INFECTION AND IMMUNITY, Mar. 1983, p. 1252-1259
0019-9567/83/031252-08$02.00/0

Vol. 39, No. 3

Relationship Between Cecal Population Levels of Indigenous Bacteria and Translocation to the Mesenteric Lymph Nodes

EARL K. STEFFEN[1] AND RODNEY D. BERG[2]*

Veterinary Medical Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri-Columbia, Columbia, Missouri 65211[1]; and Department of Microbiology and Immunology, Louisiana State University Medical Center, School of Medicine in Shreveport, Shreveport, Louisiana 71130[2]

Received 8 November 1982/Accepted 13 December 1982

Translocation is defined as the passage of viable bacteria from the gastrointestinal tract to the mesenteric lymph nodes (MLN) and other organs. The extent of translocation of certain indigenous, oxygen-tolerant bacteria from the cecum to the MLN, spleen, liver, kidney, and peritoneal cavity were determined in diassociated or triassociated gnotobiotic mice. Minimal bacterial translocation occurred to the spleen, liver, kidney, or peritoneal cavity. However, most bacterial strains readily translocated to the MLN. The percentage of the total population of each bacterial strain in the ceca was compared with the percentage of the total population of that strain in the MLN. There was a direct relationship between the numbers of a particular bacterial strain populating the ceca of diassociated or triassociated mice and the numbers of viable bacteria of this strain present in the MLN. Thus, the cecal population level of a particular bacterial strain determined the numbers of viable bacteria of this strain translocating to the MLN. The translocation of these bacterial strains from the gastrointestinal tract is an important first step in the pathogenesis of infection caused by members of the normal intestinal microflora.

Various terms have been used to describe the passage of substances from the gastrointestinal (GI) tract across the intestinal epithelium to the bloodstream and other organs. Persorption (24) has been used to describe the passage of large solid particles across the intestinal epithelium, and transmural migration (19) or resorption (15) describes the passage of viable bacteria across this barrier. Transmural migration implies that certain properties of the bacteria promote their migration through the intestinal mucosa, and resorption implies a process somewhat analogous to absorption. Little is known of the mechanisms whereby bacteria or viruses cross the intestinal epithelium. Keller and Engley (14) appear to be the first to use the term "translocation" to describe the passage of phage particles through the intestinal epithelium. Wolochow et al. (27) subsequently employed the term translocation to describe the passage of viable bacteria from the GI tract to the lymph and mesenteric lymph nodes (MLN) of rats, and Fuller and Jayne-Williams (9) used it to describe the passage of viable bacteria from the GI tract to the livers of chickens. Consequently, we also used the term translocation to describe the passage of viable indigenous bacteria from the GI tract to the MLN and other organs (3). This term implies nothing more than the passage of bacteria from one location, e.g., the GI tract, to another, e.g., the MLN, without suggesting anything concerning the mechanisms involved.

The translocation of viable bacteria from the GI tract to the MLN and other organs is undoubtedly an initial step in the pathogenesis of many bacterial diseases. However, we have concentrated our studies on the mechanisms whereby certain members of the indigenous or normal flora are able to translocate across the intestinal barrier. Bacterial translocation does not occur in healthy, specific pathogen-free (SPF) mice (3); the indigenous bacteria either do not pass through the GI mucosa, are killed in transit, or are killed by macrophages in organs such as the MLN. Bacterial translocation from the GI tract occurs, however, in mice with a depressed immune system. Indigenous bacteria translocate from the GI tract to the MLN, spleen, liver, and kidney of athymic (nu/nu) mice but not from the GI tracts of heterozygous (nu/+) or thymus-grafted (nu/nu) mice (17, 18). Furthermore, bacterial translocation from the GI tract occurs in SPF mice which have undergone neonatal thymectomy (18). Thus, the immune system of the host is one mechanism operating to confine certain indigenous bacteria to the GI tract.

