inflammation, the adaptive immune system can limit the presence of live bacteria in the periphery. Collectively, these data suggest that ILCs are essential to promote anatomical containment of *Alcaligenes* to lymphoid tissues and limit the induction of systemic inflammation in lymphocyte-replete hosts.

Loss of containment of commensal bacteria and chronic systemic inflammation is associated with several chronic human diseases (*6*–*8*). To determine whether these diseases were also associated with a loss of containment of *Alcaligenes* spp., we analyzed serum samples from cohorts of pediatric Crohn's disease patients or chronically hepatitis C virus (HCV)–infected adults for the presence of *Alcaligenes*-specific IgG. In comparison to age-matched controls, serum from pediatric Crohn's disease patients and plasma from cirrhotic HCV-infected individuals awaiting liver transplantation exhibited significantly elevated levels of relative IgG specific for *Alcaligenes* spp. (Fig. 4, I and J). Although further analysis of HCV-infected individuals with and without cirrhosis demonstrated no correlations between *Alcaligenes*-specific IgG levels and patient age or serum alanine transaminase (fig. S11, A and B), there were significant correlations between plasma levels of *Alcaligenes*-specific IgG and laboratory measures of liver disease, including increased serum bilirubin and international normalized ratio (INR) of prothrombin time as well as decreased serum albumin and platelets (Fig. 4, K to N).

Mammals have evolved multiple immunologic and physiologic mechanisms to promote the anatomical containment of commensal bacteria to intestinal sites, including promoting physical barriers (via epithelial cell tight junctions), biochemical barriers (via production of mucus layers and antimicrobial peptides), and immunologic barriers (via IgA-mediated immune exclusion; intraepithelial lymphocytes; and innate pathways involving phagocytosis, Toll-like receptor–mediated sensing, and oxidative bursts) (*1*, *2*, *18*, *19*, *25*). The demonstration that depletion of ILCs results in the selective dissemination and survival of *Alcaligenes* spp. in peripheral tissues of mice indicates that, in addition to established pathways that nonselectively maintain intestinal barrier function, more discriminatory processes may have evolved to promote the selective anatomical containment of phylogenetically defined communities of lymphoid-resident commensal bacteria (fig. S12). It is notable that *Alcaligenes* spp. has recently been identified as a dominant lymphoid-resident commensal species colonizing the PPs and mLNs of mammals (*4*). Moreover, peripheral dissemination of *Alcaligenes* spp. has been reported in patients with HIV infection, cancer, and cystic fibrosis (*26*–*29*). The identification of a pathway through which IL-22–producing ILCs can prevent dissemination of lymphoid-resident *Alcaligenes* spp. and limit systemic inflammation highlights the selectivity of immune-mediated containment of defined commensal bacterial species and could offer therapeutic strategies to limit inflammation associated with multiple debilitating chronic human diseases.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6086/1321/DC1
Materials and Methods
Figs. S1 to S12
Tables S1 and S2
Reference (*30*)

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Regulated Virulence Controls the Ability of a Pathogen to Compete with the Gut Microbiota

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The virulence mechanisms that allow pathogens to colonize the intestine remain unclear. Here, we show that germ-free animals are unable to eradicate *Citrobacter rodentium*, a model for human infections with attaching and effacing bacteria. Early in infection, virulence genes were expressed and required for pathogen growth in conventionally raised mice but not germ-free mice. Virulence gene expression was down-regulated during the late phase of infection, which led to relocation of the pathogen to the intestinal lumen where it was outcompeted by commensals. The ability of commensals to outcompete *C. rodentium* was determined, at least in part, by the capacity of the pathogen and commensals to grow on structurally similar carbohydrates. Thus, pathogen colonization is controlled by bacterial virulence and through competition with metabolically related commensals.

