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Commensal Bacteria Influence *Escherichia coli* O157:H7 Persistence and Shiga Toxin Production in the Mouse Intestine

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The presence of commensal flora reduced colonization of *Escherichia coli* O157:H7 and production of Shiga toxin (Stx) in the murine intestine. Stx production was not detected in mice colonized with *E. coli* that were resistant to the Shiga toxin phage, but it was detected in mice colonized with phage-susceptible *E. coli*.

Escherichia coli O157:H7 causes approximately 73,000 cases of food-borne illness each year in the United States (12). Diarrheal disease can progress to hemorrhagic colitis and, in about 10% of cases, hemolytic uremic syndrome develops. Shiga toxin (Stx), an AB_5 toxin, is a major virulence factor of *E. coli* O157:H7. The A-subunit has *N*-glycosidase activity and inhibits protein synthesis by cleaving a specific adenine in the host cell rRNA, ultimately leading to cell death (5). The A-subunit is delivered to target cells by the B-pentamer. Pathogenic strains of *E. coli* O157:H7 can produce one or both of two antigenically distinct forms of Stx, Stx1 and Stx2 (17).

Both Stx1 and Stx2 are encoded on distinct lambda-like phages lysogenized in the *E. coli* O157:H7 chromosome (9, 14, 18). The phage late gene promoter controls expression of Stx. As a consequence, the Stx genes are not expressed during lysogeny. However, when the phages enter the lytic cycle, the late gene promoter is activated, and new phage particles and Stx are produced and released from the cell by bacterial lysis (25). Under laboratory growth conditions, a few bacteria will spontaneously enter the lytic cycle, resulting in low levels of phage and Stx production. However, some conditions will induce lytic phage growth in the majority of the bacteria in the population. For example, treatment with antibiotics such as the quinolones can induce the phage lytic cycle in *E. coli* O157:H7 (11), resulting in high levels of Stx production both in vitro and in a mouse model of disease (10, 27). Furthermore, epidemiological studies suggest that antibiotic treatment can increase the risk of developing hemolytic uremic syndrome (26), likely due to higher levels of Stx production.

Lytic infection of commensal *E. coli* has been shown to influence Stx production. Incubation of phage-susceptible laboratory strains of *E. coli* with the Stx-encoding phage in vitro resulted in lytic phage growth and high levels of Stx in the culture supernatant (8). Similar results were seen in vivo. Mice were coinfected with an *E. coli* strain lysogenized with Stx phage and either a phage-susceptible or phage-resistant laboratory strain of *E. coli.* Higher levels of fecal Stx were recovered when mice were coinfected with the phage-sensitive strain (8).

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These studies suggest that human intestinal flora could also influence Stx production and, consequently, the severity of human disease. In a survey of human fecal isolates, about 10% of the *E. coli* strains were found to be susceptible to Stx phage (7). Interestingly, one fecal *E. coli* strain was found to actually neutralize the effects of Stx2 on Vero cells (6, 7). In this study, we modified the streptomycin-treated mouse model of *E. coli* O157:H7 infection (24) to assess the impact of nonpathogenic commensal *E. coli* on *E. coli* O157:H7 survival and, importantly, Stx production.

Streptomycin-treated mouse model of *E. coli* O157:H7 infection. The streptomycin-treated mouse model used was similar to that described previously (8). ECOR strains were obtained from the Michigan State University STEC Center ECOR collection (http://foodsafe.msu.edu/whittam/ecor/index.html). Spontaneous streptomycin-resistant mutants were selected for all strains (Table 1). Briefly, male 6-week-old CD-1 mice were given streptomycin in their drinking water to reduce the presence of facultative intestinal flora. Commensal *E. coli* was established by inoculating the mice with 10^9 phage-resistant, phage-susceptible, or toxin-neutralizing *E. coli* cells, and colonization was allowed to establish for 1 week. A control group did not receive commensal *E. coli*. The mice were challenged with 10^6 streptomycin-resistant *E. coli* O157:H7 strain 185 cells on day zero. All mice were housed separately. To determine the extent of bacterial survival starting on day zero, all the feces in the cage were collected daily, weighed, and diluted 1:5 (wt/vol) in phosphate-buffered saline. Bacterial load was determined by plating serial dilutions on MacConkey agar plates containing selective antibiotics. Persistent colonization of all *E. coli* strains had occurred as evidenced by recovery of the organisms for weeks after inoculation (data not shown).

A total of four trials were performed, two in each study. In the first study (trials 1 and 2), mice received *E. coli* O157:H7 and/or commensal *E. coli*. In the second study (trials 3 and 4), mice were treated with ciprofloxacin in addition to receiving the same strains as in the first study.