Control of the population levels of certain

indigenous bacteria by other antagonistic members of the normal flora is another mechanism operating to confine indigenous bacteria to the GI tract. Bacterial translocation from the GI tract to the MLN and other organs readily occurs in gnotobiotic mice monoassociated with various indigenous bacteria (3). Further study has demonstrated that the abnormally high population levels of these bacteria in the ceca of monoassociated gnotobiotes (4), or antibiotic-decontaminated SPF mice subsequently colonized with a certain bacterial species (1), promote their translocation from the GI tract. Since bacterial translocation occurs as readily in antibiotic-decontaminated mice as in germfree mice colonized with certain bacteria, it appears that abnormally high cecal population levels of bacteria promote their translocation rather than some attribute of germfree mice such as a thinner lamina propria or a reduced immunological response. Bacterial translocation from the GI tract also occurs in SPF mice treated orally with either penicillin, clindamycin, or metronidazole (2). Each of these antibiotics disrupts the ecology of the normal flora in the GI tract, allowing certain antibiotic-resistant enteric bacteria, such as *Escherichia coli*, to attain abnormally high population levels and to translocate to the MLN and other organs.

Although bacterial translocation occurs under conditions in which certain indigenous bacteria reach abnormally high population levels in the GI tract, it is not known whether this phenomenon is strictly a function of high bacterial levels in the GI tract or whether some bacterial strains can translocate even though their population levels are not abnormally high. Germfree mice were diassociated or triassociated with various species of indigenous bacteria, and the population levels in both the ceca and MLN were determined to obtain information concerning the relationship between the numbers of bacteria in the ceca and the numbers of bacteria translocating to the MLN. There was a direct relationship between the percentage of the total cecal population of a particular bacterial strain and the percentage of the total MLN population of that strain. Therefore, the cecal population level of a particular strain of indigenous bacteria determined the numbers of viable bacteria of this strain translocating to the MLN.

MATERIALS AND METHODS

Animals. Germfree and gnotobiotic outbred Swiss Crl:CD-1(ICR)BR mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were housed in Trexler-type vinyl isolators (Germfree Supply Division, Standard Safety Equipment Co., Palatine, Ill.). Isolators were sterilized with a 2% peracetic acid solution (FMC Corp., Buffalo, N.Y.) containing 0.1% Bio-Soft-N-300 detergent (TEA linear alkylate sulfonate, 60% active; Stepan Chemical Co., Northfield, Ill.). Mice were housed in polypropylene cages with San-i-Cel bedding (Paxton Processing Co., Inc., St. Louis, Mo.) and were fed autoclavable Purina Laboratory Chow 5010 (Ralston Purina Co., St. Louis, Mo.). The food, water, bedding, and cages were packed in a bulk sterilizing chamber (Hoeltge, Inc., Cincinnati, Ohio) and sterilized with a vacuum autoclave (American Sterilizer Co., Erie, Pa.). Sterility testing was performed by standard procedures (26).

Bacteria. *E. coli* C25 is a streptomycin-resistant strain originally isolated from the feces of a healthy human (8). *E. coli* IND, *Klebsiella pneumoniae* SPF9, *Proteus mirabilis* SPF8, *Pseudomonas* species 2, *Staphylococcus epidermidis* C64, *Streptococcus faecalis* SPF10, and *Lactobacillus brevis* SPF7 were isolated from the ceca of SPF mice. All strains except for *S. faecalis* SPF10 were present at cecal population levels greater than 10^8 CFU/g (wet weight) of cecal contents. Bacterial counts were determined on either MacConkey agar (Difco Laboratories, Detroit, Mich.) or blood agar base (Difco) supplemented with 5% (vol/vol) sterile defibrinated sheep blood (Edge Diagnostics, Memphis, Tenn.). All strains were grown at 37°C. Gram-negative bacteria were identified by using the API 20E system (Analytab Products, Plainview, N.Y.) (20). Gram-positive cocci were identified by standard procedures (12). *L. brevis* SPF7 was grown in 5% CO_2 and identified according to methods described by Holdeman et al. (11).

Association of gnotobiotic mice. Bacterial cultures were incubated overnight in brain heart infusion (BHI) (Difco) and transferred aseptically to autoclaved glass test tubes with sterile rubber stoppers. The exterior surfaces of the tubes and stoppers were sterilized with 2% peracetic acid before being transferred into the isolators. Germfree mice were associated with bacteria by inoculating their drinking water to a final bacterial concentration of approximately 10^8 CFU/ml and by placing cultures onto the food pellets. The water and food were replaced after 24 h. Mice were diassociated with the secondary strain, *E. coli* C25, after 3 weeks of monoassociation with a primary bacterial strain. Triassociated mice were inoculated simultaneously with all three strains. All bacterial strains readily colonized the GI tracts of the mice.

