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Translocation of Certain Indigenous Bacteria from the Gastrointestinal Tract to the Mesenteric Lymph Nodes and Other Organs in a Gnotobiotic Mouse Model

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Viable bacteria were not cultured from the mesenteric lymph nodes, spleens, or livers of specific-pathogen-free (SPF) mice. Viable enteric bacteria, primarily indigenous *Escherichia coli* and lactobacilli, were present in the mesenteric lymph nodes of gnotobiotic mice inoculated intragastrically with the whole cecal microflora from SPF mice but not in the nodes of control SPF mice similarly inoculated. These indigenous *E. coli* also were cultured from the mesenteric lymph nodes of 96% of gnotobiotic mice monoassociated with *E. coli* but from none of the mesenteric lymph nodes of SPF mice inoculated with the *E. coli*. Furthermore, viable *E. coli* were detected in the mesenteric lymph nodes of these monoassociated gnotobiotes for as long as 112 days after inoculation. Indigenous *Lactobacillus acidophilus* also translocated to the mesenteric lymph nodes of gnotobiotic mice monoassociated with *L. acidophilus*. Apparently, there are mechanisms active in SPF mice inhibiting translocation of indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes, spleens, and livers, whereas these mechanisms are either absent or reduced in gnotobiotic mice. Indigenous *E. coli* maintained higher population levels in the gastrointestinal tracts of the gnotobiotes compared with their population levels in SPF mice, suggesting that high bacterial population levels might promote translocation of certain bacteria from the gastrointestinal lumen to the mesenteric lymph nodes. Gnotobiotic and SPF mice, therefore, provide experimental models for determining the nature of the mechanisms operating to confine indigenous bacteria to the gastrointestinal tract in normal, healthy animals.

The intestinal absorption of immunoglobulins and other proteins occurs in mammals for a short period after birth (3). Immunoglobulins and other macromolecules pass through the epithelial barrier of the newborn intestinal mucosa with relative ease. After a short period of time, however, this absorption suddenly ceases. In mice and rats this cessation occurs at approximately 17 to 21 days after birth (30). Thus, the intestinal epithelium of the adult mammal, unlike that of the newborn, has been regarded as an impenetrable barrier to the passage of macromolecules. Recent evidence, however, suggests that the intestinal epithelium of adult mammals is permeable to very small amounts of macromolecular substances (2, 16, 41-43).

Certain bacteria also have been found to pass through the intact intestinal epithelium of the adult mammal. Bacterial translocation is defined as the passage of viable bacteria from the gastrointestinal tract through the epithelial mucosa into the lamina propria and then to the mesenteric lymph nodes and possibly other organs. Pathogenic bacteria, such as certain *Salmonella* species, readily penetrate the gastrointestinal epithelium of mice and appear in the mesenteric lymph nodes (13, 35, 36). Nonindigenous *Escherichia coli* (18, 26, 38), *Klebsiella pneumoniae* (38), *Pseudomonas* (38), and *Clostridium perfringens* (12) also disseminate from the gastrointestinal tract to other organs in antibiotic-treated mice. Very little is known, however, concerning translocation from the gastrointestinal tract by bacteria comprising the indigenous flora. Schaedler et al. (32) could not detect viable indigenous lactobacilli, anaerobic streptococci, or bacteroides in the spleens, livers, or lungs of gnotobiotic mice allowed to ingest these normal flora bacteria. Berg and Savage (8), however, detected a viable indigenous *Bacteroides* species in the mesenteric lymph nodes of gnotobiotic mice monoassociated with this organism. Mae-

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jima and Tajima (27) detected viable bacteria of several types in the mesenteric lymph nodes, livers, lungs, and kidneys of gnotobiotic mice monoassociated with these various organisms. Hale and Hill (22) cultured lactobacilli from the livers of both conventional and specific-pathogen-free (SPF) mice, and Gordon et al. (21) detected enteric bacteria in the mesenteric lymph nodes of conventional mice. Neilsen et al. (28) in a more recent study did not detect viable bacteria in the livers of conventional mice. Thus, it has not been determined conclusively that viable bacteria of the normal flora translocate from the gastrointestinal lumen to other organs in healthy conventional mice or even in gnotobiotic mice colonized with particular microbes.

We examined the mesenteric lymph nodes, spleens, and livers of healthy SPF mice for the presence of viable indigenous bacteria. Germfree and SPF mice also were inoculated intragastrically with a whole cecal flora from SPF mice or with specific bacterial isolates and their mesenteric lymph nodes tested for the presence of viable bacteria. The results of these experiments are discussed in terms of the hypothetical mechanisms or barriers present in healthy conventional mice preventing bacterial translocation from the gastrointestinal tract. These mechanisms appear to be absent or reduced in gnotobiotic mice allowing bacterial translocation from the gastrointestinal tract.

