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Inhibition of *Escherichia coli* Translocation from the Gastrointestinal Tract by Normal Cecal Flora in Gnotobiotic or Antibiotic-Decontaminated Mice

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Escherichia coli C25 maintained population levels of 10^9 to 10^{10} per g of cecum and translocated to 100% of the middle mesenteric lymph nodes in gnotobiotic mice monoassociated with *E. coli* C25. Intragastric inoculation of these mice with the cecal contents from specific-pathogen-free mice reduced the population levels of *E. coli* C25 to 10^6 per g of cecum and completely inhibited translocation to the mesenteric lymph nodes. Intragastric inoculation with heat-treated, Formalin-treated, or filtered cecal contents did not reduce the population levels of *E. coli* C25 or reduce the incidence of translocation of *E. coli* C25 to the mesenteric lymph nodes. Thus, viable bacteria apparently are required in the cecal contents inocula to reduce the population levels and the incidence of translocation of *E. coli* C25. Treatment with streptomycin plus bacitracin decreased the anaerobic bacterial levels in these gnotobiotic mice, allowing increased population levels of *E. coli* C25 and increased translocation to the mesenteric lymph nodes. *E. coli* C25 also translocated to the mesenteric lymph nodes of specific-pathogen-free mice treated with streptomycin and bacitracin before colonization with *E. coli* C25. The high cecal population levels of *E. coli* C25 in these antibiotic-decontaminated specific-pathogen-free mice apparently overwhelm any barrier to translocation exerted by the immunologically developed lamina propria of the specific-pathogen-free mice. Inoculation of gnotobiotic mice with a cecal flora also reduced the population levels of an indigenous strain of *E. coli*, with a concomitant inhibition of translocation of the indigenous *E. coli* to the mesenteric lymph nodes. Thus, bacterial antagonism of the gastrointestinal population levels of certain indigenous bacteria, such as *E. coli*, by other members of the normal bacterial flora appears to be an important defense mechanism confining bacteria to the gastrointestinal tract.

Bacterial translocation is defined as the passage of viable bacteria from the gastrointestinal tract through the epithelial mucosa to the mesenteric lymph nodes and possibly other organs such as the spleen and liver (4). Berg and Garlington (4) reported that viable bacteria of the indigenous gastrointestinal flora could not be cultured from the mesenteric lymph nodes, spleens, or livers of specific-pathogen-free (SPF) mice. Certain types of these indigenous bacteria, predominantly *Escherichia coli* and *Lactobacillus acidophilus*, are cultured, however, from these organs of gnotobiotic mice inoculated intragastrically with the whole cecal microflora from SPF mice. No viable bacteria are cultured from these organs of control SPF mice also inoculated intragastrically with a whole cecal flora. Indigenous *E. coli* is cultured from the mesenteric lymph nodes of 96% of gnotobiotic mice monoassociated with this organism, but in none of the mesenteric lymph nodes of control SPF mice also inoculated with this *E. coli* strain (4). Thus, there are defense mechanisms active in adult SPF mice that inhibit certain indigenous microbes from translocating from the gastrointestinal tract to the mesenteric lymph nodes, spleens, or livers, whereas these mechanisms are either absent or reduced in gnotobiotic mice colonized with these bacteria.

One of the defense mechanisms that appears to inhibit the translocation of certain viable bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs of mice is the control of certain bacterial population levels in the gastrointestinal tract by the presence of an antagonistic normal flora. Berg and Owens (5) compared the incidence of translocation of viable *E. coli* C25 from the gastrointestinal tract to the mesenteric lymph nodes of gnotobiotic mice colonized with *E. coli* C25 with the incidence of translocation in gnotobiotic mice colonized with *E. coli* C25 plus the whole cecal flora

from SPF mice. *E. coli* C25 maintains population levels of 10^9 to 10^{10} per g of cecum in the monoassociated gnotobiotic mice with 100% incidence of translocation to the mesenteric lymph nodes. The population levels of *E. coli* C25 are reduced drastically to 10^6 to 10^7 per g of cecum in gnotobiotic mice associated with both *E. coli* C25 and the cecal flora from SPF mice. As the population levels of *E. coli* C25 decrease due to the action of the antagonistic cecal flora, the incidence of translocation of viable *E. coli* C25 to the mesenteric lymph nodes also decreases from 100 to 0%. A decrease in the numbers of viable *E. coli* C25 per mesenteric lymph node accompanies this decrease in cecal population levels of *E. coli* C25. Thus, the unusually high population levels of *E. coli* C25 in the gastrointestinal tracts of monoassociated gnotobiotic mice appear to promote translocation of viable *E. coli* C25 to the mesenteric lymph nodes. Bacterial antagonism of *E. coli* population levels in the gastrointestinal tracts of conventional mice, therefore, could be an important defense mechanism confining these *E. coli* to the gastrointestinal tract.