Enterohemorragic *Escherichia coli* (EHEC) and enteropathogenic *E. coli* (EPEC) are important causes of diarrhea and mortality worldwide (*1*, *2*). These Gram-negative bacteria attach to and colonize the intestinal tract by inducing attaching and effacing (AE) lesions on the intestinal epithelium (*1*, *2*). The genomes of AE pathogens harbor the locus of enterocyte

effacement (LEE) that is critical for these bacteria to colonize their hosts and cause pathology (*3*, *4*). Infection with *Citrobacter rodentium*—a natural pathogen of mice that is used to model human infections with EPEC and EHEC (*5*, *6*)—is associated with a significant but reversible decrease in the number of total commensals in the colon (*7*, *8*). To assess the role of the microbiota in this enteric infection, germ-free (GF) and specific pathogen–free (SPF) C57BL/6 mice were orally inoculated with *C. rodentium*. The pathogen colonized the intestines of SPF mice, reaching maximal concentrations in the feces on days 7 to 10 post infection, followed by a decline by day 12, and becoming undetectable by day 22 (Fig. 1A). In contrast, GF mice harbored 10 times as much *C. rodentium* on days 7 to 10 post infection, and unlike SPF mice, they were unable to clear *C. rodentium* even by day 42 when the experiments were terminated (Fig. 1A). It was remarkable that all GF infected mice remained alive despite high and persistent pathogen burdens (Fig. 1B). Notably, the recruitment of neutrophils, inflammatory macrophages, and $CD3^+$ T cells in response to infection was similar in SPF and GF mice (fig. S1). Consistently, histological analysis revealed comparable pathology scores on day 12 post infection, which declined on day 22 in both GF and SPF mice (fig. S1). Furthermore, expression of antimicrobial peptides including RegIIIγ, β-defensin-1, β-defensin-3, and β-defensin-4 was comparable in the colons of infected SPF and GF mice (fig. S2).

The expression of most LEE genes in *C. rodentium* is controlled by Ler, a member of the H-NS protein family (*3*, *4*, *9*, *10*). *Ler* was expressed in the feces on day 7 post infection in both GF and SPF mice (Fig. 2A). It was noteworthy that expression of *ler* and *tir*, a Ler-regulated gene, were both down-regulated on day 12 after infection in both GF and SPF mice and were not expressed at day 42 in GF mice, despite robust pathogen colonization (Fig. 2A and fig. S3). To monitor the expression of *ler* in the intestine, we engineered a bioluminescent reporter *C. rodentium* strain in which the *ler* promoter was fused to the *lux*CDABE operon of *Photorhabdus luminescens* (fig. S4) (*11*). *Ler* expression was detected in the feces on day 5, but down-regulated by days 7 to 12 post infection (Fig. 2B). Furthermore, *ler* expression was visualized on day 5 post infection in the ileum, cecum, and distal colon but was down-regulated on day 14 in both SPF and GF mice (Fig. 2C). The *ler* luminescent signal in GF mice was ~10 times that in SPF mice, consistent with higher pathogen load in GF mice. If the *C. rodentium* were harvested from infected GF mice at day 21 and reinoculated into SPF mice, the pathogen robustly grew in the intestines and elicited colonic inflammation (fig. S5).

Because expression of virulence LEE genes is essential for pathogen colonization in the intestines of SPF mice (*4*), we asked whether LEE virulence was required for colonization in GF mice. To assess this, we orally infected GF and SPF mice with wild-type (WT) *C. rodentium* and isogenic strains deficient in Ler (Δ*ler*) or in EscN (Δ*escN*), a Ler-regulated adenosine triphosphatase required for injection of virulence factors via the type III secretion system (T3SS) into host cells (*4*). As expected, WT, but not the Δ*ler* or Δ*escN* strains, grew in the intestine of SPF mice (Fig. 2D). Notably, the Δ*ler* and Δ*escN* mutants grew robustly in GF mice, reaching numbers similar to those of the WT pathogen (Fig. 2D and fig. S6). Thus, Ler-dependent LEE virulence is not required for intestinal *C. rodentium* growth in the absence of the microbiota. To determine whether Ler and the T3SS regulate the ability of the microbiota to outcompete the pathogen, we orally infected GF mice with WT or the Δ*ler* or the Δ*escN* mutant *C. rodentium*, and the infected GF mice were colonized with commensals by cohousing them at either day 3 or day 21 post infection with SPF mice. Notably, the burden of WT *C. rodentium* in GF mice harboring the pathogen for 21 days declined significantly after 3 days of cohousing and was further reduced by 5 to 6 logs by day 7, similarly to the decline observed in mice infected with the Δ*ler* or the Δ*escN* mutant for 3 and 21 days (Fig. 2E and fig. S6). In contrast, the microbiota could not outcompete the WT bacterium on day 3 post infection when GF mice were cohoused with SPF mice (Fig. 2E), consistent with the observation that *ler* expression is active in WT *C. rodentium* at day 3 post infection (fig. S7).