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 polystyrene cages (Maryland Plastics, New York, N.Y.) with stainless-steel lids covered with individual filter tops (Econ-filter cover, Scientific Products, 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 polystyrene 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.). These mice were fed Purina Laboratory Chow 5010, autoclavable (Ralston Purina Co.), and used San-I-Cel laboratory animal bedding. The food, water, and bedding were vacuum sterilized in a bulk sterilizer chamber (Hoeltge, Inc., Cincinnati, Ohio) with a 28-inch (ca. 70.82 cm) vacuum cycle in an AMSCO automatic sterilizer adapted with a vacuum pump.

Bacteria. The mouse cecal microflora for inoculating CD-1 germfree and 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.) (3) maintained at less than 10 parts of oxygen per million 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 Trypticase soy broth (Difco Laboratories, Detroit, Mich.) prepared with 0.3 M phosphate buffer (pH 7.5) containing 0.05% dithiothreitol (Sigma Chemical Co., St. Louis, Mo.). The cecal suspension was mixed vigorously on a Vortex Genie Mixer (Scientific Products) 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 cecal suspensions then were removed from the anaerobic glove box, and the outsides were sterilized with peracetic acid and placed into the vinyl isolators containing germfree or SPF mice. Groups of mice were inoculated intragastrically with the cecal suspension using 2.5-inch, (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 previously described (6).

Indigenous *E. coli* and *Lactobacillus acidophilus* were isolated from the mesenteric lymph nodes of the gnotobiotic mice inoculated intragastrically with the cecal suspension from SPF mice. *E. coli* were isolated on selective Tergitol-7 agar (Difco Laboratories) (31) incubated aerobically at 37°C, and *L. acidophilus* were isolated on selective 10 A agar (31) incubated in 10% carbon dioxide at 37°C. *E. coli* was identified by using API E 20 identification strips (Analytab Products, Inc., Plainview, N.Y.) (33). *L. acidophilus* and other aerobic and facultatively anaerobic bacteria isolated from the mesenteric lymph nodes of gnotobiotic or SPF mice were identified by standard procedures (11, 33). *E. coli* or *L. acidophilus* were placed in sterile glass ampoules; the outsides of the ampoules were sterilized with 2% peracetic acid and added to the food pellets and drinking water of the germfree mice.

Indigenous *Bacteroides fragilis* were isolated from the cecal contents of an SPF mouse placed in the anaerobic glove box. The cecal contents were plated on prereduced enriched Trypticase soy agar containing 0.002% palladium chloride (3) incubated at 37°C in the anaerobic glove box. *B. fragilis* was identified by using the Minitek anaerobic system (Baltimore Biological Laboratories, Cockeysville, Md.) (23). Germfree mice were inoculated intragastrically with *B. fragilis* cultures prepared as described above for the cecal suspension inoculum.

Testing for translocation of anaerobic bacteria. Mice were killed by cervical dislocation and placed in the anaerobic glove box. Their abdomens were soaked with 70% alcohol; an incision was made through the skin by using 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 brain heart infusion broth (BHI) (Difco Laboratories) and incubated anaerobically to test for any bacterial contamination of the viscera. The data obtained from the few mice exhibiting bacterial contamination of the viscera were discarded. The middle mesenteric lymph node draining the jejunum, ileum, and cecum was located in the mesentery of the ascending colon and excised with another set of sterile instruments. The spleen, kidney, and liver then were excised by using a sterile set of instruments for each organ.

The organs were transferred in the anaerobic glove box to grinding tubes (Tri R Instruments, Rockville Center, N.Y.) containing prereduced, enriched Trypticase soy broth (3). The organs were homogenized with Teflon grinders (Tri R Instruments), and the grinding tubes containing the homogenates were incubated at 37°C for at least 3 days. After incubation, the organ homogenates were Gram stained, and 0.1 ml was spread with sterile glass L-rods onto enriched Trypticase soy agar plates able to support the growth of a wide variety of strictly anaerobic bacteria (3). These enriched Trypticase soy agar plates also were incubated in the anaerobic glove box at 37°C for at least 3 days. Any bacteria present in high numbers in the broth containing the organ homogenate but unable to grow on the enriched Trypticase soy agar plates should be seen in the Gram stain. Hypothetically, however, only one viable bacterium present in the organ homogenate should produce a positive culture after incubation by using these culturing procedures.