These experiments strongly suggest that bacterial antagonism of *E. coli* gastrointestinal populations inhibits the translocation of viable *E. coli* to the mesenteric lymph nodes. There are possible mechanisms other than bacterial antagonism, however, that might account for the reduction in translocation of *E. coli* C25 in these gnotobiotic mice inoculated with *E. coli* C25 plus the cecal contents. For example, a biochemical factor in the cecal flora inoculum, other than viable antagonistic bacteria, might inhibit the in vivo growth of *E. coli* C25, thereby reducing its gastrointestinal population levels and, subsequently, the incidence of translocation to the mesenteric lymph nodes. Germfree mice possess a much "thinner" lamina propria, with one-tenth the number of immunoglobulin A-producing plasma cells when compared with conventional mice (8). Therefore, translocation of *E. coli* C25 might occur in gnotobiotic mice monoassociated with *E. coli* C25, but not in those colonized with *E. coli* C25 plus the antagonistic cecal flora, because antigens of the complex cecal flora stimulate an increase in the numbers of lymphocytes in the lamina propria. A thicker lamina propria with greater numbers of lymphocytes might present a more efficient "anatomical barrier" as well as a more efficient "immune barrier" to bacterial translocation from the gastrointestinal tract. Streptomycin-resistant bacterial mutants may not colonize gnotobiotic mice as effectively as the streptomycin-sensitive parental strain (2). Therefore, our demonstration of a decrease in the gastrointestinal population levels and a reduction in the translocation of streptomycin-resistant *E. coli* C25 after colonization of gnotobiotic mice with a cecal flora might not be applicable to the streptomycin-sensitive *E. coli* strain indigenous to CD-1 SPF mice.

Experiments described in this paper demonstrate that: (i) viable bacteria, rather than a biochemical factor, in the cecal flora inoculum appear to reduce the *E. coli* population levels in gnotobiotic mice, with concomitant inhibition of translocation of these *E. coli* to the mesenteric lymph nodes; (ii) a cecal flora inoculum also can inhibit the population levels of a streptomycin-sensitive strain of indigenous *E. coli* in gnotobiotic mice, with concomitant reduction in the incidence of translocation of this indigenous *E. coli* to the mesenteric lymph nodes; and (iii) high population levels of *E. coli* in the ceca can overwhelm any possible immunological or anatomical barrier to translocation of *E. coli* to the mesenteric lymph nodes in SPF mice.

MATERIALS AND METHODS

Animals. SPF (CD-1) mice were purchased from Charles River Breeding Laboratories, Wilmington, Mass. The mice were housed under barrier-sustained conditions with automatically controlled temperature, humidity, and light conditions. The SPF mice were kept in autoclaved polypropylene cages (Maryland Plastics, New York, N.Y.) with stainless-steel lids covered with individual filter tops (Scientific Products, Inc., Grand Prairie, Tex.). The mice were maintained with Purina Laboratory Chow (Ralston Purina Co., Inc., St. Louis, Mo.) and acidified water (0.001 N HCl) given ad libitum. Bedding consisted of San-I-Cel laboratory animal bedding (Paxton Processing Co., Inc., Paxton, Ill.).

Germfree and gnotobiotic mice (CD-1 strain, Charles River Breeding Laboratories) were housed in autoclaved polypropylene cages with stainless-steel wire lids inside Trexler-type flexible vinyl isolators (Germfree Supply Division, Standard Safety Equipment Co., Palatine, Ill.) sterilized with 2% peracetic acid (FMC Corp., Buffalo, N.Y.) containing 0.1% Bio-Soft-N-300 (TEA linear alkylate sulfonate, 60% active; Stepan Chemical Co., Northfield, Ill.). The mice were fed autoclavable Purina Laboratory Chow 5010 (Ralston Purina Co.). Their cages contained San-I-Cel laboratory animal bedding. The food, water, and bedding were vacuum sterilized in a bulk sterilizer chamber (Hoeltge, Inc., Cincinnati, Ohio) in an automatic sterilizer (American Sterilizer Co., Erie, Pa.) adapted with a vaccum pump to deliver a 28-in. (ca. 70-cm) vacuum cycle.

Bacteria. An indigenous *E. coli* strain was isolated from the mesenteric lymph nodes of gnotobiotic mice inoculated intragastrically with a suspension of cecal contents from SPF CD-1 mice as previously described (3). Nonindigenous streptomycin-resistant *E. coli* C25 was isolated originally from the feces of a healthy human (11, 17). The *E. coli* strains were cultured overnight in brain heart infusion (Difco Laboratories, Detroit, Mich.), centrifuged, and suspended to the

desired concentrations in sterile normal saline. The bacterial suspensions were transferred to glass tubes, and the outsides were sterilized with 2% peracetic acid for transfer into the vinyl germfree isolators. Germfree mice or antibiotic-treated SPF mice were inoculated with these *E. coli* strains by placing the viable cultures on their food and in their drinking water.