Influence of commensal *E. coli* on survival of *E. coli* O157:H7. In trial 1, replication of *E. coli* O157:H7 strain 185 occurred in the mice lacking commensal *E. coli*, and on days 1 to 3, about 10^8 to 10^9 CFU/g feces was recovered (Fig. 1). For comparison with the other mice in the same trial, these results are plotted on all three panels of Fig. 1 ("185 alone" data). The impact of commensal *E. coli* on survival of *E. coli* O157:H7 was

TABLE 1. Strains used in this study

Bacterial strain	Description[a]	Source or reference
E. coli O157:H7 strain 185	Clinical isolate PT-32 transformed with pBBR1MCS-2; *stx1*$^+$ *stx2*$^+$; Str^r Kan^r	8 and this study
Nonpathogenic *E. coli* strains[b]		
183-ϕS	*E. coli* strain ECOR-13 transformed with pBBR1MCS-5; Str^r Gent^r; amplifies Stx in vitro when infected with phage from 185	15 and this study
158-ϕR	*E. coli* strain ECOR-54 transformed with pBBR1MCS-1; Str^r Cm^r; resistant to phage from 185	15 and this study
160-TN	Human fecal *E. coli* isolate FI-29 transformed with pBBR322; Str^r Amp^r Tc^r; resistant to phage from 185; neutralizes Stx2 in vitro	6, 8, and this study
DH5α	K-12; colicin B sensitive	Protein Express
DM1187(pCLB1)	Constitutively expresses colicin B	4
Vibrio harveyi strains		
BB120	AI-1$^+$ AI-2$^+$	21
BB152	AI-1$^-$ AI-2$^+$	21
BB170	Sensor 1$^-$ sensor 2$^+$	21

[a] Str^r, streptomycin resistant; Kan^r, kanamycin resistant; Cm^r, chloramphenicol resistant; Gent^r, gentamicin resistant; Amp^r, ampicillin resistant; Tc^r, tetracycline resistant.
[b] ϕS, susceptible to phage infection; ϕR, resistant to phage infection; TN, Stx neutralizer.

examined in mice colonized with phage-resistant (Fig. 1A), phage-susceptible (Fig. 1B), or toxin-neutralizing (Fig. 1C) *E. coli*. All three strains had become established at similar levels, with about 10^7 to 10^8 CFU/g feces (Fig. 1, day 0). Infection with *E. coli* O157:H7 strain 185 did not affect survival of the phage-resistant strain, 158-ϕR (Fig. 1A, days 1 to 3); however, reduced survival of the phage-susceptible strain, 183-ϕS (Fig. 1B), and the Stx-neutralizing strain, 160-TN (Fig. 1C), was observed on day 3.

The presence of any strain of commensal *E. coli* reduced survival of *E. coli* O157:H7 (Fig. 1A to C) compared to the mice lacking commensal *E. coli* (Fig. 1A to C). For the phage-resistant strain, a statistically significant ($P < 0.05$) reduction in *E. coli* O157:H7 survival was seen on days 1 to 3 (Fig. 1A). For the phage-susceptible strain (Fig. 1B) and Stx-neutralizing strain (Fig. 1C), the difference was statistically significant ($P < 0.05$) only on days 1 and 2, corresponding to the times when the commensal bacteria were present in high numbers.

A second trial was performed, and very similar results were obtained (data not shown). The presence of any strain of commensal *E. coli* significantly reduced the ability of *E. coli* O157:H7 to survive in the mouse intestine. Interestingly, in trial 2 the phage-susceptible strain colonized very poorly; on day zero, only about 10^5 CFU/g of feces was recovered. However, even colonization at this low level reduced survival of *E. coli* O157:H7 by more than 100-fold.

Autoinducer production. *E. coli* can communicate in a cell-density-dependent manner through the process of quorum sensing (21, 22), mediated by the signaling molecules autoinducer 2 (AI-2) and AI-3. AI-3 has been shown to upregulate expression of the locus of enterocyte effacement genes for intimate attachment by *E. coli* O157:H7 (20), and production of AI-3 by commensal *E. coli* has been proposed to enhance intestinal colonization by *E. coli* O157:H7. Production of both AI-2 and AI-3 in *E. coli* requires the LuxS enzyme (20). We examined the ability of the *E. coli* strains used in this study to produce AI-2 as a marker for LuxS activity and production of AI-3. Bioluminescence by the *Vibrio harveyi* reporter strain BB170 (sensor 1$^-$ sensor 2$^+$) was used to measure AI-2 production as described previously (19, 21). Light production was measured using a Luminoskan Ascent luminometer (Labsystems, Franklin, MA). Culture supernatant from the *V. harveyi* control strains BB120 (AI-1$^+$ AI-2$^+$) and BB152 (AI-1$^-$ AI-2$^+$) induced a greater-than-10-fold increase in bioluminescence in *V. harveyi* reporter strain BB170, whereas supernatant from the negative control, *E. coli* DH5α, did not cause a substantial induction in bioluminescence (Table 2). The three nonpathogenic *E. coli* strains and the *E. coli* O157:H7 strain used in this study all produced AI-2 (Table 2), as evidenced by a nearly 10-fold increase in bioluminescence. These results suggest that the contribution of autoinducer produced by commensal *E. coli* on enhancing colonization of *E. coli* O157:H7 is negligible compared to the strong inhibitory effects exerted by commensal *E. coli.*