Testing for translocation of bacteria. Mice were sacrificed by cervical dislocation, and the cranial and caudal mesenteric lymph node, spleen, liver, kidney, and cecum were excised aseptically as previously described (3). The peritoneal cavity was swabbed before and after the removal of the mesenteric lymph nodes and cultured in BHI to detect the presence of bacteria in the peritoneal cavity and to detect contamination after excision of the mesenteric lymph node. The spleens, livers, and kidneys were each homogenized in 5 ml of BHI and incubated overnight. The ceca were each homogenized in 9.0 ml of BHI and serially diluted in sterile saline. The numbers of viable bacteria per gram (wet weight) of cecum were determined by spreading 0.1-ml aliquots of the appropriate dilutions onto either MacConkey or blood agar. The organ homogenates from mice associated with a gram-positive strain were plated on blood agar. The MLN were homogenized in small, tapered grinding tubes (Tri-R Instruments, Inc., Rockville Centre, N.Y.) containing 0.5 ml of BHI. Aliquots of 0.1 and 0.2 ml of

TABLE 1. Incidences of bacterial translocation to various organs of diassociated mice[a]

Primary bacterial strain	Presence of primary strain in[b]:					Presence of *E. coli* C25 secondary strain in[b]:				
	MLN	Spleen	Liver	Kidney	Peritoneal cavity	MLN	Spleen	Liver	Kidney	Peritoneal cavity
E. coli IND	8/8	0/8	0/8	0/8	0/8	5/8	0/8	0/8	0/8	0/8
K. pneumoniae SPF9	1/4	0/4	0/4	0/4	2/7	2/4	0/4	0/4	0/4	2/6
P. mirabilis SPF8	1/6	0/6	0/6	0/6	0/6	5/6	0/6	0/6	0/6	0/6
Pseudomonas sp. strain 2	2/5	0/5	0/5	0/5	1/6	4/5	0/5	0/5	0/5	1/6
S. epidermidis C64	6/7	0/7	0/7	0/7	0/8	3/7	1/7	1/7	1/7	0/8
S. faecalis SPF10	0/5	0/5	0/5	0/5	0/5	5/5	1/5	0/5	0/5	0/5
L. brevis SPF7	2/6	0/6	0/6	0/6	0/6	6/6	2/6	2/6	1/6	0/6
Total (% positive)	49	0	0	0	7	73	10	7	5	7

[a] Determined for the primary strain and for *E. coli* C25 secondary strain after 3 weeks of monoassociation with primary strain and 1 additional week of diassociation with *E. coli* C25.

[b] Expressed as number of organs positive/number tested.

MLN homogenate each were spread onto either MacConkey or blood agar plates as described above. Sterile BHI (1 ml) was then added to the remaining broth in the grinding tube containing the mesenteric lymph node homogenate and incubated at 37°C overnight. Each overnight culture of MLN, spleen, liver, or kidney homogenate was subcultured onto the appropriate agar medium to determine whether viable bacteria were present. Hypothetically, only one viable bacterium in the organ homogenate will produce a positive culture after incubation under these culturing procedures. The period of time between sacrificing a mouse and completion of the grinding and plating procedures never exceeded 15 min.

Statistical analyses. The incidences of translocation in groups of mice were compared by using the chi-square test. The two-sided Student *t* test was used to evaluate differences in numbers of bacteria in various organs. *P* values less than 0.05 were considered significant.

RESULTS

The incidences of translocation to the MLN, spleen, kidney, liver, and peritoneal cavity of mice monoassociated with a primary bacterial strain for 3 weeks and then diassociated with the *E. coli* C25 secondary strain for an additional week are shown in Table 1. The primary strains were cultured from 49% of the MLN, and *E. coli* C25 was cultured from 73% of the MLN. The incidences of translocation to the MLN of the primary strains ranged from 0% for *S. faecalis* SPF10 to 100% for *E. coli* IND, whereas the incidences of translocation of the *E. coli* C25 secondary strain ranged from 43 to 100% of the MLN. The primary strains did not translocate to the spleen, liver, or kidney; *E. coli* C25 translocated to these organs at very low incidences. The primary strains and *E. coli* C25 each translocated to only three of the peritoneal cavities. Thus, these bacterial strains translocated to the MLN at much higher incidences than to the spleen, liver, kidney, or peritoneal cavity.