Testing for translocation of aerobic bacteria. Mice were killed, and their mesenteric lymph nodes, spleens, and livers were removed as described above. The organs were transferred to grinding tubes containing sterile BHI to detect aerobic bacteria or sterile 10 A broth to detect lactobacilli. The organs were homogenized, and the BHI tubes were incubated aerobically, whereas the 10 A broth tubes were incubated in 10% carbon dioxide at 37°C. After incubation, 0.1 ml of the organ homogenate was spread on blood agar plates to detect aerobic bacteria, Tergitol-7 agar plates to detect *E. coli*, or 10 A agar plates to detect lactobacilli. The organ homogenates also were Gram stained to confirm that bacteria present in the homogenates grew on the agar plates. The aerobic bacteria and lactobacilli were identified by API E 20 identification strips (33) and other standard procedures (11).

The numbers of viable *E. coli* were determined in the organs of gnotobiotic mice monoassociated with indigenous *E. coli*. The organs were removed aseptically and homogenized as previously described. Tenfold and one hundred-fold dilutions were made in tubes of sterile BHI. Portions (0.1 ml) of each dilution and 0.1 ml of the undiluted organ homogenate were spread on Tergitol-7 agar plates and incubated aerobically at 37°C. Population levels of *E. coli* were computed as $\log_{10}$ numbers of viable bacteria per organ. The remainder of the organ homogenate also was incubated, Gram stained, and spread on Tergitol-7 agar plates as described above.

Bacterial population levels in the gastrointestinal tract. Gnotobiotic mice were killed and placed in an anaerobic glove box, and the ilea and ceca were removed aseptically. The organs were homogenized in sterile grinding tubes containing prereduced, enriched Trypticase soy broth as described (3). Dilutions then were made in tubes of sterile normal saline, and 0.1 ml of the various dilutions and 0.1 ml of the remaining organ homogenate were plated on prereduced, enriched Trypticase soy agar. After at least 3 days of incubation, the population levels were determined as $\log_{10}$ numbers of viable anaerobes per organ.

The dilution tubes were removed from the anaerobic glove box, and 0.1 ml of each dilution was plated on blood agar, Tergitol-7 agar, or 10 A agar. The blood agar and Tergitol-7 agar plates were incubated aerobically at 37°C, and the 10 A agar plates were incubated in 10% carbon dioxide at 37°C.

In some experiments only indigenous *E. coli* or lactobacilli were quantitated in the gastrointestinal tracts of SPF or gnotobiotic mice. The mice were killed and the stomachs and ceca were removed aseptically. The stomachs and ceca from mice inoculated with lactobacilli were homogenized in grinding tubes containing sterile 10 A broth, and the ceca from mice inoculated with *E. coli* were homogenized in grinding tubes containing sterile BHI. Dilutions were made in normal saline, and 0.1 ml of each dilution was plated on either Tergitol-7 agar or 10 A agar. Tergitol-7 agar was incubated aerobically at 37°C, and 10 A agar was incubated in 10% carbon dioxide at 37°C. Population levels then were calculated as $\log_{10}$ numbers of viable bacteria per organ.

RESULTS

Lack of bacterial translocation in SPF mice. It is not known whether viable bacteria of the normal intestinal flora of SPF mice remain confined to the gastrointestinal tract or whether they also can be found in other organs of these mice. Consequently, adult SPF mice were killed by cervical dislocation and placed in an anaerobic glove box. The middle mesenteric lymph nodes draining the jejunum, ileum, cecum, and ascending colon were excised as soon as possible by sterile techniques (13). Histological examination of hematoxylin-and-eosin-stained frozen sections prepared from the relatively firm, pale yellow, nodular tissue removed from the mesentery of the ascending colon confirmed that this rounded, oblong body was the major mesenteric lymph node. Spleens and sections of the liver also were removed from these mice. The organs were homogenized in grinding tubes containing enriched Trypticase soy broth, and 0.1 ml of each homogenate was spread on enriched Trypticase soy agar plates. One-half of the remaining organ homogenate in enriched Trypticase soy broth also was incubated in the anaerobic glove box, Gram stained, and plated on enriched Trypticase soy agar to detect low num-

bers of anaerobes not detected on the initial organ plates. The remaining one-half of the organ homogenate was removed from the anaerobic glove box and 0.1 ml was plated on a variety of agar media supporting the growth of aerobic bacteria. The remaining organ homogenate also was incubated aerobically, Gram stained, and plated on the agar media. Viable bacteria were not detected in any of the bacterial cultures of the mesenteric lymph nodes, spleens, or livers from these mice (Table 1). Thus it appears that viable indigenous bacteria do not translocate through the mucosal barrier of the intestinal tract to these organs in healthy, adult SPF mice.