C. rodentium induces marked inflammation in the distal colon, which requires LEE virulence factors including the T3SS (*4*). If the T3SS promotes outgrowth of *C. rodentium* by enabling the pathogen to colonize a niche at or near the epithelium, it would be expected that impaired colonization of the Δ*escN* mutant could not be rescued when inflammation is provided by other means. Oral administration of dextran sulfate sodium (DSS), a chemical that directly damages the colonic epithelium (*12*), enhanced the colonization of the WT bacterium by 3 logs but not that of the Δ*escN* mutant (fig. S8). In addition, coinfection of the Δ*escN* mutant with WT *C. rodentium*, which induces inflammation, did not rescue colonization by the T3SS mutant strain (fig. S8). These results indicate that inflammation promotes colonization of WT *C. rodentium* not in the absence of a functional T3SS. The adaptive immune system—including immunoglobulin G (IgG) production and T helper 17 responses against the pathogen—are important for eradication of *C. rodentium* (*13*–*16*). It was noteworthy that the production of IgG, IgM, and IgA against *C. rodentium* was similar in GF and SPF mice (fig. S9). Furthermore, the generation of colonic interleukin-17 (IL-17)– and interferon-γ–producing T cells in response to *C. rodentium* was comparable in SPF and GF mice (fig. S10).

C. rodentium colonizes and infects the surface of the intestinal epithelium, a site largely devoid of commensals (*17*). At day 5 post infection, Tir, a marker of *C. rodentium* attachment to the epithelium, was seen on the intestinal surface of GF mice infected with WT *C. rodentium*, but not those with the Δ*ler* strain (fig. S11). In contrast, Tir labeling was not detected on day 21 post infection on the intestinal surface of mice infected with either WT or mutant *C. rodentium* (fig. S11). Consistently, the WT pathogen was associated with the epithelium, whereas the Δ*ler* mutant localized to the intestinal lumen on day 5 post infection as determined by dual-fluorescence in situ hybridization (FISH) (Fig. 3A). On day 21 post infection, however, neither WT nor Δ*ler* *C. rodentium* bacteria were detected on the epithelial surface; instead, they localized to the intestinal lumen (Fig. 3A). Similarly, transmission electron microscopy revealed *C. rodentium* at or near the intestinal epithelium on day 5, but not on day 21, post infection (Fig. 3B). As expected, Δ*ler* *C. rodentium* did not localize at or near the epithelium on day 5 post infection

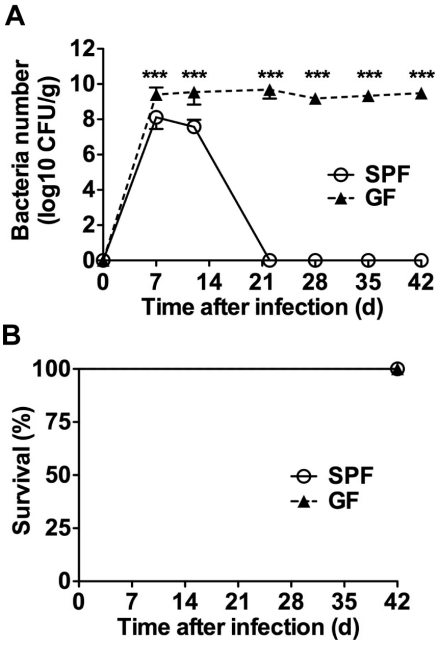

Fig. 1. The microbiota is required for eradication of *C. rodentium*. (**A** and **B**) SPF and GF mice ($n = 7$) were infected orally with 1×10^9 colony-forming units (CFU) of *C. rodentium*, and pathogen load in feces (A) and mouse survival (B) were determined over the indicated time. Data points are means ± SD. Results are representative of at least three independent experiments. ***$P < 0.001$, Mann-Whitney *U* test.

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(Fig. 3B). Consistent with these results, *ler-lux*–expressing WT *C. rodentium* found attached to the cecal and colonic epithelium were abundant in the early phase of infection but dramatically decreased later during infection, even though pathogen burdens in the feces were comparable (Fig. 3C and fig. S12). These results indicate that the localization of the pathogen differs in the early and late phase of infection, and this is controlled by LEE virulence gene expression.