Colicin production. The ability of commensal *E. coli* to reduce survival of *E. coli* O157:H7 could be due to competition for nutrients or colonization sites. Alternatively, the commensal *E. coli* could actively kill the *E. coli* O157:H7 by production of toxins such as colicins. The strains were tested for colicin production. None of the nonpathogenic *E. coli* produced colicins active against the indicator strains of DH5α or *E. coli* O157:H7 strain 185. However, both DH5α and *E. coli* O157:H7 strain 185 were killed by colicin B, which was produced by strain DM1187(pCLB1) (4). These results suggest that competition with the commensal *E. coli*, not active bactericidal activity, was likely responsible for the reduced survival of *E. coli* O157:H7.

Influence of commensal *E. coli* on Stx production. Fecal Stx levels were measured by enzyme-linked immunosorbent assay according to the manufacturer's instructions (Premier EHEC ELISA; Meridian Bioscience, Inc., Cincinnati, OH). In both trials 1 and 2, Stx was frequently detected in the feces of mice lacking commensal *E. coli* (Fig. 2). Increased production of Stx in the absence of commensal *E. coli* is likely due to the higher intestinal survival levels of *E. coli* O157:H7 in the absence of commensal *E. coli*.

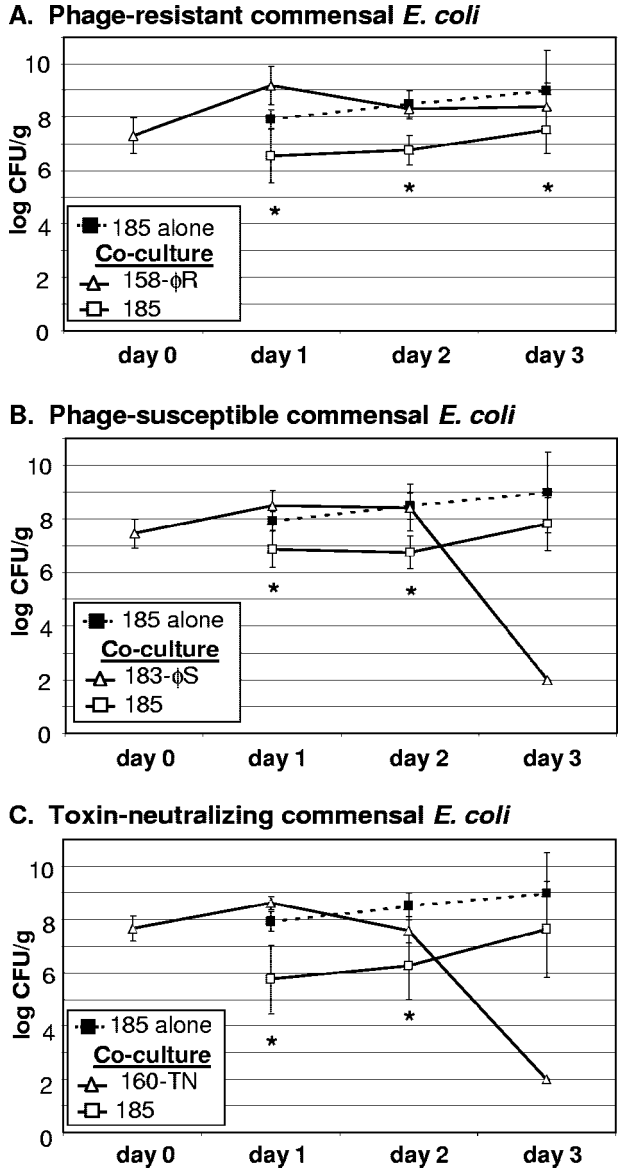

FIG. 1. Survival of *E. coli* O157:H7 and commensal *E. coli* in the murine intestine (trial 1). Fecal recovery of *E. coli* O157:H7 (closed squares) from mice lacking commensal *E. coli* challenged with 10^6 *E. coli* O157:H7 strain 185 cells is shown in all three panels. Additionally, in each panel fecal recovery of bacteria from mice inoculated with 10^9 commensal *E. coli* cells (open triangles) and challenged 1 week later with 10^6 *E. coli* O157:H7 strain 185 cells (open squares) is shown. An asterisk represents statistically significant ($P < 0.05$; Student's *t* test) differences in *E. coli* O157:H7 colonization when inoculated alone (closed squares) compared to when commensal *E. coli* strains were present (open squares). (A) Fecal bacterial recovery from mice inoculated with phage-resistant *E. coli* strain 158-φR and *E. coli* O157:H7 strain 185. (B) Fecal bacterial recovery from mice inoculated with phage-susceptible *E. coli* strain 183-φS and *E. coli* O157:H7 strain 185. (C) Fecal bacterial recovery from mice inoculated with Stx-neutralizing *E. coli* strain 160-TN and *E. coli* O157:H7 strain 185.