The mouse cecal microflora for inoculating gnotobiotic mice monoassociated with indigenous *E. coli* or *E. coli* C25, or antibiotic-treated SPF mice, was obtained from SPF CD-1 mice. Three SPF mice were killed by cervical dislocation and placed in an anaerobic glove box (Coy Manufacturing Co., Ann Arbor, Mich.) (1) maintained at less than 10 parts of oxygen per 10^6 parts of an atmosphere consisting of 5% carbon dioxide, 10% hydrogen, and 85% nitrogen. The oxygen level inside the anaerobic glove box was monitored daily with a Trace Oxygen Analyzer (Lockwood and McLorie, Inc., Horsham, Pa.). The mouse ceca were removed aseptically, cut into small pieces, and placed in 50 ml of sterile prereduced tryptic soy broth (Difco) prepared with 0.3 M phosphate buffer (pH 7.5) containing 0.05% dithiothreitol (Sigma Chemical Co., St. Louis, Mo.). The cecal suspensions were mixed vigorously on a Vortex mixer (Scientific Products, Grand Prairie, Tex.) and allowed to settle. The supernatant containing the cecal microflora was transferred inside the anaerobic glove box to sterile glass tubes and stoppered with sterile soft-rubber stoppers. The stoppered tubes containing the suspensions of cecal contents were then removed from the anaerobic glove box, the outsides were sterilized with peracetic acid, and the tubes were placed into the vinyl isolators containing the gnotobiotic mice monoassociated with *E. coli* C25 or indigenous *E. coli*. The mice were inoculated intragastrically with 0.5 ml of the cecal contents with 2.5-in. (ca. 6.35-cm), 22-gauge, stainless-steel feeding needles with 2-mm stainless-steel bulbs on their tips (Popper and Sons, Inc., New Hyde Park, N.Y.) as described previously (3).

Antibiotic-decontaminated SPF mice also were inoculated intragastrically with the cecal contents, using feeding needles. Antibiotic-decontaminated SPF mice were inoculated with *E. coli* C25 by placing viable cultures on their food and in their drinking water.

Treatment of cecal contents inocula. Three SPF mice were killed by cervical dislocation and placed in an anaerobic glove box. The cecal contents were removed from the mice in the anaerobic glove box, and a suspension was prepared in reduced tryptic soy broth as described above. Portions of the cecal contents suspension were removed from the anaerobic glove box and either heated at 65°C for 2 h or treated with 2.0% Formalin (J. T. Baker Chemical Co., Phillipsburg, N.J.) for 16 h followed by washing three times with sterile 0.15 M NaCl. Another portion of the cecal contents suspension was passed through a 0.22-μm filter (Gelman Instruments Co., Ann Arbor, Mich.). These treated cecal contents were all determined to be sterile by testing on blood agar (Difco) incubated aerobically and on enriched tryptic soy agar (Difco) incubated anaerobically. The treated cecal contents were placed in sterile glass tubes, stoppered with sterile soft-rubber stoppers, and transferred to germfree isolators as described above. The nontreated cecal contents were transferred to sterile glass tubes and stoppered inside the anaerobic chamber as already described.

Germfree mice were monoassociated with *E. coli* C25 1 week before inoculation with the cecal contents preparations. On day 0, four groups of these monoassociated gnotobiotic mice, each containing 10 mice, were inoculated intragastrically (0.5 ml) with filtered, heat-treated, Formalin-treated, or nontreated cecal contents. The three groups of gnotobiotic mice receiving treated cecal contents were intragastrically inoculated (0.5 ml) again 2 and 4 days later with the treated cecal contents. One week after the beginning of the cecal contents inoculations, all mice in the four groups were sacrificed and tested for translocation of viable *E. coli* C25 to the mesenteric lymph nodes. The numbers of viable *E. coli* C25 per gram of cecum also were determined.

Antibiotic treatment of SPF and gnotobiotic mice. SPF mice, 8 weeks old, were killed, and the number of anaerobic bacteria per gram of cecum was determined. The mice also were tested for viable *E. coli* C25 in their mesenteric lymph nodes. Other SPF mice of the same age were given drinking water containing 4 mg of streptomycin sulfate per ml (Pfizer, Inc., New York, N.Y.) and 4 mg of bacitracin per ml (The Upjohn Co., Kalamazoo, Mich.) for 4 days. The decontaminated SPF mice were transferred to sterile cages after the 4 days of antibiotic treatment. Fecal samples from these mice were cultured in the anaerobic glove box for strictly anaerobic bacteria by diluting in prereduced normal saline and plating on enriched tryptic soy agar containing 1 mg of polymyxin B (Pfizer) per ml to inhibit *E. coli* C25. Fecal samples also were cultured aerobically on blood agar (Difco) and Tergitol-7 agar (Difco) (6, 7). Gram stains of fecal samples taken after 4 days of antibiotic treatment showed very few bacteria. The total population levels of the bacteria cultured aerobically on blood agar and Tergitol-7 agar, and then cultured anaerobically on enriched tryptic soy agar, were less than 10 bacteria per g of feces.

On day 4, decontaminated SPF mice were tested for translocation of *E. coli* C25 to the mesenteric lymph nodes. The number of anaerobic bacteria per gram of cecum also was determined. The mice then were inoculated with streptomycin-resistant *E. coli* C25 by placing viable cultures on their food and in their drinking water. Antibiotic treatment was continued for 3 more days for a total of 7 days and then discontinued. Again, the mice were tested for translocation of *E. coli* C25 and cecal population levels of bacteria were determined. The decontaminated mice then were inoculated intragastrically with cecal contents from SPF mice as already described.