The intestine harbors a large number of bacterial species (*18*). To determine whether commensal bacteria exhibit different abilities to outcompete *C. rodentium*, we orally infected GF mice with *C. rodentium* and on day 21 post infection the mice were colonized with either *E. coli* or one of two different anaerobic *Bacteroides* species, all isolated from the intestines of SPF mice (*19*). The burden of *C. rodentium* in GF mice declined to ~1/200th by day 3 and to less than 1/500th by day 14 upon colonization with *E. coli*, but not at all with *B. thetaiotaomicron* or *B. vulgatus* (Fig. 4A). Secondary administration of *E. coli* to GF mice already colonized with *B. thetaiotaomicron* or

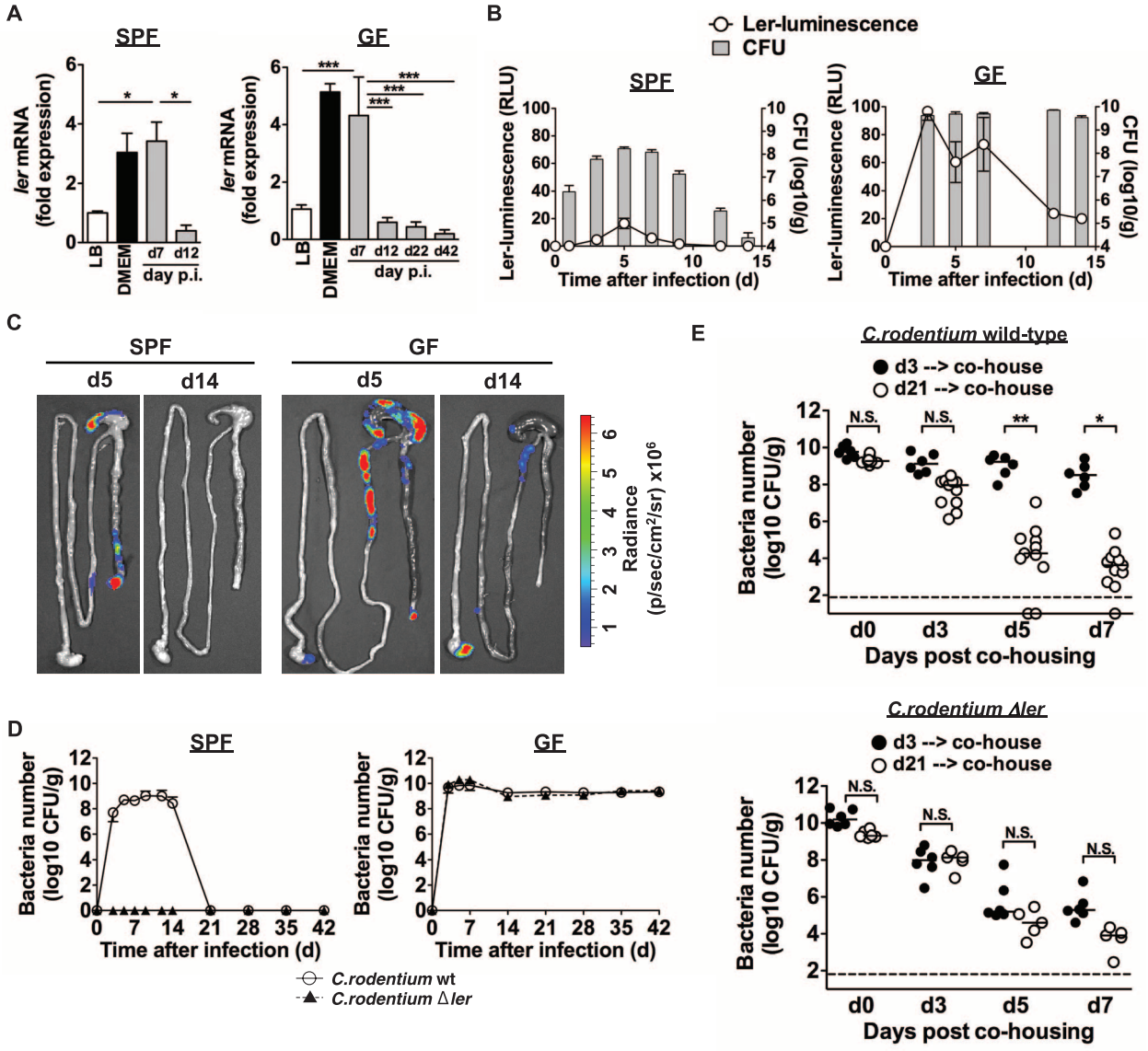

Fig. 2. Expression and role of *ler* during *C. rodentium* infection in SPF and GF mice. (**A**) *ler* mRNA levels were determined by quantitative polymerase chain reaction in fecal pellets of SPF and GF mice infected with *C. rodentium* at the indicated days post infection. Expression was normalized to that of the kanamycin-resistance gene carried by the *C. rodentium* strain. Control experiments were performed by determining *ler* mRNA levels in *C. rodentium* grown under inducing Dulbecco's modified Eagle's medium (DMEM) and repressing Luria broth (LB) in vitro culture conditions (*4*, *10*). Data represent mRNA expression relative to that in *C. rodentium* cultured in LB medium. Results are means ± SD of individual mice ($n = 3$). Results are representative of at least two experiments. $^{*}P < 0.05$, $^{***}P < 0.001$. (**B**) Expression of *ler* in fecal pellets of SPF and GF mice infected with the reporter *ler-lux C. rodentium* strain at the indicated day post infection. Results show luminescence (relative light units) and CFU of *ler-lux C. rodentium* in the same samples. Data are expressed as means ± SD of individual mice ($n = 4$). Results are representative of at least two experiments. (**C**) Bioluminescent imaging of *ler* expression in the intestines of SPF and GF mice infected with the *ler-lux C. rodentium* strain. Imaging was performed on day 5 and 14 post infection, and the signal was quantified on the basis of the color scale shown below. Results are representative of three individual mice. (**D**) SPF and GF mice ($n = 5$) were infected orally with 10^9 CFU of WT and Δ*ler* mutant *C. rodentium*, and pathogen load in feces was determined over the indicated time. Data points are means ± SD. Results are representative of at least two experiments. (**E**) GF mice were infected with WT and Δ*ler* mutant *C. rodentium*. At day 3 or day 21 post infection, mice were cohoused with SPF mice (1:1). Pathogen load was determined in feces on the indicated days after cohousing. Dots represent individual mice. Results are representative of at least three experiments.