While the presence of any of the strains of commensal *E. coli* caused a similar reduction in survival of *E. coli* O157:H7 (Fig. 1), the different strains of commensal *E. coli* were observed to exert differential effects on Stx production. Fecal Stx was never recovered from mice colonized with the phage-resistant strain, 158-φR (Fig. 2A and B). On day 2 of trial 1, significantly less ($P < 0.05$) toxin was recovered from mice colonized with the phage-resistant strain, 158-φR, and the phage-resistant toxin-neutralizing strain, 160-TN (Fig. 2A), compared to mice lacking commensal *E. coli* (Fig. 2A). In contrast, Stx was recovered from mice colonized with the phage-susceptible strain (183-φS) on all 3 days. On day 2, the amount of Stx recovered from the mice colonized with the phage-susceptible strain was not statistically different from that in the mice lacking commensal *E. coli*. About 50-fold fewer *E. coli* O157:H7 microorganisms were recovered from mice colonized with the phage-susceptible *E. coli* compared to the mice lacking commensal *E. coli* (Fig. 1B), and the increased toxin production by fewer *E. coli* O157:H7 cells could be due to lytic infection of the phage-susceptible strain by the *E. coli* O157:H7 Stx-encoding phage.

Fecal toxin recovery from the second trial (Fig. 2B) was similar to that seen in the first trial. Stx was most commonly detected in the feces of mice lacking commensal *E. coli*. Similarly, Stx was rarely recovered from mice colonized with phage-resistant (158-φR) or phage-resistant, toxin-neutralizing (160-TN) *E. coli*. However, unlike trial 1, for the mice colonized with the phage-susceptible strain (183-φS), fecal Stx was detected only for one mouse on day 3. The phage-susceptible strain survived poorly in trial 2, with only about 10^5 CFU/g, compared to between 10^7 and 10^8 CFU/g for trial 1, and the failure to observe amplified Stx production could have been due to low numbers of phage-susceptible bacteria. However, as noted above, even this low colonization level significantly reduced survival of *E. coli* O157:H7.

Overall, in these two trials, Stx was detected in 17 of 36 fecal samples from mice lacking commensal *E. coli*. Stx was not detected in fecal samples from mice colonized with the phage-resistant strain, 158-φR. Stx was detected in 1 of 36 fecal samples from mice colonized with the phage-resistant, toxin-neutralizing strain, 160-TN. In contrast, Stx was detected in 8 of 36 stool samples for mice colonized with the phage-susceptible strain, 183-φS. Thus, the sensitivity or

TABLE 2. AI-2 production[a] by *E. coli*

Source of supernatant	Fold increase in light[b]
V. harveyi strains	
BB120 (AI-1$^+$ AI-2$^+$)	11.1
BB152 (AI-1$^-$ AI-2$^+$)	14.3
E. coli strains (non-O157:H7)	
DH5α	1.3
158-φR	14.4
160-TN	11.2
183-φS	8.1
E. coli O157:H7 strain 185	10.5

[a] AI-2 production in test strain supernatants was assessed by bioluminescence after incubation with the reporter strain, *V. harveyi* BB170 (sensor 1$^-$ sensor 2$^+$) for 4.5 hours. *V. harveyi* strains were grown in autoinducer bioassay medium. *E. coli* strains were grown in Luria-Bertani broth with 0.5% glucose.

[b] Values represent the fold increase in light produced by *V. harveyi* cells incubated with test strain supernatants over incubation with medium alone.