Gnotobiotic mice colonized with *E. coli* C25 plus a normal cecal flora from SPF mice were treated with streptomycin (4 mg/ml) and bacitracin (4 mg/ml) in sterile water. The outsides of sterile screw-top flasks containing the antibiotic solutions were sterilized with 2.0% peracetic acid and transferred into the vinyl isolators housing the gnotobiotic mice. The antibiotic solutions served as drinking water for 7 days.

Testing for translocation of *E. coli* strains. Mice were killed by cervical dislocation and placed in

the anaerobic glove box. Their abdomens were soaked wtih 70% alcohol, an incision was made through the skin with sterile scissors, and the skin covering the abdomen was reflected. An incision then was made through the peritoneum with another pair of sterile scissors. The abdominal wall was reflected, exposing the peritoneal cavity. The exposed viscera were swabbed with a sterile cotton-tipped applicator stick, which then was placed in a tube of sterile tryptic soy broth and incubated aerobically to test for any bacterial contamination of the viscera. The middle mesenteric lymph node was located in the mesentery of the ascending colon and excised with another set of sterile instruments. The middle mesenteric lymph node was placed in a sterile grinding tube containing 3.0 ml of brain heart infusion. The nodes were homogenized with Teflon grinders (Tri-R Instruments, Rockville Center, N.Y.) and removed from the anaerobic glove box. The lymph node homogenate was incubated at 37°C for 24 h and then Gram stained and cultured on Tergitol-7 agar containing streptomycin to detect *E. coli* C25 and on Tergitol-7 agar without streptomycin to detect the indigenous *E. coli*. Hypothetically, as few as one viable *E. coli* organism in the mesenteric lymph node will produce a positive culture after incubation utilizing these culturing procedures.

RESULTS

Translocation of viable *E. coli* C25 to the mesenteric lymph nodes in gnotobiotic mice colonized with *E. coli* C25 followed by inoculation with either nontreated cecal contents from SPF mice or cecal contents treated to remove viable bacteria. Germfree mice were colonized with streptomycin-resistant *E. coli* C25 for 1 week before oral inoculation with SPF mouse cecal contents passed through 0.22-μm filters, heated for 2 h at 65°C, or treated with 2.0% Formalin for 16 h. Control, gnotobiotic mice monoassociated with *E. coli* C25 received oral inoculations of nontreated cecal contents from SPF mice. The gnotobiotic mice receiving filtered, heat-treated, or Formalin-treated cecal contents exhibited approximately 10^9 viable *E. coli* C25 per g of cecum, and their mesenteric lymph nodes also contained viable *E. coli* C25 (Table 1). Conversely, only approximately 10^6 viable *E. coli* C25 per g of cecum were cultured from gnotobiotic mice receiving oral inoculations of nontreated cecal contents from SPF mice. The mesenteric lymph nodes from these latter gnotobiotic mice did not contain viable *E. coli* C25. Inhibition of the population levels or translocation of *E. coli* C25 did not appear to be due to some biochemical factor in the cecal contents inoculum other than viable bacteria. Thus, viable bacteria apparently are required in the cecal contents inoculum from SPF mice to reduce the gastrointestinal population levels of *E. coli* C25 and to reduce the incidence of translocation of *E. coli* C25 to the mesenteric lymph nodes.

TABLE 1. *Incidence of bacterial translocation and cecal population levels of E. coli C25 in gnotobiotic mice monoassociated with E. coli C25 and then inoculated with SPF cecal contents treated to remove viable bacteria*

Cecal contents	*E. coli* C25 per g of cecum[a]	Incidence of translocation to mesenteric lymph nodes[b]
Nontreated	1.6×10^6 (2.0×10^5–6.89×10^6)	0/10
Filtered[c]	7.28×10^9 (3.96×10^9–1.03×10^{12})	6/6
Heat treated[d]	8.38×10^9 (1.07×10^7–5.74×10^{12})	8/8
Formalin treated[e]	4.26×10^9 (1.85×10^9–7.86×10^9)	8/8

[a] Median numbers of *E. coli* C25 per gram of cecum; ranges in parentheses.

[b] Number of mesenteric lymph nodes containing viable *E. coli* C25 over number of mesenteric lymph nodes tested.

[c] Cecal contents passed through 0.22-μm filter.

[d] 65°C for 2 h.

[e] Treated with 2.0% Formalin for 16 h followed by washing three times with sterile 0.15 M NaCl.