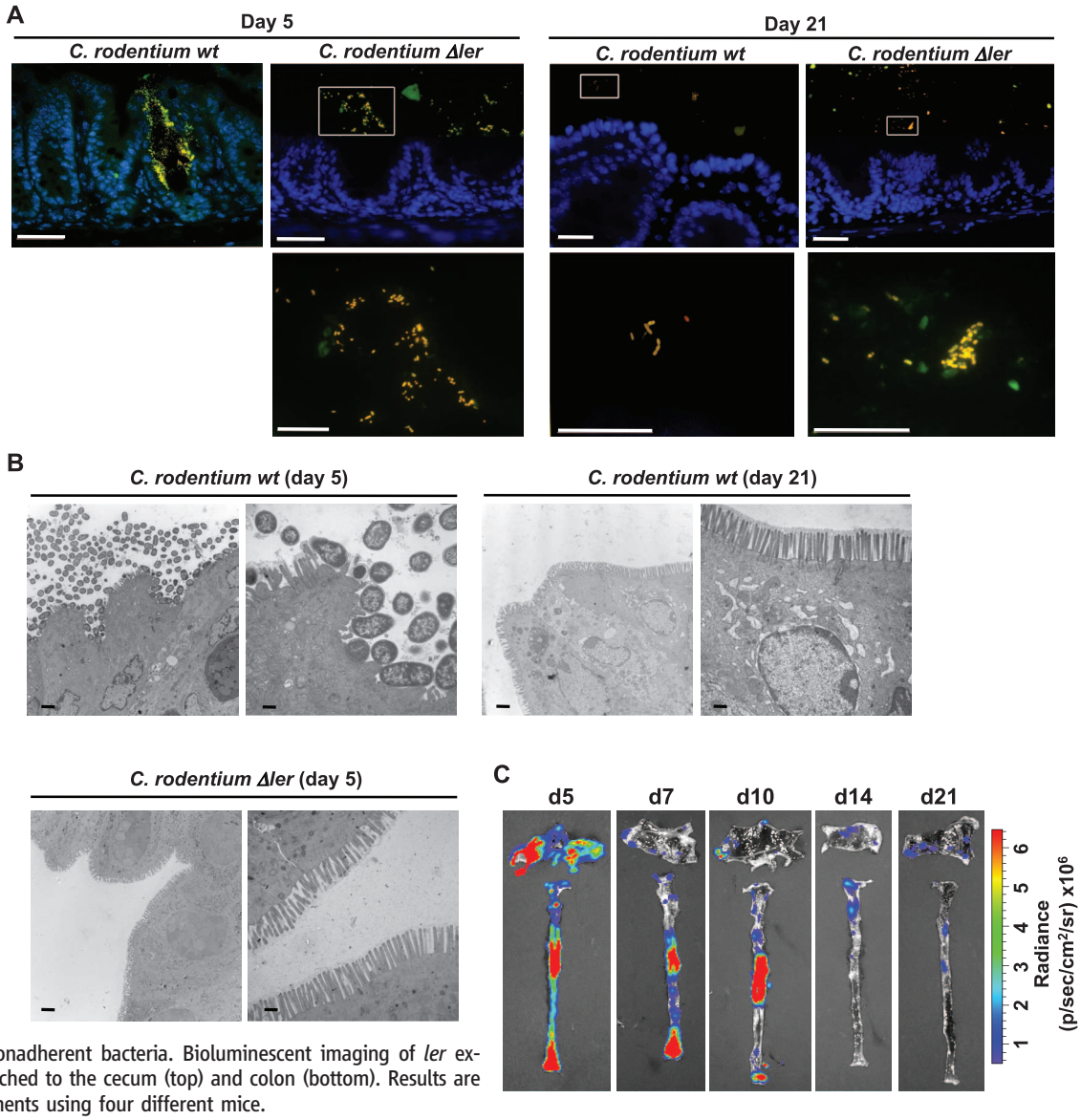

Fig. 3. Localization of *C. rodentium* to intestinal niches is mediated by LEE-encoded virulence factors. (**A**) Dual FISH staining with DNA probes that label virtually all true bacteria (EUB338, red) and the γ-Proteobacteria class to which *C. rodentium* belongs (GAM42a, green). Pathogenic bacteria (i.e., EUB338$^+$/GAM42a$^+$ cells) are yellow. (Bottom) High-magnification images corresponding to boxed areas (top). Note the yellow color on all the bacteria stained in the cecum infected with the Δ*ler* mutant, which indicates that all bacteria in the lumen are *C. rodentium*. Scale bars: 50 μm (top), 20 μm (bottom). Results are representative of two experiments. (**B**) Transmission electron micrographs of cecum from infected GF mice at day 5 and day 21 post infection with WT *C. rodentium* and at day 5 with the Δ*ler* mutant. Original magnification: 3400× (left) and 13,500× (right). Scale bars: 2 μm (left) and 500 nm (right). Results are representative of two experiments. (**C**) GF mice were infected orally with WT *C. rodentium* carrying the *ler-lux* fusion. Cecum and colonic tissues were collected at the indicated day and then washed with phosphate-buffered saline to remove nonadherent bacteria. Bioluminescent imaging of *ler* expression of *C. rodentium* attached to the cecum (top) and colon (bottom). Results are representative of two experiments using four different mice.