The population levels of the primary strains in the ceca and MLN of the diassociated mice are shown in Table 2. The mean cecal population levels of each primary strain in the diassociated mice were about 10^9 per gram (wet weight). The cecal populations of the gram-negative strains were higher than those of the gram-positive strains. The cecal population levels of three of the four gram-negative primary strains decreased significantly after diassociation with *E. coli* C25 when compared with their population levels after 3 weeks of monoassociation. The cecal population of only one of the three strains of gram-positive bacteria decreased significantly after diassociation. Colonization of the monoassociated mice with *E. coli* C25 also decreased the population levels of the primary strains in the MLN as compared with their population levels in monoassociated mice. However, only

TABLE 2. Population levels of bacteria in the ceca and MLN of diassociated mice[a]

Primary bacterial strain	Cecum		MLN	
	Log_{10} population level of primary strain ± SE[b]	Log_{10} population level of *E. coli* C25 ± SE[b]	Population level of primary strain	Population level of *E. coli* C25
E. coli IND	9.94 ± 0.06 (−0.95)[c,d]	9.02 ± 0.07 (−0.82)[e,f]	11 (−32)[c]	7 (−36)[e,f]
K. pneumoniae SPF9	9.83 ± 0.05 (−0.69)[d]	9.50 ± 0.07 (−0.34)[c]	2 (−54)[d]	12 (−31)
P. mirabilis SPF8	8.94 ± 0.71 (−0.60)[d]	9.72 ± 0.69 (−0.12)	2 (−42)	23 (−20)
Pseudomonas species 2	9.17 ± 0.14 (−0.33)	9.74 ± 0.14 (−0.10)	2 (−26)	24 (−19)
S. epidermidis C64	8.51 ± 0.14 (−0.83)[d]	9.95 ± 0.06 (+0.11)	4 (−16)	14 (−29)
S. faecalis SPF10	7.86 ± 0.12 (−0.04)	10.12 ± 0.17 (+0.28)	0 (0)	37 (−6)
L. brevis SPF7	8.69 ± 0.22 (+0.03)	10.10 ± 0.10 (+0.26)	2 (−8)	186 (+143)
Mean	8.99 (−0.49)	9.74 (−0.10)	3 (−25)	43

[a] Mice were monoassociated for 3 weeks with the primary bacterial strain and then were colonized with *E. coli* C25 secondary strain for 1 week. The mice were tested for bacterial translocation from the GI tract at the end of the 4 weeks.

[b] CFU per gram wet weight.

[c] Values in parentheses represent differences between population levels of primary bacterial strains in monoassociated mice compared with population levels of strains in mice diassociated with both primary strain and *E. coli* C25.

[d] Significant decrease from 3-week monoassociation values (two-sided Student's t test, $P < 0.05$).

[e] Values in parentheses represent differences between population levels of *E. coli* C25 after 1 week of monoassociation and after 1 week of diassociation.

[f] Significant decrease from 1-week *E. coli* C25 monoassociation values (two-sided Student's t test, $P < 0.05$).

TABLE 3. Percentage of total population of primary strain and secondary bacterial strains in the ceca and MLN of diassociated mice[a]

Primary bacterial strain	% of total population			
	Primary strain		*E. coli* C25 secondary strain	
	Cecum	MLN	Cecum	MLN
E. coli IND	89	60	11	40
K. pneumoniae SPF9	67	15	33	85
P. mirabilis SPF8	13	8	87	92
Pseudomonas species 2	20	8	80	92
S. epidermidis C64	4	22	96	78
S. faecalis SPF10	<1	0	>99	100
L. brevis SPF7	5	1	95	99
Mean	28	16	72	84

[a] Determined for the primary strain and the *E. coli* C25 secondary strain after 3 weeks of monoassociation with the primary strain and 1 additional week of diassociation with *E. coli* C25.

the population levels of *K. pneumoniae* SPF9 decreased significantly in the MLN of diassociated mice.

E. coli IND and *K. pneumoniae* SPF9 were the only primary strains predominant over *E. coli* C25 in the ceca (Table 3). *E. coli* IND also was present in the MLN at population levels greater than those of *E. coli* C25. However, *K. pneumoniae* SPF9 was not the predominant organism in the MLN. *E. coli* C25 was predominant in both the ceca and MLN in the five other diassociation combinations. Thus, with the exception of *K. pneumoniae* SPF9, the percentage of the total cecal population of each of the primary strains was similar to the percentage of the total MLN populations of those strains.

