a much larger collection of colitogenic bacteria.

Materials and Methods

Bacterial Strains, Plasmids, and Growth Conditions. AIEC strain LF82 was used in this study (10). Bacterial strains and plasmids are listed in Table S1. Details are provided in *SI Materials and Methods*.

Gene Inactivation and Complementation. Isogenic mutants were generated with PCR products using the method described by Datsenko and Wanner (47), with modifications for pathogenic *E. coli* (48). Primers for gene manipulation and verification are listed in Table S2. More details are provided in *SI Materials and Methods*.

RT-qPCR of Bacterial Cultures. RNA was isolated and RT-qPCR was performed as described in *SI Materials and Methods*. The RT-qPCR primers are listed in Table S2.

TEM Analysis. Bacterial cells were visualized using a JOEL 1200 EX transmission electron microscope, as detailed in *SI Materials and Methods*.

Plate-Based Bacterial Motility Assays. Bacterial motility assays are described in detail in *SI Materials and Methods*.

Animal Husbandry. Gnotobiotic BALB/cByJ mice harboring the ASF consortium (49) were bred and maintained in gnotobiotic isolators. SPF BALB/cByJ *T-bet*$^{-/-}$*Rag2*$^{-/-}$, *Il10*$^{-/-}$, WT, and ex-ASF mice were housed in a barrier facility. All animal experiments were approved and conducted in accordance with guidelines of Harvard Medical School's Standing Committee on Animals and the National Institutes of Health. Details of the ASF consortium and maintenance of ex-ASF mice are provided in *SI Materials and Methods*.

Infection of ex-ASF Mice with LF82. On transfer from gnotobiotic isolators to conventional housing, 8-wk-old ex-ASF mice were orally inoculated with 10^8 CFU of LF82 WT or Δ*qseC*, as described in *SI Materials and Methods*.

16S rRNA Gene Surveys of Stool and Sequence Analysis. DNA was isolated from stool, amplified for the V4 region of the 16S rRNA gene, and sequenced using an Illumina MiSeq instrument. Reads were processed and analyzed with QIIME (37). Details are provided in *SI Materials and Methods*.

In Vivo Competition. JAX BALB/cByJ (7- to 8-wk-old) mice were inoculated with 1:1 cultures of 10^9 CFU of LF82 pNG162:Δ*qseC::kan* or Δ*qseC*/pNG162:Δ*qseC::kan*, as described in *SI Materials and Methods*.

DSS Treatment of ex-ASF Mice. ex-ASF mice (5- to 7-wk-old) underwent a 7-d DSS intervention, as described in detail in *SI Materials and Methods*.

LED209 Interventions. Mice were orally administered an equal volume of LED209 (0.4 mg/mouse), vehicle, or water (sham). Details are provided in *SI Materials and Methods*.

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Histology. Sections were examined and degree of colitis was scored as described previously (50) and in *SI Materials and Methods*.

qPCR Analysis of *Enterobacteriaceae* in Stool. DNA was isolated from stool and qPCR was performed, as described in *SI Materials and Methods*.

Luminal CA Measurements by HPLC. CAs were measured as described previously (28) and in *SI Materials and Methods*.

In Vitro LED209 Stimulation and RT-qPCR for Cytokine Gene Expression. MLNs and spleens were harvested from 5- to 7-wk-old JAX BALB/cByJ mice to enrich for CD11c^{+} dendritic cells and CD4^{+} cells, respectively (*SI Materials and Methods*).

Statistical Analysis. All statistical tests were performed in Graphpad Prism 6.0h. Averages are reported as mean ± SEM except for fold change, for which averages are mean ± fractional SD. Absence of error bars indicates minimal SEM or SD.

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