B. vulgatus also reduced the number of *C. rodentium* in the feces to ~1/500th (Fig. 4A). In contrast, colonization of mice harboring *E. coli* with *B. thetaiotaomicron* or *B. vulgatus* was not effective in further reducing the burden of *C. rodentium* in their feces (Fig. 4A). Assessment of total *Enterobacteria* (*E. coli* and *C. rodentium*) and *Bacteroides* on day 14 post colonization with individual commensal bacteria showed comparable numbers of bacteria in the mouse feces (Fig. 4B). Before inoculation of commensal bacteria day 0 (d0), all *Enterobacteria* detected in the feces were *C. rodentium* (Fig. 4C). In contrast, >99% of the total *Enterobacteria* in the *E. coli*–inoculated group were *E. coli* after 14 days of colonization (Fig. 4C). We next analyzed the growth abilities of each of these species on a custom carbohydrate growth array that contains most of the common mono- and polysaccharides present in plant and animal tissue (Fig. 4D and table S1). Both *C. rodentium* and *E. coli* exhibited optimal growth on monosaccharides (Fig. 4D). In contrast, both *B. thetaiotaomicron* and *B. vulgatus* exhibited broad abilities to catabolize both mono- and polysaccharides (Fig. 4D). Because γ-Proteobacteria, such as *E. coli*, specifically accumulate after *C. rodentium* infection (*7*), these results suggest that this microbial change may benefit the host by increasing the number of commensals that can outcompete the pathogen. To further assess whether competition for glycans between commensals and *C. rodentium* is important for pathogen eradication, GF mice fed a conventional maintenance diet containing both mono- and polysaccharides were orally infected with *C. rodentium*, and on day 21 post infection, the infected GF mice were colonized with *B. thetaiotaomicron* for 7 days. On day 7 post colonization with *B. thetaiotaomicron*, the mice were divided into two groups that were fed the conventional diet or a simple sugar diet containing monosaccharides but not polysaccharides. The burden of *C. rodentium* in the GF mice fed the simple sugar diet rapidly declined to ~1/200th by day 3, whereas no decline was observed when the mice were fed the conventional diet (Fig. 4E). The results were not explained by the simple sugar diet alone, because in the absence of colonization with *B. thetaiotaomicron*, the number of *C. rodentium* in feces was comparable between animals fed with the conventional and simple sugar diets (Fig. 4F). These results suggest that, while mice ingest the simple sugar diet, *B. thetaiotaomicron* cannot use polysaccharides and is forced to compete with *C. rodentium* for available monosaccharides or another common nutrient that both *C. rodentium* and *B. thetaiotaomicron* rely on in the absence of dietary polysaccharides. These findings clarify the tenuous nature of AE pathogens, in terms of their survival and/or colonization within hosts, and therefore, nutrient or probiotic shifting of the microbiota to promote commensals that directly compete with pathogens for food sources may be a useful therapeutic approach.