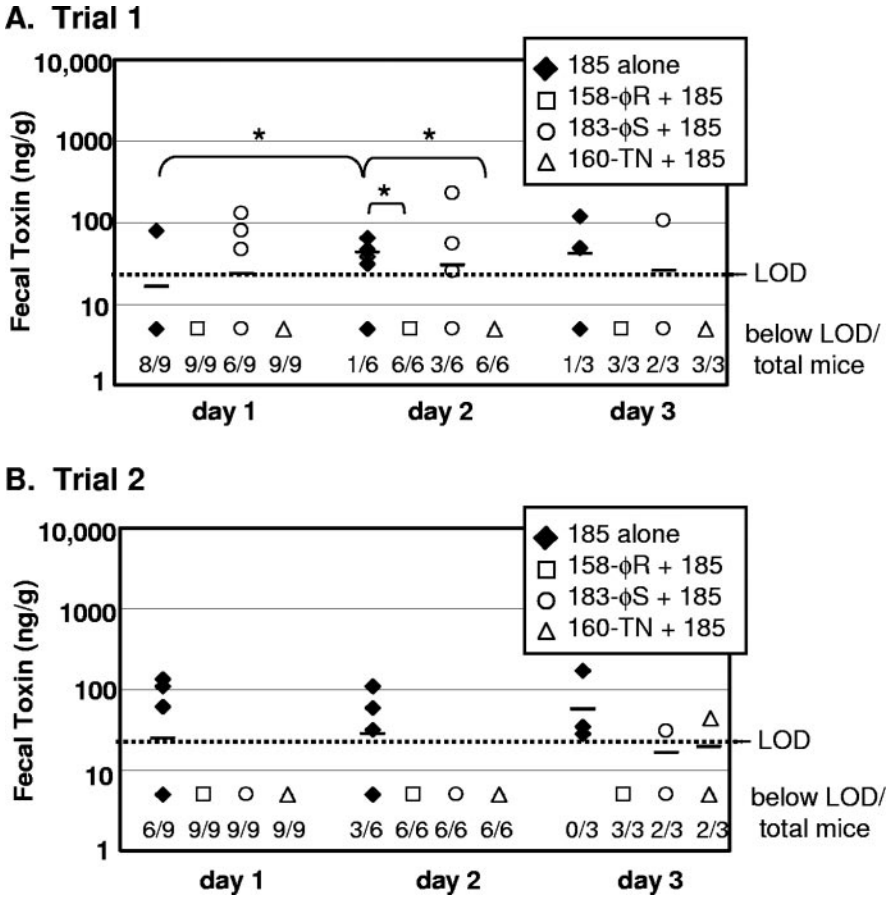

FIG. 2. Fecal toxin recovery from mice infected with *E. coli* O157:H7 in the presence or absence of commensal *E. coli* (trials 1 and 2). The limit of detection (LOD) of Stx, 25 ng/g feces, is indicated by the dotted line. Symbols above the dotted line represent fecal toxin recovery from a single mouse. A single symbol below the dotted line represents the mice lacking detectable Stx, and the numbers indicate the number of mice lacking fecal Stx divided by the total number of mice in the group. The geometric mean is indicated by the bar. An asterisk denotes statistical significance ($P < 0.05$; Student's t test) of Stx recovery between the groups indicated by the bracket. (A) Toxin recovery from trial 1, corresponding to colonization data in Fig. 1. (B) Toxin recovery from trial 2.

resistance to the Stx phage can influence Stx production in the intestinal environment.

Influence of ciprofloxacin on survival of *E. coli* O157:H7. Treatment with the antibiotic ciprofloxacin has been shown to induce the phage lytic cycle and result in increased Stx production in vitro and in vivo in mice infected with *E. coli* O157:H7 (27). We examined the influence of phage-susceptible and phage-resistant commensal *E. coli* on ciprofloxacin-induced Stx production. All of the strains used in this study were equally susceptible to ciprofloxacin (MIC, 0.015 μg/ml). Preliminary studies established that a 10-μg intraperitoneal dose of ciprofloxacin would allow the examination of the role of ciprofloxacin on phage induction with little effect on bacterial viability in vivo (data not shown). An experimental protocol similar to that described in trial 1 was used to study the effects of ciprofloxacin on *E. coli* O157:H7 survival and Stx production, except a single dose of 10 μg of ciprofloxacin was administered to mice (intraperitoneally) on day 2 post-*E. coli* O157:H7 infection.

In the first ciprofloxacin trial (trial 3), in the absence of commensal *E. coli*, *E. coli* O157:H7 strain 185 survived at very high levels, between 10^9 and 10^{10} CFU/g feces (Fig. 3). As observed in Fig. 1, the presence of any strain of commensal *E. coli* significantly reduced ($P < 0.05$) the survival of *E. coli* O157:H7 strain 185 (Fig. 3A to C). Recovery of all strains of *E. coli* was slightly reduced by ciprofloxacin treatment (Fig. 3, day 3). Results for the second ciprofloxacin trial (trial 4) were similar to those for trial 3 (data not shown).

Influence of ciprofloxacin on Stx production. As observed in trials 1 and 2, commensal *E. coli* affected Stx production in trials 3 and 4. Significantly more ($P < 0.05$) Stx was recovered from the feces of mice lacking commensal *E. coli* than from mice colonized with nonpathogenic *E. coli* on every day in both trials (Fig. 4). The impact of ciprofloxacin treatment can be seen on day 3. In trial 3 (Fig. 4A), in the mice lacking commensal *E. coli* a 60-fold increase in Stx was observed, with a mean value of 10,437 ng/g recovered on day 3 compared to a mean value of 175 ng/g on day 2, and this increase was statistically significant (Fig. 4).