The finding that the numbers of a bacterial strain populating the ceca affected the numbers of these particular bacteria translocating to the MLN of diassociated gnotobiotic mice prompted further experiments to provide more evidence of a direct relationship between the cecal population levels of particular bacteria and their numbers in the MLN. Germfree mice were triassociated with three different strains of coliform bacteria, *E. coli* IND, *K. pneumoniae* SPF9, and *P. mirabilis* SPF10. There was very little or no translocation of any of these strains to the spleen, liver, kidney, or peritoneal cavity (Table 4). However, *E. coli* IND was cultured from 77% of the MLN, *K. pneumoniae* was cultured from 78% of the MLN, and *P. mirabilis* SPF8 was cultured from 55% of the MLN from these triassociated mice. These differences in the incidences of translocation were not statistically significant.

K. pneumoniae SPF9 was the predominant organism in the ceca of the triassociated mice, followed by *E. coli* IND and then *P. mirabilis* (Table 5). The cecal populations of these strains in monoassociated mice were compared with their cecal populations in triassociated mice to determine whether any of the strains were inhibited. *K. pneumoniae* SPF9 was inhibited only slightly in the ceca, whereas *P. mirabilis* SPF8 and *E. coli* IND were inhibited by an average of 1.61 and 1.43 $\log_{10}$, respectively.

K. pneumoniae SPF9 was the predominant organism in both the ceca and the MLN of the triassociated mice (Table 5). *E. coli* IND was the next most populous organism in these organs. *P. mirabilis* SPF8 was present at the lowest numbers in both the ceca and MLN compared with the other two strains. The relative population level of each bacterial strain in the MLN therefore reflected the relative population level of that strain in the ceca of the triassociated mice.

The percentage of the total population of each bacterial strain in the cecum was compared with the percentage of the total population of each strain in the MLN (Table 6). No statistically significant differences in the percentage of the total cecal population of each primary strain compared with the percentage of the total MLN population of each primary strain were observed in 18 of the 21 sets of values. In one trial after 3 weeks of triassociation, the percentage of the total population of *E. coli* IND in the MLN was greater than the percentage of the total population of *E. coli* IND in the cecum, and the percentage of the total population of *K. pneumoniae* SPF9 also was greater in the cecum than in the MLN. The percentage of the total population of *K. pneumoniae* SPF9 in the ceca also was significantly greater than its percentage of the total population in the MLN after 8 weeks of triassociation. However, the average percentages in the cecum and MLN of these three strains in all seven triassociation trials were not significantly different. Thus, the percentage of the total population of each bacterial strain was similar in both the ceca and MLN for each of the strains tested.

DISCUSSION

This study demonstrates a direct relationship between the numbers of a particular bacterial strain populating the ceca of diassociated or triassociated mice and the numbers of viable bacteria of this strain translocating to the MLN. Although the cranial and caudal MLN drains both the ileum and cecum (6), previous results demonstrate that translocation of indigenous bacteria from the GI tract to the MLN is associ-

ated with high populations of these bacteria in the ceca rather than the ilea in both monoassociated gnotobiotic mice (3) and antibiotic-decontaminated SPF mice (1) colonized with certain bacteria. These indigenous bacteria do not reach population levels in the ilea as high as those in the ceca of gnotobiotic mice, and the mass of contents is greater in the cecum than in the ileum. Therefore, bacteria in the ileum may not reach the critical population levels required to promote their translocation from the GI tract.

There is no evidence available as to the route by which these indigenous bacteria cross the GI epithelial barrier. Translocation could be due to pinocytosis of bacteria by mucosal epithelial cells, to passive absorption of bacteria, or to certain properties or activities of the bacteria. *Salmonella typhimurium* and pathogenic *E. coli* readily translocate through the epithelial cells lining the GI tract (21–23). Bacteria reaching the lamina propria most likely enter the lymphatics, since the blood capillaries are reported to be impenetrable to particles the size of bacteria (21). Once through the epithelial lining of the GI tract, the indigenous bacteria apparently travel by the lymphatics to the MLN and then may disseminate to other organs.