Bacterial translocation in gnotobiotic mice inoculated with a whole cecal microflora. Mechanisms appear to be active in adult SPF mice which prevent extralumenal translocation of the bacteria comprising their indigenous intestinal microflora. These inhibitory mechanisms may be absent or reduced in germfree mice for reasons described below. Therefore, germfree mice were inoculated intragastrically with bacterial suspensions prepared from the entire ceca of adult SPF mice to determine whether or not extralumenal bacterial translocation would occur in this animal model. The germfree mice were inoculated with the entire cecal flora since it was not known which bacterial species might be able to translocate from the gastrointestinal lumen. Elaborate precautions were taken to eliminate oxygen contamination of the cecal suspensions and thereby allow colonization of the mice with strictly anaerobic bacteria as well as other members of the indigenous flora. A control group of SPF mice also was inoculated with this cecal suspension, whereas another control group of SPF mice was not inoculated. The groups of SPF mice were housed throughout the experiment in germfree isolators similar to those housing the gnotobiotic mice. Mesenteric lymph nodes from mice of each group were cultured aerobically and anaerobically 7 days after the intragastric inoculations. The ceca and portions of the distal ilea from the gnotobiotic mice also were cultured on both enriched and selective media to confirm colonization by the various bacterial types and to determine their population levels. Translocation of indigenous bacteria from the gastrointestinal lumen to the mesenteric lymph node occurred in 11 of 17 gnotobiotic mice inoculated with the whole cecal flora (Table 1). *Lactobacillus* and *E. coli* were the predominant bacteria cultured from these mesenteric lymph nodes. Interestingly, no strictly anaerobic bacteria were detected in these mesenteric lymph nodes, even though these anaerobes were the most abundant bacterial type in the cecal suspension inoculum and colonized the ilea and ceca at high population levels (Table 2). Viable bacteria were not detected in the mesenteric lymph nodes of the unchallenged SPF mice nor in the mesenteric lymph nodes of the SPF mice intragastrically inoculated with the cecal flora.

Table 1. Bacterial translocation from the gastrointestinal tract to the mesenteric lymph nodes in gnotobiotic mice inoculated intragastrically with the whole cecal flora from SPF mice.

Mice	Intragastric* inoculation with normal cecal flora	Positive MLN** Number of mice	Bacterial type in positive MLN Number of MLN tested
Specific pathogen-free	–	0/15	None
	+	0/10	None
Gnotobiotes	+	11/17	8/17 *E. coli* 7/17 *Lactobacillus* 3/17 *S. faecalis* 1/17 *K. pneumoniae* 1/17 *P. mirabilis*

* Cecal flora inoculum prepared from the ceca of SPF mice in an anaerobic glove box.

** MLN indicates mesenteric lymph nodes. The MLN were tested 1 week after intragastric inoculation with the normal cecal flora. One MLN draining the cecum and ileum was tested per mouse.

Bacterial translocation in gnotobiotic mice monoassociated with certain indigenous bacteria. Germfree mice provide an animal model for demonstrating translocation of indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes. Viable bacteria, however, were not detected in all the mesenteric lymph nodes of gnotobiotic mice inoculated with the cecal suspension. Furthermore, primarily *E. coli* and *Lactobacillus* translocated to the mesenteric lymph nodes even though a broad spectrum of bacterial species was present in the cecal suspension inoculum and many of these bacteria reached high population levels in the gastrointestinal tracts of the gnotobiotes. Perhaps indigenous bacteria will translocate to the mesenteric lymph nodes of germfree mice more readily if the mice are inoculated with only one of these bacterial strains rather than the whole cecal flora. *E. coli*, for example, might reach higher population levels in gnotobiotic mice monoassociated with *E. coli* without an antagonistic normal flora than in these gnotobiotes given the entire cecal flora. Consequently, viable indigenous *E. coli* and *L. acidophilus* were isolated from the mesenteric lymph nodes of the gnotobiotic mice inoculated with the whole cecal microflora. Cultures of either *E. coli* or *L. acidophilus* then were inoculated onto the food and into the drinking water of groups of germfree and SPF mice. The mesenteric lymph nodes, spleens, and portions of the livers from mice of each group were cultured 3, 7, or 14 days after inoculations. No *E. coli* were detected in the mesenteric lymph nodes, spleens, or livers of the challenged SPF mice, whereas 96% of the mesenteric lymph nodes of the gnotobiotic mice monoassociated with *E.*

Table 2. Colonization of germfree mice with indigenous bacteria after intragastric inoculation of the whole cecal flora from SPF mice.