Ciprofloxacin treatment had less of an impact on Stx production in mice colonized with commensal *E. coli* (Fig. 4). Stx was rarely detected in mice colonized with the phage-resistant *E. coli* strain, 158-φR, or the phage-resistant, toxin-neutralizing strain, 160-TN. In contrast, in trial 3 on day 3, fecal Stx was

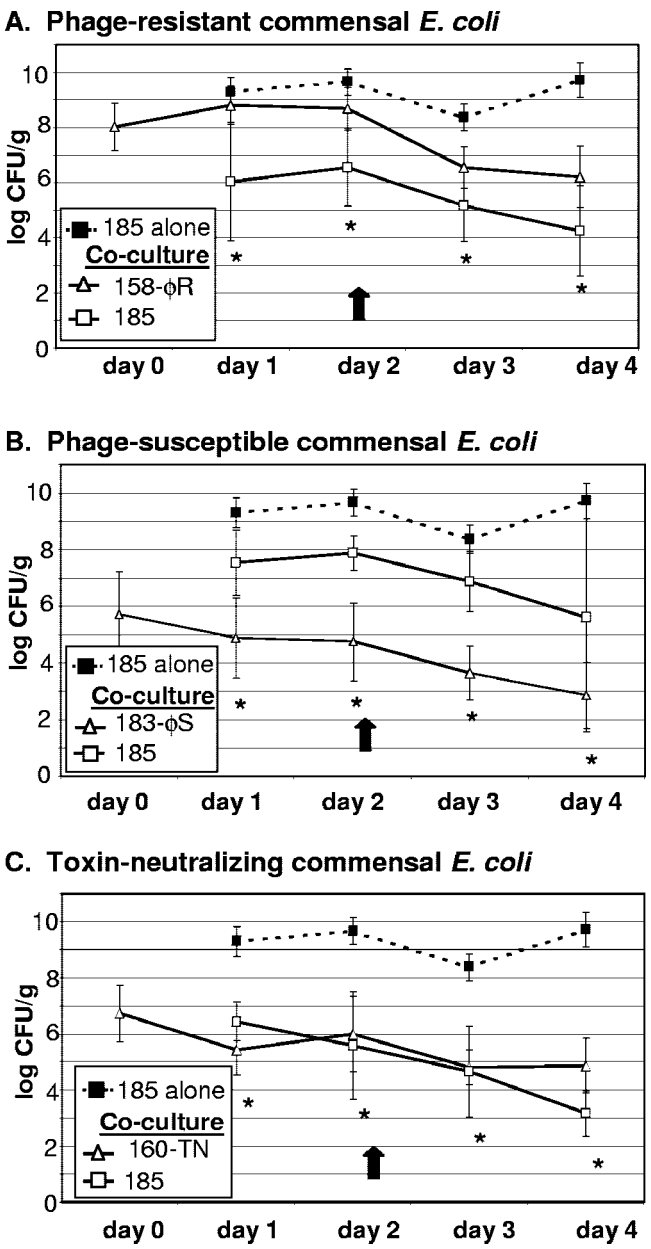

FIG. 3. Effect of ciprofloxacin on survival of *E. coli* O157:H7 and nonpathogenic *E. coli* in the murine intestine (trial 3). Mouse infection studies were performed as described for Fig. 1. On day 2, each mouse received 10 μg ciprofloxacin by intraperitoneal injection (black arrow). An asterisk represents a statistically significant ($P < 0.05$; Student's *t* test) difference in *E. coli* O157:H7 colonization when inoculated alone (closed squares) compared to when commensal *E. coli* strains were present (open squares).

detected in six of eight mice colonized with the phage-susceptible *E. coli* strain (183-φS), and the amount of Stx recovered on day 3 after ciprofloxacin treatment was significantly greater ($P < 0.05$) than that recovered before ciprofloxacin treatment on day 1 or 2 (Fig. 4). Furthermore, on day 3 the amount of toxin recovered from the mice colonized with the phage-susceptible strain was significantly ($P < 0.05$) greater than that recovered from mice colonized with either of the phage-resistant strains (Fig. 4). However, there was still significantly more ($P < 0.05$) Stx in the feces of mice lacking commensal *E. coli* compared to mice with phage-susceptible *E. coli.*

In trial 4 (Fig. 4B), the response to ciprofloxacin was not as dramatic as observed in trial 3. Fecal Stx recovery in the mice lacking commensal *E. coli* in trial 4 was higher after ciprofloxacin treatment than before ciprofloxacin treatment; however, it was not as high as observed in trial 3. Less fecal Stx was recovered from the mice with commensal *E. coli* compared to the mice lacking commensal *E. coli* on all days.