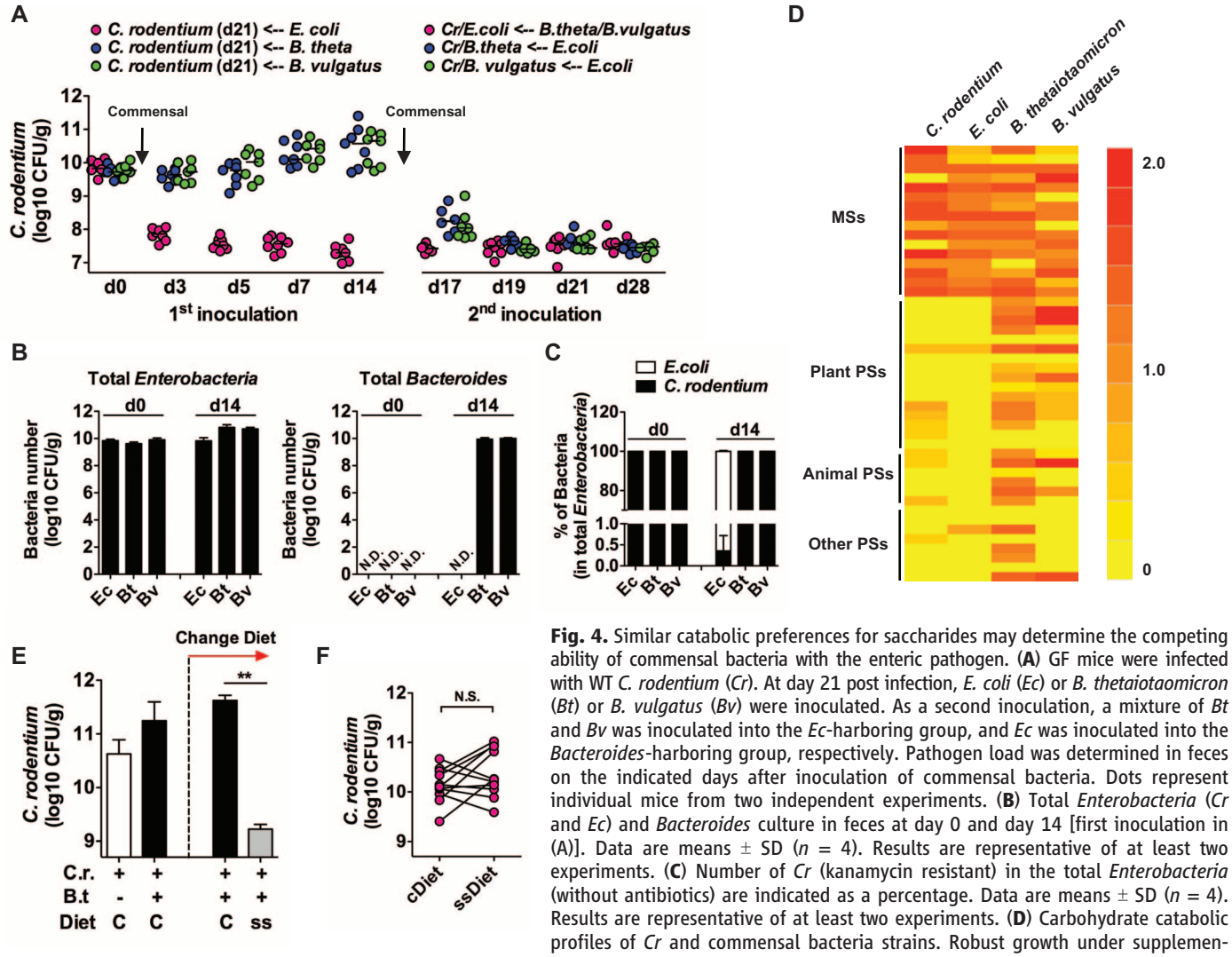

Fig. 4. Similar catabolic preferences for saccharides may determine the competing ability of commensal bacteria with the enteric pathogen. (**A**) GF mice were infected with WT *C. rodentium* (*Cr*). At day 21 post infection, *E. coli* (*Ec*) or *B. thetaiotaomicron* (*Bt*) or *B. vulgatus* (*Bv*) were inoculated. As a second inoculation, a mixture of *Bt* and *Bv* was inoculated into the *Ec*-harboring group, and *Ec* was inoculated into the *Bacteroides*-harboring group, respectively. Pathogen load was determined in feces on the indicated days after inoculation of commensal bacteria. Dots represent individual mice from two independent experiments. (**B**) Total *Enterobacteria* (*Cr* and *Ec*) and *Bacteroides* culture in feces at day 0 and day 14 [first inoculation in (A)]. Data are means ± SD (n = 4). Results are representative of at least two experiments. (**C**) Number of *Cr* (kanamycin resistant) in the total *Enterobacteria* (without antibiotics) are indicated as a percentage. Data are means ± SD (n = 4). Results are representative of at least two experiments. (**D**) Carbohydrate catabolic profiles of *Cr* and commensal bacteria strains. Robust growth under supplementation of monosaccharides (MSs) or polysaccharides (PSs) indicated as red, and no growth indicated as yellow. Raw data are provided in table S1. (**E**) GF mice were infected with WT *Cr*. At day 21 post infection, *Bt* was inoculated. On day 7 post colonization with *Bt*, the mice were divided into two groups that were fed a conventional maintenance diet (C) or a simple sugar diet (ss). Pathogen load was determined in feces on day 3 after diet switching. Results are means ± SD of individual mice (initially n = 10, and divided into two groups n = 5 each). Results are representative of two experiments. (**F**) *Cr* monoassociated GF mice (day 21) were fed a simple sugar diet (ssDiet) for 7 days. Pathogen load was compared before and after switching to ssDiet from conventional diet (cDiet). Dots represent individual mice and are representative of three independent experiments $^{**}P < 0.01$. N.S. denotes not significant.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/science.1222195/DC1
Materials and Methods
Figs. S1 to S12
Table S1
References (*20*–*22*)

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Regulated Virulence Controls the Ability of a Pathogen to Compete with the Gut Microbiota

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Establishing an Enteric Infection

Complex and highly regulated interactions are required to keep the peace between the bacteria that reside in our gut and the immune system. How do pathogenic bacteria, such as the strains of *Escherichia coli* that cause gastroenteritis, get a foothold to establish an infection, and what is the role of resident bacteria in this process? **Kamada *et al.*** (p. 1325, published online 10 May; see the Perspective by **Sperandio**) infected mice orally with *Citrobacter rodentium* and found that mice with normal commensal microflora, which were better able to contain the infection than mice that lacked the commensals, which were not able to clear the infection.

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