Bacterial types*	No. of viable** bacteria in cecal flora inoculum	Ileum		Cecum	
		3 days ***	7 days	3 days	7 days
Total anaerobes	7.9	9.5**** (8.4-10.0)	8.5 (7.9-9.0)	11.0 (10.7-11.3)	10.0 (8.6-10.4)
Total aerobes	5.3	8.9 (7.2-9.5)	8.5 (7.6-9.0)	9.3 (9.1-9.5)	8.6 (8.0-9.1)
E. coli	4.4	8.8 (6.7-9.5)	7.6 (5.0-8.1)	9.2 (9.1-9.5)	8.5 (7.0-9.1)
Lactobacillus	5.5	8.8 (7.7-9.2)	8.1 (7.4-8.4)	8.7 (8.5-8.7)	8.2 (7.6-8.7)

* Total anaerobes cultured on enriched Trypticase Soy agar, total aerobes cultured on blood agar, *E. coli* cultured on selective Tergitol-7 agar, and lactobacilli cultured on selective 10 A agar.

** Log_{10} numbers of viable bacteria in cecal flora inoculum.

*** Days after intragastric inoculation when mice were tested for colonization.

**** Mean log_{10} numbers of viable bacteria per gram of tissue cultured at 3 or 7 days following the intragastric inoculations. Ranges in parentheses; 5 mice per test.

coli contained viable *E. coli* (Table 3). Only 1 of 24 spleens and 3 of 24 livers from the gnotobiotic mice contained viable *E. coli*. Thus, indigenous *E. coli* translocated from the gastrointestinal tract more readily in monoassociated gnotobiotic mice than in gnotobiotes given the entire cecal flora in the preceding experiment. Furthermore, culturing the mesenteric lymph nodes appears to be a more sensitive method of detecting bacterial translocation within a few days after inoculation than is culturing either the livers or spleens.

L. acidophilus was detected 3 and 7 days after inoculation in all the mesenteric lymph nodes of the gnotobiotes monoassociated with *L. acidophilus* and in 5 of 8 mesenteric lymph nodes 14 days after inoculation. Thirty percent of the mesenteric lymph nodes of the SPF mice inoculated with *L. acidophilus* also contained *L. acidophilus*. Interestingly, a higher percentage of the spleens and livers of gnotobiotes monoassociated with *L. acidophilus* were positive than were positive in the gnotobiotes monoassociated with *E. coli*. *B. fragilis* alone would not colonize the germfree mice. Consequently, the germfree mice were inoculated with *E. coli* 1 week before inoculation with *B. fragilis*. Viable *E. coli* were detected in all the mesenteric lymph nodes of these diassociated gnotobiotic mice as expected, but, surprisingly, none of the mesenteric lymph nodes contained viable *B. fragilis*.

Quantitation of viable *E. coli* in mesenteric lymph nodes, spleens, and livers of gnotobiotes monoassociated with indigenous *E. coli*. Viable *E. coli* in the mesenteric lymph nodes, spleens, or livers of gnotobiotic mice monoassociated with *E. coli* were quantitated to determine whether or not there was a reduction in numbers of viable bacteria in these organs with increasing time after inoculation. The median numbers of viable *E. coli* in the mesenteric lymph nodes did not decrease with time after inoculation, although a few mice did exhibit greater numbers of viable *E. coli* in the mesenteric lymph nodes at 3 or 7 days after inoculation than at 14 days after inoculation (Table 4). These results also demonstrate that only low numbers of viable *E. coli* are present in mesenteric lymph nodes.

Population levels of indigenous bacteria in the gastrointestinal tract related to incidence of bacterial translocation. The population levels of *E. coli* and *L. acidophilus* also were determined in the gastrointestinal tracts of the same SPF and gnotobiotic mice that were tested for bacterial translocation reported in Table 3. Greater numbers of *E. coli* were present in the ceca of the gnotobiotes monoassociated with *E. coli* than in the ceca of SPF mice inoculated with *E. coli* (Table 5). This difference was not due to the greater cecal size of the gnotobiotes since there were approximately 10,000 times more *E. coli* in the ceca of gnotobiotes than in the ceca of the SPF mice. *Lactobacillus* population levels in either the stomachs or ceca of the inoculated SPF mice, however, were not much reduced compared with their population levels in the monoassociated gnotobiotes. Thus, the higher population levels of *E. coli* in the ceca of the monoassociated gnotobiotes compared with the inoculated SPF mice

Table 3. Comparisons between SPF and gnotobiotic mice tested for bacterial translocation from the gastrointestinal tract to the mesenteric lymph node, spleen, and liver.