Elimination by antibiotic treatment of the inhibitory effect of the cecal flora on translocation of *E. coli* C25 to the mesenteric lymph nodes in gnotobiotic mice. It was of interest to determine whether translocation of viable *E. coli* C25 to the mesenteric lymph nodes could occur again in gnotobiotic mice that had once received the cecal contents inoculation to inhibit cecal population levels and translocation of *E. coli* C25. It seemed possible that the bacterial antigens of the cecal contents inoculum might stimulate an increase in the number of lymphocytes in the lamina propria and thereby provide a more efficient immunological or anatomical barrier to bacterial translocation. Gnotobiotic mice monoassociated with *E. coli* C25 for 1 week exhibited very high gastrointestinal populations of *E. coli* C25 (10^{13} to 10^{14} per g of cecum), and all of their mesenteric lymph nodes contained viable *E. coli* C25 (Fig. 1). These monoassociated gnotobiotic mice then were inoculated intragastrically with cecal contents from SPF mice. When tested 1 week later, the numbers of anaerobic bacteria had increased to approximately 10^{10} per g of cecum and the *E. coli* C25 populations had decreased to approximately 10^5 to 10^6 per g of cecum. This decrease in cecal population levels of *E. coli* C25 was accompanied by a complete inhibition of translocation of viable *E. coli* C25 to the mesenteric lymph nodes. At this point, the gnotobiotic mice

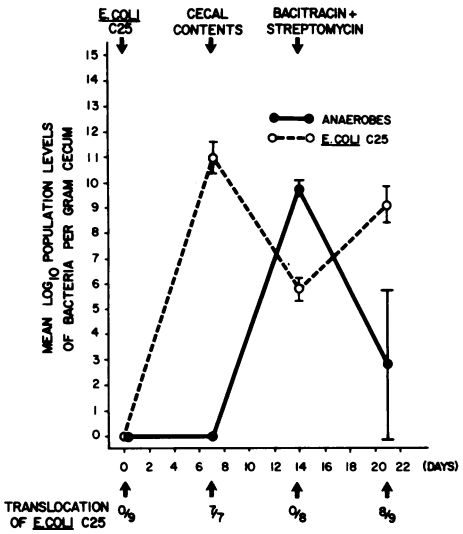

FIG. 1. *Oral antibiotic treatment of gnotobiotic mice removes the inhibition by the cecal flora of translocation of viable E. coli C25 to the mesenteric lymph nodes. The incidence of translocation of E. coli C25 is presented as the number of mesenteric lymph nodes with viable E. coli C25 over the number of mesenteric lymph nodes tested. One middle mesenteric lymph node is tested per mouse. The population levels of E. coli C25 and anaerobic bacteria are presented as the mean log_{10} bacteria per gram of cecum, with brackets denoting standard error; seven to nine mice were tested per point.*

colonized with *E. coli* C25 plus the antagonistic cecal flora were given bacitracin plus streptomycin in their drinking water. These antibiotic-treated gnotobiotic mice were tested 1 week later for bacterial population levels in their ceca and for bacterial translocation to their mesenteric lymph nodes. The numbers of anaerobic bacteria were reduced to 10^3 per g of cecum, whereas the *E. coli* C25 populations again increased to approximately 10^9 per g of cecum (Fig. 1). The incidence of translocation of viable *E. coli* C25 to the mesenteric lymph nodes also increased from zero of eight to eight of nine mice tested. Thus, the colonization of gnotobiotic mice for 1 week with a cecal bacterial flora did not stimulate a permanent barrier to the translocation of viable *E. coli* C25 to the mesenteric lymph nodes. The inhibition of translocation of *E. coli* C25 by an antagonistic flora in gnotobiotic mice is reversible when the antagonistic flora is removed by antibiotic treatment. These results lend further support to bacterial antagonism of the population levels of certain bacteria as a mechanism confining these bacteria to the gastrointestinal tract.

Promotion of translocation of *E. coli* C25 to the mesenteric lymph nodes by high cecal population levels of *E. coli* C25 in antibiotic-decontaminated SPF mice. One week of colonization of gnotobiotic mice with a cecal bacterial flora may not be enough time to stimulate an effective immunological or anatomical barrier to bacterial translocation. Consequently, these experiments were repeated in SPF mice to determine whether high cecal population levels of *E. coli* C25 would promote bacterial translocation even though the SPF mice had been exposed to a cecal flora since birth and exhibited a thick lamina propria compared with germfree mice. Adult SPF mice were given bacitracin plus streptomycin in their drinking water for 4 days to remove the antagonistic cecal flora (Fig. 2). The SPF mice exhibited nearly 10^{10} anaerobic bacteria per g of cecum before antibiotic treatment and no anaerobic bacteria after 4 days of antibiotic treatment. At this point the mice were inoculated with streptomycin-resistant *E. coli* C25, and the antibiotic treatment was continued for 3 more days for a total of 7 days. On day 7 of antibiotic treatment the mice were tested for cecal population levels of *E. coli* C25 and anaerobic bacteria and also for translocation of *E. coli* C25 to the mesenteric lymph nodes. The population levels of *E. coli* C25 increased to approximately 10^{11} per g of cecum as the anaerobic bacteria were eliminated by the antibiotic treat-