The intestinal environment is very complex, and many factors, including the presence of commensal bacteria, can influence the colonization of enteric pathogens such as *E. coli* O157:H7. The administration of nonpathogenic bacteria, or probiotics, into the intestine has been suggested as a method to prevent future colonization by enteropathogens and protect against disease (reviewed in reference 2), and bacterial species such as *Bifidobacteria breve* (1), *Clostridium butyricum* (23), and *Lactobacillus rhamnosus* (16) inhibit growth of *E. coli* O157:H7 in the intestine.

Nonpathogenic *E. coli* can also reduce colonization by *E. coli* O157:H7. Miranda et al. (13) demonstrated that a human K-12 *E. coli* isolate could out-compete *E. coli* O157:H7 strain EDL933 in the murine intestine, and they proposed that competition for glycolytic substrates was responsible. In this study, commensal *E. coli* did not out-compete *E. coli* O1457:H7, but it did cause a modest decrease in their numbers. Reduced recovery of the *E. coli* O157:H7 strain was not due to autoinducer or colicin production. In studies carried out to 18 days, the *E. coli* O157:H7 strain was still recovered at high levels (10^5 to 10^7 CFU/g feces) when commensal *E. coli* was present. The *E. coli* O157:H7 strain used in this study was a clinical isolate, and it may be particularly well adapted to survive in the intestine.

Commensal *E. coli* may not always exert a beneficial effect. While all of the strains of nonpathogenic *E. coli* characterized in this study had similar abilities to reduce colonization of *E. coli* O157:H7, their impacts on Stx production were not the same. Production of Stx was more commonly observed in mice colonized with phage-sensitive *E. coli* than in mice colonized with phage-resistant *E. coli*, suggesting that lytic infection of the phage-susceptible strain contributed to the Stx production. Phage-susceptible strains of *E. coli* are not uncommon; about 10% of human fecal *E. coli* isolates were found to be susceptible to lytic infection and produce Stx when incubated with the phage (7).

We also examined the influence of a toxin-neutralizing strain on Stx production in vivo. In previous studies, an *E. coli* isolate from a healthy child was shown to neutralize the cytotoxic effects of Stx2 on Vero cells, but it did not neutralize the related toxin, Stx1 (6). Stx1 and Stx2 share 55% amino acid homology, and strains of *E. coli* O157:H7 can produce Stx1, Stx2, or both (17). However, the ability to produce Stx2 has been associated with progression to severe disease, including hemolytic uremic syndrome (3). Since Stx2 is more toxic than Stx1, the toxin-neutralizing strain could be useful for preventing severe disease in humans. Both 160-TN, the toxin neutralizing strain, and 158-φR, the non-toxin-neutralizing strain, are resistant to the phage. A detectable level of Stx was rarely observed in mice colonized with either phage-resistant strain, and it was not possible to assess if toxin neutralization was