Mice	Indigenous bacteria inoculated	Mesenteric lymph node				Spleen				Liver			
		3*	7	14	Total	3	7	14	Total	3	7	14	Total
Specific pathogen-free	*E. coli*	0/15**	0/15	0/10	0/40	0/10	0/10	0/10	0/30	0/10	0/10	0/10	0/30
Monoassociated gnotobiotes	*E. coli*	22/23	22/23	8/8	52/54	0/8	1/8	0/8	1/24	0/8	3/8	0/8	3/24
Specific pathogen-free	*L. acidophilus*	3/10	3/10	3/10	9/30	2/10	0/10	0/10	2/30	1/10	2/10	0/10	3/30
Monoassociated gnotobiotes	*L. acidophilus*	8/8	8/8	5/8	21/24	3/8	2/8	2/8	7/24	2/8	4/8	2/8	8/24
Diassociated gnotobiotes	*E. coli*	ND***	4/4	10/10	14/14	ND	1/5	2/11	3/16	ND	1/5	2/11	3/16
	and *B. fragilis*	ND	0/4	0/10	0/14	ND	1/5	1/11	2/16	ND	1/5	2/11	3/16

* Days following bacterial inoculation when organs were tested for viable bacteria.

** Number of positive organs per number of mice tested.

*** ND denotes not done.

might account for the 96% incidence of translocation of *E. coli* to the mesenteric lymph nodes of the gnotobiotes versus the 0% incidence of *E. coli* translocation in the SPF mice. Also, the nearly similar population levels of *Lactobacillus* in the ceca or stomachs of the gnotobiotic mice compared with the SPF mice may explain why the differences in the incidence of *Lactobacillus* translocation to the mesenteric lymph nodes between the SPF and gnotobiotic mice are not as dramatic as the differences in incidence of translocating *E. coli*.

Translocation of indigenous *E. coli* in gnotobiotic mice colonized with *E. coli* for an extended time. It was of interest to determine whether or not bacterial translocation from the gastrointestinal tract to these organs ceases after a certain period of time. Consequently, translocation of *E. coli* to the mesenteric lymph nodes, spleens, and livers of gnotobiotic mice monoassociated with *E. coli* was tested periodically for a total of 112 days after inoculations of the mice (Table 6). Viable *E. coli* were not detected in the livers by day 14 after inoculation. The numbers of positive spleens were too low to determine with certainty whether or not these organs also contained viable *E. coli* during this test period. Viable *E. coli*, however, were present in the mesenteric lymph nodes during the entire 112-day test period without causing any observable disease symptoms.

DISCUSSION

Viable bacteria were not cultured from the mesenteric lymph nodes, spleens, or livers of SPF mice. Viable bacteria were cultured, however, from the mesenteric lymph nodes, etc., of gnotobiotic mice inoculated intragastrically with

Table 4. Quantitation of viable indigenous *E. coli* in the mesenteric lymph nodes, spleens, and livers of gnotobiotic mice monoassociated with *E. coli*.

Days following inoculation of germfree mice	Mesenteric lymph node		Spleen		Liver	
	Positive MLN Number of mice	Viable *E. coli* per positive MLN	Positive spleens Number of mice	Viable *E. coli* per positive spleen	Positive livers Number of mice	Viable *E. coli* per positive liver
3	22/23	100 (100-3,700)	0/8	0	0/8	0
7	22/23	100 (100-3,700)	1/8	>560,000	3/8	1000 (100->560,000)
14	8/8	100 (<100-200)	0/8	0	0/8	0

* Median number of viable bacteria per organ; ranges in parentheses.

Table 5. Bacterial population levels in ceca and stomachs compared between SPF and gnotobiotic mice inoculated intragastrically with indigenous *E. coli* or *L. acidophilus*.

Mice	Indigenous* bacteria inoculated	Organ tested	Time after inoculation in days		
			3	7	14
Specific pathogen-free	*E. coli*	cecum	5.9 (5.4-6.1)	5.9 (5.6-6.2)	ND
Monoassociated gnotobiotes	*E. coli*	cecum	9.8 (9.3-10.3)	9.9 (9.2-10.3)	ND
Specific pathogen-free	*L. acidophilus*	stomach	8.0 (6.6-8.7)	6.6 (6.0-7.9)	7.8 (6.6-8.6)
Monoassociated gnotobiotes	*L. acidophilus*	stomach	7.5 (5.7-8.4)	8.8 (8.7-9.1)	8.4 (8.1-8.7)
Specific pathogen-free	*L. acidophilus*	cecum	7.8 (7.3-8.3)	7.8 (6.3-8.7)	8.1 (7.7-8.8)
Monoassociated gnotobiotes	*L. acidophilus*	cecum	8.7 (8.4-8.9)	8.4 (7.5-8.8)	8.3 (7.9-8.8)

* Mice inoculated by placing viable bacterial cultures on the food pellets and in the drinking water.