Peyer's patches appear to concentrate certain antigens inoculated intragastrically into mice (5, 13). They also may serve as collection points for the passage of *Salmonella enteritidis* from the GI tract to the MLN of SPF mice (6). It is not known, however, whether indigenous bacteria also concentrate in Peyer's patches during translocation. Peyer's patches are overlaid with specialized epithelial cells, called M-cells, specifically adapted for the absorption of particulates from the GI lumen (5, 16). However, the majority of Peyer's patches are located in the small intestine, and our data indicate a relationship between cecal bacterial populations and translocation to the MLN rather than a relationship between ileal populations and bacterial translocation.

Morphological and immunological abnormalities of the germfree mouse might still be present after only 1 or 3 weeks of association with these indigenous bacteria. The lamina propria of the germfree mouse is considerably thinner than that of conventional mice (10). Germfree mice also contain fewer immunoglobulin A-producing cells in their lamina propria and exhibit underdeveloped Peyer's patches compared with conventional mice (7). *E. coli*, however, translocates at high rates from the GI tract to the MLN of antibiotic-decontaminated SPF mice colonized with *E. coli* (1). These mice exhibit a lamina propria of normal thickness and have been stimulated immunologically from birth by antigens of normal flora bacteria. Therefore, the high inci-

TABLE 4. Incidences of bacterial translocation to various organs of triassociated mice

Weeks of triassociation	*E. coli* IND					*K. pneumoniae* SPF9					*P. mirabilis* SPF8				
	MLN	Spleen	Liver	Kidney	Peritoneal cavity	MLN	Spleen	Liver	Kidney	Peritoneal cavity	MLN	Spleen	Liver	Kidney	Peritoneal cavity
1	6/10[a]	0/10	0/10	0/10	0/10	8/10	0/10	0/10	0/10	0/10	6/10	0/10	0/10	0/10	0/10
1	5/9	0/10	0/10	0/10	0/10	8/9	0/10	1/10	0/10	0/10	3/9	0/10	0/10	0/10	0/10
3	7/10	0/10	0/10	0/10	0/10	8/10	0/10	0/10	0/10	0/10	7/10	0/10	0/10	0/10	0/10
3	9/10	0/10	0/10	0/10	0/10	7/10	1/10	0/10	0/10	0/10	4/10	0/10	0/10	0/10	0/10
5	8/10	0/10	0/10	0/10	0/10	9/10	1/10	0/10	0/10	0/10	6/10	0/10	0/10	0/10	0/10
8	8/10	1/10	1/10	0/10	0/10	6/10	1/10	1/10	1/10	0/10	5/10	0/10	0/10	0/10	0/10
12	10/10	0/10	1/10	0/10	0/10	8/10	0/10	0/10	0/10	0/10	7/10	0/10	1/10	0/10	0/10
% Positive	77	1	3	0	0	78	4	3	1	0	55	0	1	0	0

[a] Number of organs positive for bacteria/number tested.
[b] Not significantly different from MLN translocation rates of *E. coli* IND and *K. pneumoniae* SPF9 (chi-square test).

TABLE 5. Cecal and MLN populations of triassociated mice

Weeks of triassociation[a]	Mean $\log_{10}$ cecal population[b]			Differences from $\log_{10}$ cecal population in monoassociated mice[b]			Mean MLN population[b]			Differences from MLN cecal population in monoassociated mice[b,d]		
	E.c.	*K.p.*	*P.m.*	*E.c.*	*K.p.*	*P.m.*	*E.c.*	*K.p.*	*P.m.*	*E.c.*	*K.p.*	*P.m.*
1	9.60	10.04	8.94	−1.62	−0.45	−1.22	25	15	5	−32	−30	−117
1	9.50	9.77	8.99	−1.72	−0.72	−1.17	49	36	31	−8	−9	−91
3	9.12	9.74	8.80	−1.62	−0.73	−1.74	5	73	17	−52	+28	−105
3	9.26	9.75	8.97	−1.48	−0.72	−1.57	42	26	20	−15	−19	−102
5	9.13	9.54	8.57	ND[e]	ND	ND	32	24	20	−25	−21	−102
8	9.22	9.82	9.00	ND	ND	ND	20	28	10	−37	−17	−112
12	9.45	9.80	9.20	ND	ND	ND	46	87	15	−11	+42	−107
Mean	9.33	9.78	8.92	−1.61	−0.66	−1.43	31	41	17	−26	−4	−105
	±0.19[c]	±0.15	±0.20	±0.14	±0.10	±0.28	±16	±27	±8	±16	±27	±8

[a] Ten mice per experiment.
[b] *E.c.*, *K.p.*, and *P.m.* denote *E. coli* IND, *K. pneumoniae* SPF9, and *P. mirabilis* SPF8, respectively.
[c] Mean ± standard deviation.
[d] Mean MLN populations after 1 and 3 weeks of monoassociation.
[e] ND, Not determined.

dences of bacterial translocation in gnotobiotic mice cannot be explained solely on the basis of anatomical or immunological deficiencies, but are due to the abnormally high cecal population levels achieved by these bacteria in the absence of bacterial antagonism by other members of the normal flora.