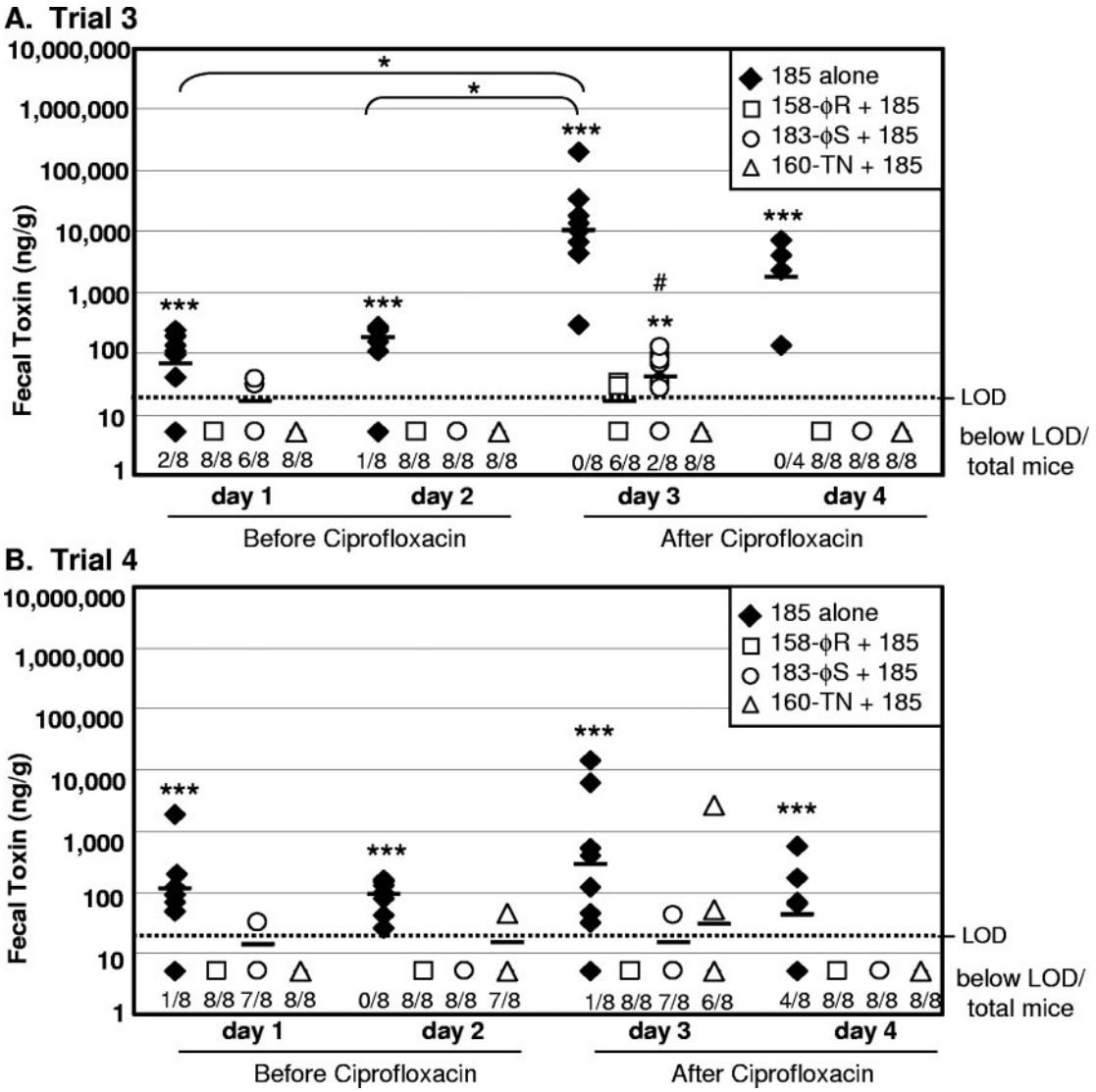

FIG. 4. Effect of ciprofloxacin treatment on fecal toxin recovery from mice infected with *E. coli* O157:H7 in the presence or absence of commensal *E. coli* (trials 3 and 4). Mice were inoculated with nonpathogenic *E. coli* and/or *E. coli* O157:H7, and on day 2, each mouse received 10 μg ciprofloxacin by intraperitoneal injection. Data represent fecal toxin recovery as described in the legend for Fig. 2. The geometric mean is indicated by the bar. The limit of detection (LOD) was 25 ng/g feces (dotted line). A single asterisk denotes statistical significance ($P < 0.05$; Student's *t* test) in the strain 185 group between the days indicated by the bracket. A triple asterisk indicates significantly more ($P < 0.05$; Student's *t* test) Stx was recovered from mice infected with strain 185 in the absence of commensal *E. coli* compared to the mice colonized with any of the three commensal strains of *E. coli* on the same day. A double asterisk denotes statistically significant ($P < 0.05$; Student's *t* test) Stx recovery from mice colonized with the phage-sensitive strain (183-φS) compared to mice colonized with either the phage-resistant strain (158-φR) or the phage-resistant, toxin-neutralizing strain (160-TN) on the same day. A pound sign denotes a statistically significant ($P < 0.05$; Student's *t* test) increase in Stx in the 183-φS plus 185 group on day 3 compared to toxin recovery in that group for days 1, 2, and 4. (A) Toxin recovery from trial 3, corresponding to colonization data in Fig. 3; (B) toxin recovery from trial 4.

occurring. The mice were treated with ciprofloxacin to increase Stx production; however, even after ciprofloxacin treatment, very little Stx was recovered from mice colonized with either phage-resistant strain. These studies suggest that phage resistance may be more important than toxin neutralization.

The intestinal environment cannot be replicated in vitro, and animal studies are absolutely essential for understanding human disease. Overall, we observed a tremendous variability with respect to colonization by the nonpathogenic *E. coli* and pathogenic *E. coli* O157:H7 and an even greater variability with respect to production of Stx. Fecal Stx levels ranged from below the limit of detection (25 ng/g) to about 1,000 ng/g for untreated mice, and in one case, treatment with ciprofloxacin resulted in over 200,000 ng/g. The composition of the intestinal bacteria exerts a strong influence on production of Stx, and we have shown that even low numbers of commensal *E. coli* can substantially reduce colonization by *E. coli* O157:H7. While we verified that streptomycin treatment eliminated endogenous *E. coli* as evidenced by lack of bacterial growth on MacConkey agar plates, streptomycin-resistant microbes such as bacterial

anaerobes or even fungal species could be present and could also influence colonization by *E. coli* and Stx production.

Epidemiologic studies strongly suggest that the amount of Stx produced in the intestine during an *E. coli* O157:H7 infection is important in determining the severity of disease (26). The health implications of an extremely high toxin burden are profound and may determine whether infection by *E. coli* O157:H7 will lead to self-limiting diarrheal disease or life-threatening complications, such as hemolytic uremic syndrome. Understanding the complex factors that influence Stx production could hold the key to treating this important disease.

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