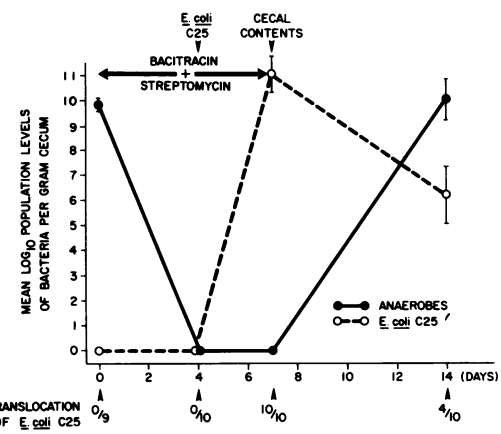

FIG. 2. *High population levels of E. coli C25 in the ceca of antibiotic-decontaminated SPF mice promote translocation of viable E. coli C25 to the mesenteric lymph nodes. The incidence of translocation of E. coli C25 is presented as the number of mesenteric lymph nodes with viable E. coli C25 over the number of mesenteric lymph nodes tested. One middle mesenteric lymph node is tested per mouse. The population levels of E. coli C25 and anaerobic bacteria are presented as the mean log_{10} bacteria per gram of cecum, with brackets denoting standard error; nine to ten mice were tested per point.*

ment. The increase in *E. coli* C25 cecal population levels promoted translocation of viable *E. coli* C25 to all of the mesenteric lymph nodes (10 of 10). The antibiotic treatment then was discontinued, and the SPF mice were inoculated intragastrically with a whole cecal flora. The mice were tested again 1 week later for translocation of *E. coli* C25, and the cecal population levels of the strictly anaerobic bacteria were determined. The numbers of anaerobic bacteria increased again to 10^{10} per g of cecum, and the levels of *E. coli* C25 decreased from 10^{11} per g of cecum to 10^6 to 10^7 per g of cecum (Fig. 2). Accompanying this decrease in cecal population levels of *E. coli* C25, the incidence of translocation of viable *E. coli* C25 to the mesenteric lymph nodes decreased from 10 of 10 to 4 of 10. Thus, bacterial antagonism of *E. coli* C25 by anaerobic bacteria with a concomitant decrease in the translocation of *E. coli* C25 to the mesenteric lymph nodes occurs in SPF mice as well as in gnotobiotic mice. The very high population levels of *E. coli* C25 in the ceca of these antibiotic-decontaminated SPF mice apparently overwhelm any immunological or anatomical barrier in SPF mice to translocation of *E. coli* C25 to the mesenteric lymph nodes.

Reduction in the gastrointestinal population levels of an indigenous *E. coli* strain by a cecal flora inoculum, with concomitant reduction in the incidence of translocation of this *E. coli* strain to the mesenteric lymph nodes. Germfree mice were colonized with an indigenous *E. coli* strain susceptible to streptomycin. After 1 week, one group of these gnotobiotic mice monoassociated with indigenous *E. coli* was inoculated intragastrically with the cecal contents from SPF mice. The other group of monoassociated gnotobiotic mice served as controls and was not inoculated with cecal contents. At various intervals from 1 to 14 days after the inoculation with cecal contents, both the experimental and the control gnotobiotic mice were tested for the translocation of viable indigenous *E. coli* to the mesenteric lymph nodes. The population levels of indigenous *E. coli* and the strictly anaerobic bacteria also were determined in both the ceca and ilea of these mice. The population levels of indigenous *E. coli* in the monoassociated gnotobiotic mice remained constant at 10^{10} to 10^{11} per g of cecum during the 1- to 14-day test intervals (Table 2). The population levels of indigenous *E. coli* in the ilea also remained fairly constant throughout the 14 days. Viable indigenous *E. coli* were detected in all of the mesenteric lymph nodes of these mice at all test intervals.

Different results were obtained from the gnotobiotic mice colonized with indigenous *E. coli* for 1 week before intragastric inoculation with the cecal contents (Table 2). The anaerobic bacteria of the cecal contents inoculum readily colonized the mice and stabilized at population levels of approximately 10^8 per g of ileum and

TABLE 2. *Decrease in the gastrointestinal population levels of indigenous E. coli by bacterial antagonism with concomitant reduction in the incidence of translocation of viable indigenous E. coli to the mesenteric lymph nodes of gnotobiotic mice*

Days after inoculation of gnotobiotic mice with cecal contents[a]	Gnotobiotic mice inoculated with only indigenous *E. coli*			Gnotobiotic mice inoculated with indigenous *E. coli* plus cecal contents from SPF mice				
	Population levels of indigenous *E. coli*		Incidence of translocation of indigenous *E. coli*	Population levels of anaerobic bacteria		Population levels of indigenous *E. coli*		Incidence of translocation of indigenous *E. coli*
	Ileum	Cecum		Ileum	Cecum	Ileum	Cecum	
1	8.5[b] (8.0–9.7)	10.8 (9.6–11.4)	5/5[c]	8.3 (8.0–8.6)	11.2 (10.5–12.5)	8.4 (8.0–8.6)	9.8 (9.6–9.9)	5/5
2	9.6 (8.6–10.1)	11.4 (10.1–12.3)	5/5	8.8 (8.4–9.4)	9.6 (8.6–10.3)	7.0 (6.7–7.1)	7.7 (7.2–8.3)	5/5
3	7.3 (6.3–8.1)	10.7 (9.6–11.2)	4/4	8.4 (8.2–8.7)	9.1 (8.6–9.7)	7.8 (6.9–8.3)	7.9 (7.3–8.4)	4/5
4	8.5 (7.9–9.5)	11.3 (10.9–11.9)	5/5	8.2 (7.9–8.4)	9.1 (8.6–10.2)	7.3 (6.6–8.2)	7.1 (6.4–8.3)	2/5
7	7.6 (7.1–8.3)	10.1 (9.9–10.2)	5/5	8.2 (7.7–8.5)	8.9 (8.2–9.8)	7.6 (7.2–7.9)	7.4 (7.2–7.6)	0/5
14	9.2 (8.8–10.4)	11.0 (10.3–11.7)	5/5	7.7 (7.4–8.0)	9.3 (8.8–10.5)	7.3 (6.3–8.0)	7.2 (6.6–7.6)	0/5

[a] Germfree mice were monoassociated with the indigenous *E. coli* strain for 1 week before inoculation with the cecal contents from SPF mice.