Similar proportions of bacteria in both the ceca and MLN of gnotobiotic mice imply that the different bacterial strains translocate at similar rates and have comparable survival rates in the MLN. Although there may be a common mechanism whereby different bacteria translocate, it is unlikely that these bacteria would be equally resistant to the defense mechanisms of the host. The absence of viable cells of *S. faecalis* SPF10 in the MLN of both monoassociated and diassociated mice suggests that this strain does not translocate in high numbers to the MLN, since it would be expected to survive host defenses at least as well as the other gram-positive bacteria tested. Current studies on the survival rates of different strains of translocating bacteria in the MLN should provide information indicating whether the presence of viable bacteria in the MLN is a reflection of their survival in the MLN.

Various studies demonstrate that overgrowth of the intestines by certain bacteria promotes their translocation from the GI tract to other organs. Van der Waaij et al. (25) reported bacterial translocation to the MLN of antibiotic-decontaminated mice subsequently colonized with certain bacteria. We also have found that indigenous bacteria readily translocate from the GI tracts of antibiotic-decontaminated mice subsequently colonized with indigenous bacteria and from the GI tracts of gnotobiotic mice monoassociated with these bacteria (1, 4). Bacterial translocation from the GI tract also occurs in SPF mice treated orally with penicillin, clindamycin, or metronidazole (2). Thus, disruption of the ecology of the normal bacterial flora in the GI tract allows certain enteric bacteria to attain abnormally high population levels and to translocate to the MLN and other organs. Bacterial

TABLE 6. Percentage of total population of bacterial strains in the ceca and MLN of triassociated mice

Weeks of triassociation	*E. coli* IND		*K. pneumoniae* SPF9		*P. mirabilis* SPF8	
	Cecum	MLN	Cecum	MLN	Cecum	MLN
1	26	34	68	56	6	10
1	32	24	58	66	10	11
3	21	22	70	55	9	23
3	21	51[b]	66[c]	39	12	10
5	28	20	63	61	7	20
8	18	41	71[c]	44	11	15
12	28	31	65	55	15	10
Mean	25	32	66	54	10	14

[a] Ten mice were tested per experiment.
[b] Significantly greater percentage in MLN versus cecum (two-sided Student's t test, $P < 0.05$).
[c] Significantly greater percentage in cecum versus MLN (two-sided Student's t test, $P < 0.05$).

translocation is an important first step in the pathogenesis of opportunistic infections caused by certain members of the normal GI flora. Many of the bacterial species found to translocate from the GI tracts of the gnotobiotic mice in this study are often the etiological agents of these infections. The results presented here indicate that the extent of translocation of certain indigenous bacteria is directly related to their population levels in the GI tract.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of Martha Kiser Smith.

This investigation was supported by Public Health Service grants AI14235 and AI15826 from the National Institute of Allergy and Infectious Diseases and by American Cancer Society grant PDT-135.