** Median $\log_{10}$ numbers of viable bacteria per organ; ranges in parentheses, 8-15 mice per test.

Table 6. Viable *E. coli* detected in the mesenteric lymph nodes for an extended time period following monoassociation of gnotobiotic mice with indigenous *E. coli*.

Organ tested	Colonization time in days						
	3	7	14	21	28	56	112
Mesenteric lymph node	14/15*	14/15	10/12	3/5	5/5	3/5	5/5
Spleen	0/8	1/8	1/12	0/5	2/5	1/5	0/5
Liver	0/8	3/8	0/12	ND	ND	ND	ND

* Number of positive organs over the number of mice tested.

the whole cecal flora from SPF mice. Furthermore, viable indigenous *E. coli* were detected in 96% of the mesenteric lymph nodes of gnotobiotic mice monoassociated with *E. coli*, and indigenous *L. acidophilus* in 88% of gnotobiotic mice monoassociated with *L. acidophilus*. There appear to be mechanisms active in the healthy SPF mouse which inhibit translocation of certain indigenous bacteria from the gastrointestinal tract to the spleens, livers, and especially the mesenteric lymph nodes, whereas these mechanisms are absent or reduced in gnotobiotic mice colonized with these organisms.

The anatomical, physiological, and immunological development of SPF mice is influenced profoundly by the presence of their indigenous gastrointestinal microflora. Germfree mice without indigenous gastrointestinal bacteria have not developed anatomically, physiologically, or immunologically to the degree of SPF mice. The intestinal lamina propria of SPF mice is densely infiltrated with lymphocytes and plasma cells (31). This infiltration has been termed "physiological inflammation." In fact, the morphology of the intestinal villi may be determined to a great extent by the presence of these lymphoid cells in the lamina propria. The intestinal lamina propria of germfree mice, on the other hand, contains very little lymphoid tissue (17). The lamina propria of germfree mice, or gnotobiotic mice associated for only a short time with certain bacteria, is not as thick as the lamina propria of SPF mice. Consequently, the lamina propria of these gnotobiotic mice may not be as effective a barrier to bacterial translocation as the lamina propria of SPF mice.

The indigenous bacteria present in the gastrointestinal tracts of SPF mice also have a direct impact on the host's immunological tissues. Crabbe et al. (17) demonstrated that the lamina propria of germfree mice contain only one-tenth as many plasma cells producing immunoglobulin A (IgA) as the lamina propria of conventional mice. Abrams (1) reported that germfree mice have fewer circulating polymorphonuclear leukocytes compared with conventional mice. Consequently, the undeveloped immune system of the germfree mouse may not respond to antigenic stimuli as quickly as the immune system of the conventional mouse and may not exert an inhibitory effect on bacterial translocation from the gastrointestinal tract to the mesenteric lymph nodes.

Oral immunization with certain macromolecules decreases their absorption by gastrointestinal tissue. Walker et al. (41, 42) demonstrated that the in vitro absorption of horseradish peroxidase or bovine serum albumin is inhibited selectively in everted gut sacs of rats by prior oral immunization with either of these antigens. Andre et al. (2) detected lower levels of human serum albumin in the blood of rats immunized intragastrically and then challenged intragastrically with human serum albumin than in nonimmunized rats challenged with it. Immunization also inhibits the translocation of certain pathogenic bacteria such as *Salmonella typhimurium* (39) and *S. enteritidis* (15). It is uncertain how local immune mechanisms protect the intestinal mucosa from invasion by pathogenic bacteria. Secretory immunoglobulin A specifically inhibits adherence of certain bacteria to epithelial cells, thereby preventing colonization of epithelial surfaces (20, 45). Local immune mechanisms, however, have not been shown to reduce gastrointestinal colonization by the indigenous bacteria. Nonetheless, the local immune response on mucosal surfaces and cell-mediated immunity in lymph nodes may be factors inhibiting translocation of certain indigenous bacteria from the gastrointestinal tract.