[b] Mean $\log_{10}$ viable bacteria per gram of tissue; ranges in parentheses.

[c] Number of mesenteric lymph nodes exhibiting viable indigenous *E. coli* compared with number of mice tested.

10^9 per g of cecum. The population levels of indigenous *E. coli* were reduced dramatically from nearly 10^{10} per g of cecum on day 1 after inoculation with cecal contents to 10^7 to 10^8 per g of cecum on day 2 after inoculation, where they remained throughout the experiment. The numbers of indigenous *E. coli* also were reduced in the ilea of these inoculated mice, but only approximately 10-fold compared with the >100-fold reduction in cecal population levels of *E. coli*. The decrease in gastrointestinal population levels of *E. coli* was followed by a reduction in the incidence of mesenteric lymph nodes exhibiting viable indigenous *E. coli*. The incidence of translocation of *E. coli* to the mesenteric lymph nodes was reduced from 100% positive (five of five) to 40% positive (two of five) by 4 days after inoculation with the cecal contents, and to 0% (zero of five) by 7 days after inoculation. Thus, the cecal contents inoculum decreased the gastrointestinal population levels of the indigenous *E. coli* strain and reduced from 100 to 0% the incidence of translocation of this *E. coli* strain to the mesenteric lymph nodes. Bacterial antagonism of the gastrointestinal population levels of indigenous *E. coli* by the normal bacterial flora appears to be an important defense mechanism confining these *E. coli* to the gastrointestinal tract.

DISCUSSION

Many bacteria, such as *E. coli*, maintain much greater population levels in the gastrointestinal tracts of monoassociated gnotobiotic mice than in those of conventional or SPF mice (6, 7, 13). Syed et al. (18) suggest that *E. coli* C25 population levels in the gastrointestinal tracts of mice are a function of *E. coli* C25 multiplication in the cecum and perhaps in the lower ileum. Freter and Abrams (13) reported that a collection of 95 anaerobic bacteria inoculated intragastrically into gnotobiotic mice monoassociated with *E. coli* C25 will reduce the *E. coli* C25 populations to near the normal population levels of *E. coli* strains in conventional mice. Furthermore, Freter (12) found that competition for substrates, inhibitory fatty acids at low pH, and a labile volatile inhibitor each could decrease the population levels of *E. coli* C25 under certain conditions in anaerobic continuous-flow cultures. Lee and Gemmell (15) suggest that certain volatile fatty acids, especially butyric acid, produced by anaerobic fusiform-shaped bacteria may inhibit the growth of coliforms, such as *E. coli*. Davis et al. (9), using immunofluorescence, observe that microcolonies of indigenous *E. coli* in the intestinal mucosal layer are displaced by fusiform-shaped anaerobes during the development of the normal gastrointestinal flora of infant mice. Consequently, in the experiments described here, it is probable that certain strictly anaerobic bacteria in the cecal contents inoculum from SPF mice antagonize the cecal population levels of *E. coli* C25. *E. coli* population levels are not inhibited nor is the incidence of translocation reduced in gnotobiotic mice receiving cecal contents inocula with viable bacteria removed by passage through 0.22-μm filters, by heating for 2 h at 65°C, or by treating with 2.0% Formalin for 16 h. However, the numbers of *E. coli* C25 are decreased from 10^9 to 10^6 per g of cecum, and translocation of *E. coli* C25 to the mesenteric lymph nodes is completely inhibited in gnotobiotic mice monoassociated with *E. coli* C25 followed by oral inoculation with nontreated cecal contents. Any biochemical factor in the cecal contents inoculum inhibiting population levels of *E. coli* C25 most likely would be inactivated by the heat or Formalin treatment. This biochemical factor, however, most likely would not be removed by filtering the cecal contents through 0.22-μm filters. Therefore, the reduction in cecal population levels of *E. coli* C25 with the concomitant inhibition of translocation of *E. coli* C25 to the mesenteric lymph nodes in gnotobiotic mice after inoculation with cecal contents appears to be due to the presence of viable bacteria of the cecal flora and not to an unknown biochemical factor.