LITERATURE CITED

1. **Berg, R. D.** 1980. Inhibition of *Escherichia coli* translocation from the gastrointestinal tract by normal cecal flora in gnotobiotic or antibiotic-decontaminated mice. Infect. Immun. **29**:1073–1081.
2. **Berg, R. D.** 1981. Promotion of the translocation of enteric bacteria from the gastrointestinal tracts of mice by oral treatment with penicillin, clindamycin, or metronidazole. Infect. Immun. **33**:854–861.
3. **Berg, R. D., and A. W. Garlington.** 1979. Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in a gnotobiotic mouse model. Infect. Immun. **23**:403–411.
4. **Berg, R. D., and W. E. Owens.** 1979. Inhibition of translocation of viable *Escherichia coli* from the gastrointestinal tract of mice by bacterial antagonism. Infect. Immun. **25**:820–827.
5. **Bockman, D. E., and W. Stevens.** 1977. Gut-associated lymphoepithelial tissue: bidirectional transport of tracer by specialized epithelial cells associated with lymphoid follicles. RES J. Reticuloendothel. Soc. **21**:245–253.
6. **Carter, P. B., and F. M. Collins.** 1974. The route of enteric infection in normal mice. J. Exp. Med. **139**:1189–1203.
7. **Crabbe, P. A., H. Bazin, H. Eyssen, and J. F. Heremans.** 1968. The normal flora as a major stimulus for proliferation of plasma cells synthesizing IgA in the gut. Int. Arch. Allergy Appl. Immunol. **34**:360–375.
8. **Freter, R.** 1962. *In vivo* antagonism of intestinal bacteria against *Shigella flexneri*. J. Infect. Dis. **110**:38–46.
9. **Fuller, R., and D. J. Jayne-Williams.** 1970. Resistance of the fowl (*Gallus domesticus*) to invasion by its intestinal flora. II. Clearance of translocated intestinal bacteria. Res. Vet. Sci. **11**:368–374.
10. **Gordon, H. A.** 1968. Is the germ-free animal normal? A review of its abnormalities in young and old age, p. 127–150. *In* M. E. Coates (ed.), The germ-free animal in research. Academic Press, Inc., New York.
11. **Holdeman, L. V., E. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
12. **Ivler, D.** 1974. Staphylococcus, p. 91–95. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
13. **Kagnoff, M. F.** 1977. Functional characteristics of Peyer's patch lymphoid cells. IV. Effect of antigen feeding on frequency of antigen-specific B cells. J. Immunol. **118**:992–997.
14. **Keller, R., and F. B. Engley, Jr.** 1958. Fate of bacteriophage particles introduced into mice by various routes. Proc. Soc. Exp. Biol. Med. **18**:577–580.
15. **Lykkegaard Nielsen, M., T. Justesen, S. Asnaes, J. G. Stage, and O. V. Nielsen.** 1975. Anaerobic and aerobic bacteriological study of the liver and biliary tract in healthy laboratory animals. Dan. Med. Bull. **22**:159–164.
16. **Owen, R. L., and L. J. Albert.** 1974. Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. Gastroenterology **66**:189–203.
17. **Owens, W. E., and R. D. Berg.** 1980. Bacterial translocation from the gastrointestinal tract of athymic (nu/nu) mice. Infect. Immun. **27**:461–467.
18. **Owens, W. E., and R. D. Berg.** 1982. Bacterial translocation from the gastrointestinal tracts of thymectomized mice. Curr. Microbiol. **7**:169–174.
19. **Schweinburg, F. B., A. M. Seligman, and F. Fine.** 1950. Transmural migration of intestinal bacteria. A study based on the use of radioactive *Escherichia coli*. N. Engl. J. Med. **242**:747–751.
20. **Smith, P. B., K. M. Tomfohrde, D. L. Rhoden, and A. Balows.** 1972. API system: a multitube micromethod for identification of *Enterobacteriaceae*. Appl. Microbiol. **24**:449–452.
21. **Staley, T. R., E. W. Jones, and L. D. Conley.** 1969. Intestinal monocontamination in the neonatal pig. Microbiological studies, p. 65–71. *In* E. A. Mirand and N. Back (ed.), Germfree biology. Experimental and clinical aspects. Advances in experimental medicine and biology, vol. 3. Plenum Press, New York.
22. **Takeuchi, A.** 1967. Electron microscope studies of experimental *Salmonella* infection. Am. J. Pathol. **50**:109–136.
23. **Takeuchi, A.** 1971. Penetration of the intestinal epithelium by various microorganisms. Curr. Top. Pathol. **54**:1–27.
24. **Volkheimer, G.** 1974. Passage of particles through the wall of the gastrointestinal tract. Environ. Health Perspect. **9**:215–225.
25. **van der Waaij, D., J. M. Berghuis-deVries, and J. E. C. Lekkerkerk-van der Wees.** 1972. Colonization resistance of the digestive tract and the spread of bacteria to the lymphatic organs in mice. J. Hyg. **70**:335–342.
26. **Wagner, M.** 1959. Determination of germfree status. Ann. N.Y. Acad. Sci. **78**:89–101.
27. **Wolochow, H., G. J. Hildebrand, and C. Lamanna.** 1966. Translocation of microorganisms across the intestinal wall of the rat: effect of microbial size and concentration. J. Infect. Dis. **116**:523–528.