The population level obtained in the gastrointestinal tract by a particular bacterial species also may be a critical factor determining whether or not this bacterial species will translocate to other organs. Abrams (1) found that the number of viable *S. typhimurium* translocating to the mesenteric lymph nodes after intragastric inoculation of pathogenic *S. typhimurium* was approximately 400 times greater in gnotobiotic mice than in the conventional mice. The gnotobiotic mice contained approximately 10^9 *S. typhimurium* per g of small intestine, whereas conventional mice harbored only 10^4 *S. typhimurium* per g of small intestine. *S. typhimurium* reached population levels of 10^{11} bacteria/g of tissue in the ligated ilea of either gnotobiotic or conventional mice, however, and equal numbers of viable *S. typhimurium* translocated to the mesenteric lymph nodes of both the gnotobiotic and conventional mice. Thus, when *S. typhimurium* reached high population levels in the ilea of conventional mice, translocation to the mesenteric lymph nodes occurred as readily in these mice as in the gnotobiotic mice. In our study, population levels of indigenous *E. coli* in the ceca of the gnotobiotic mice monoassociated with *E. coli* were 10,000 times greater than the *E. coli* population levels in the ceca of SPF mice inoculated with this *E. coli*. Therefore, translocation of indigenous *E. coli* to the mesenteric lymph nodes also might not occur unless the *E. coli* reach a critical high population level in mouse gastrointestinal tracts.

No strictly anaerobic bacteria were cultured from the mesenteric lymph nodes of the gnoto-

biotic mice associated with the whole cecal flora from SPF mice even though these bacteria reached high population levels in the gastrointestinal tracts of these animals. However, possibly some of the strictly anaerobic bacteria present in the cecal suspension inoculum did not colonize the gnotobiotic mice. Also, the ileum may be the site at which bacteria translocate from the gastrointestinal tract, and the population levels of strict anaerobes were lower in the ilea than in the ceca of these animals. Indigenous *B. fragilis* also was not detected in the mesenteric lymph nodes of gnotobiotes diassociated with *B. fragilis* and *E. coli*. Phagocytosis and killing by polymorphonuclear leukocytes and macrophages are of primary importance in the host defense against facultatively anaerobic bacteria such as *E. coli* (44). Casciato et al. (14) and Bjornson et al. (9) also have demonstrated the in vitro killing of *B. fragilis* by human and rabbit leukocytes. There is very little information available, nonetheless, concerning in vivo humoral and cellular host defense mechanisms against the strictly anaerobic bacteria of the indigenous flora. Consequently, it is not known why indigenous *E. coli* and *Lactobacillus* readily translocated from the gastrointestinal tract to the mesenteric lymph nodes of the gnotobiotic mice in the experiments described, whereas indigenous *B. fragilis* did not. The population levels of *B. fragilis* were not determined. Consequently, *B. fragilis* may not have reached population levels in the ileum or cecum in concentrations high enough to promote translocation. The specific characteristics of each bacterial strain also are likely to determine to some degree whether or not a particular strain will translocate through the gastrointestinal mucosa and remain viable in the mesenteric lymph nodes.

Takeuchi (35, 36) and Staley et al. (34), using electron microscopy, have described translocation of *S. typhimurium* and pathogenic *E. coli* through epithelial cells of the gastrointestinal tract. It is likely that the bacteria in the lamina propria not engulfed by phagocytes enter the lymphatics, since the blood capillaries are reported to be impenetrable to particles the size of bacteria (25). Electron microscopic studies are now in progress to describe the translocation of the indigenous *E. coli* and *L. acidophilus* from the gastrointestinal lumen to the mesenteric lymph nodes. Bockman and Stevens (10) and Kagnoff (24) reported that specialized epithelial cells overlying Peyer's patches in mice appear to concentrate horseradish peroxidase and other antigens inoculated intragastrically. Carter and Collins (13) found that the ileal Peyer's patches of mice inoculated intragastrically with *S. enteritidis* appear to be collection points for translocating *S. enteritidis*, since more *S. enteritidis* were detected in Peyer's patches than in the surrounding gut wall. Consequently, we also are examining Peyer's patches of gnotobiotic mice monoassociated with indigenous *E. coli* or *L. acidophilus* to determine whether or not Peyer's patches are collection points during translocation of these bacteria.

Certain indigenous bacteria such as *E. coli* or *L. acidophilus* readily translocate from the gastrointestinal lumen to the mesenteric lymph nodes and other organs of gnotobiotic mice colonized with these bacteria. These organisms do not readily translocate to the mesenteric lymph nodes, spleens, or livers of SPF mice. Gnotobiotic and SPF mice colonized with various indigenous bacteria, therefore, provide animal models for determining the mechanisms inhibiting bacterial translocation from the gastrointestinal tract to other organs in SPF mice but allowing bacterial translocation in gnotobiotic mice.

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