It seemed possible that translocation of *E. coli* C25 might occur in gnotobiotic mice monoassociated with *E. coli* C25, but not in those colonized with *E. coli* C25 plus the cecal flora from SPF mice, because the cecal flora bacteria might induce an increase in the numbers of intraepithelial lymphocytes and lamina propria lymphocytes. Greater numbers of lymphocytes might provide a more efficient immunological barrier to translocation of viable bacteria across the lamina propria. The mesenteric lymph nodes also might be immunologically stimulated by antigens of the bacteria in the cecal inoculum and thereby inactivate foreign bacteria more efficiently. Our experiments showed that this was not the case. The inhibition of translocation of *E. coli* C25 by a cecal flora in gnotobiotic mice is reversible when the viable anaerobic bacteria are removed by treatment with bacitracin plus streptomycin. Thus, 1 week of colonization of gnotobiotic mice with a cecal flora does not stimulate an immunological barrier to translocation of *E. coli* C25 to the mesenteric lymph nodes. Furthermore, elimination of the normal gastrointestinal flora of SPF mice by bacitracin plus streptomycin followed by colonization with *E. coli* C25 allows *E. coli* C25 to attain popula-

tion levels of 10^{11} per g of cecum and to translocate to the mesenteric lymph nodes. Thus, exposure of SPF mice for 8 weeks of life to antigens of the gastrointestinal flora does not induce an immunological barrier to translocation of *E. coli* to the mesenteric lymph nodes if the *E. coli* attain high population levels in the gastrointestinal tracts of these mice. This does not mean that there is no immunological barrier to bacterial translocation from the gastrointestinal tract. The very high population levels of *E. coli* in the gastrointestinal tracts of these antibiotic-treated SPF mice, and the gnotobiotic mice in the earlier experiments, may overwhelm a possible immunological barrier to translocation.

Germfree mice possess only one-tenth the number of immunoglobulin A-producing plasma cells in their gastrointestinal mucosa when compared with the number in conventional mice (8). The underdeveloped immune system of gnotobiotic mice recently colonized with bacteria may allow certain indigenous bacteria to cross the epithelial mucosa and remain viable in the mesenteric lymph nodes and other organs. Owens and Berg (16) could culture viable bacteria from 50% of the mesenteric lymph nodes, spleens, livers, and kidneys of athymic (nu/nu) mice, whereas heterozygous (nu/+) mice exhibit viable bacteria in only 5.2% of these organs. Grafting thymuses from nu/+ mice to nu/nu mice reduces the incidence of bacterial translocation from the gastrointestinal tract from 50% in the athymic (nu/nu) mice to 7.8% in the thymus-grafted (nu/nu) mice. Furthermore, treatment of SPF mice with immunosuppressive drugs, such as cyclophosphamide, 5-fluorouracil, methotrexate, cytosine arabinoside, or prednisone promotes translocation of viable bacteria from the gastrointestinal tract to the mesenteric lymph node, spleen, liver, and kidney (unpublished data). Thus, the host's immune response appears to be one mechanism that inhibits the translocation of certain viable bacteria from the gastrointestinal tract to other organs.

The cecal wall and lamina propria of germfree mice is extremely thin compared with that of conventional mice (14). Colonization of gnotobiotic mice for only 1 week with a cecal flora most likely does not stimulate as thick a lamina propria as that found in conventional mice. Antibiotic treatment of SPF mice to remove the normal gastrointestinal flora, however, provides an SPF mouse model with a lamina propria similar to that of nontreated SPF mice. Streptomycin-resistant *E. coli* C25 attains population levels of 10^{11} per g of cecum and translocates to the mesenteric lymph nodes in these antibiotic-treated SPF mice. Thus, any inhibition to bacterial translocation exerted by a thicker lamina propria is overcome by the large numbers of *E. coli* C25 populating the gastrointestinal tracts of the antibiotic-treated SPF mice.

Bammann et al. (2) observed that streptomycin-resistant mutants of *Streptococcus mutans* do not colonize gnotobiotic or conventional rats as effectively as do the streptomycin-sensitive parental strains. Fitzgerald and Keyes (10) also found that erythromycin- or streptomycin-resistant mutants of streptococci inoculated into hamsters disappear gradually, whereas the parent strains persist in these animals. It is postulated that protein synthesis is altered in streptomycin-resistant mutants, resulting in bacterial cells with different rates of metabolism and growth, different surface properties, and even altered membrane permeabilities (2). Therefore, streptomycin-resistant *E. coli* C25 might be able to grow successfully in the gastrointestinal tracts of monoassociated gnotobiotic mice lacking a competitive normal bacterial flora, but not as successfully in gnotobiotic mice colonized by a whole cecal flora from SPF mice. In this case, streptomycin-resistant *E. coli* C25 would be uniquely sensitive to antagonism by the normal cecal flora. If so, then the experimental results demonstrating bacterial antagonism of the populations of *E. coli* C25 by a cecal bacterial flora with a concomitant reduction in translocation to the mesenteric lymph nodes may not be a defense mechanism applicable to the streptomycin-sensitive strains of *E. coli* indigenous to the SPF mouse. Our experiments, however, demonstrate that the cecal population levels of a streptomycin-sensitive strain of indigenous *E. coli* also are reduced by a cecal flora inoculum, with a concomitant inhibition of translocation of indigenous *E. coli* to the mesenteric lymph nodes of the gnotobiotic mice. Consequently, bacterial antagonism by members of the normal cecal flora appears to be an important defense mechanism confining certain bacteria, such as indigenous *E. coli*, to the gastrointestinal tract.

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