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Microbial Interference and Colonization of the Murine Gastrointestinal Tract by *Listeria monocytogenes*

ZUZANA ZACHAR AND DWAYNE C. SAVAGE*

Department of Microbiology, University of Illinois, Urbana, Illinois 61801

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Two strains of *Listeria monocytogenes*, one that formed smooth colonies on agar surfaces and a variant of it that formed rough colonies, colonized the gastrointestinal tracts of germfree mice. Within 24 h after mice were inoculated orally with about 100 bacteria, the population levels per gram (wet weight) of tissue of both strains were 10^5 to 10^7 in the stomach and ileum and 10^8 to 10^9 in the cecum and colon, respectively. As detected in Gram-stained histological sections, in such gnotobiotes, the bacteria colonized the lumen in all areas of the tract and much of the mucus layer on the epithelial surface in the proximal colon. The strain that formed smooth colonies did not colonize the tracts of specific-pathogen-free mice, but did colonize, to the same levels as in germfree mice, the stomachs and bowels of ex-germfree mice previously associated with two members of the indigenous flora (*Bacteroides* and *Clostridium*). In the latter animals, however, the listeria did not form layers on the colonic epithelium as efficiently as they did in monoassociated gnotobiotes.

The natural history of *Listeria monocytogenes* is not well understood, although many investigators have addressed themselves to the problem (5, 6, 26, 30). Healthy carriers of *L. monocytogenes* have been documented among human and animal populations. *L. monocytogenes* has been isolated from the feces of healthy slaughter house workers (3, 12), egg product factor workers (12), office personnel (13), laboratory technicians working with *L. monocytogenes* (13), and pregnant women (10, 11). It has been isolated as well from cattle, sheep, mice, mink, chincillas, chickens, starlings, and sparrows (16).

Laboratory mice have a long history in studies with *L. monocytogenes* (6, 26). Listeric infection in mice generally manifests itself as septicemia with accompanying focal necrosis, especially in the liver, and often results in death. To achieve such results, investigators commonly inject the microorganisms intraperitoneally (6, 26). When the oral route of infection has been used by some investigators, however, less consistent results have been obtained. White Swiss mice were only mildly infected when they drank water containing the organism (17). They did not die and yielded evidence of infection only as lesions detected microscopically in their livers and spleens. By contrast mice that were fed a suspension of the bacteria died (9). In still other studies, however, the organism inoculated into the esophagus (18) of, or ingested in milk (27) by mice had no obvious effect on them.

The gastrointestinal (GI) tract has been said to play a role in the ecology of *L. monocytogenes* (5). However, the interactions of this pathogen and the indigenous microbiota of the GI tract apparently have not been examined previously. The microbial ecology of the murine GI tract has been studied extensively (21). Germfree and specific-pathogen-free (SPF) mice have been used in such studies. Germfree and SPF mice allow experiments to be performed under controlled environmental conditions (21). In addition, germfree mice provide an opportunity for constructing simplified model GI ecosystems. In this report, we demonstrate that *L. monocytogenes* will colonize and persist in the germfree murine GI tract. Further, we present some evidence on interactions of *L. monocytogenes* with the indigenous GI microflora.

MATERIALS AND METHODS

Bacteria. Two strains of *L. monocytogenes* were used in this study. A strain that produced smooth colonies on agar surfaces (*L. monocytogenes* S) was obtained from F. C. Collins, Trudeau Institute, Saranac Lake, N.Y. A strain that produced rough colonies (*L. monocytogenes* R) was isolated in our laboratory from the colon of an ex-germfree mouse that had been monoassociated with *L. monocytogenes* S for 30 weeks. The cultures were maintained in beef extract deeps at 4°C and transferred into fresh medium every 3 months (3).

Bacteroides 116-2 and *Clostridium* 109-2 were isolated in our laboratory from the cecum of a CD-1 mouse (29). The cultures were maintained in prere-

duced brain heart infusion broth and transferred weekly into fresh medium.

Characterization of the rough strain. The following biochemical and physiological tests were used in identifying and characterizing the rough strain isolate: (i) Gram stain reaction; (ii) motility at 25 and 37°C; (iii) agglutination with anti-*L. monocytogenes* S antiserum; (iv) catalase production; (v) fermentation of key sugars; (vi) gelatin liquefaction; (vii) indole production, and (ix) nitrate reduction (14). All media (except motility agar) were incubated aerobically at 37°C for 4 days. Appropriate positive controls were run in parallel.

Mice. Mice used in this investigation are described in Table 1. All mice were housed in cages inside plastic isolators and given sterile food (Wayne Laboratory Animal Diets, Allied Mills, Chicago, Ill.) and sterile tap water ad libitum.

SPF mice were checked for *L. monocytogenes* before inoculation. Fresh fecal pellets were collected from the mice and plated on tryptose-polymyxin B agar (3), MacBrides agar (14), and nalidixic acid-mannitol (NM) agar (see below). *L. monocytogenes* was not found in any of the samples.

Media. *L. monocytogenes* was grown in tryptose phosphate broth (Gibco). Bacterial suspensions were diluted in dilution broth (D broth, 2 g of tryptone [Difco], 5 g of NaCl per liter of water). D broth was also used for collecting, grinding, and diluting tissue samples. Tissue samples from SPF mice were plated on NM agar. All other tissue samples were plated on tryptose agar (Difco). NM agar (Table 2) was modified from the selective medium for *L. monocytogenes* described by Beerens and Tahon-Castel (2). The modification allows the differentiation of *L. monocytogenes* and *Streptococcus faecalis*. Both organisms are resistant to nalidixic acid and their colonial morphology is identical on nalidixic acid agar under our plating conditions. We added mannitol (0.1%) and bromothymol blue (0.08%) to the medium. *S. faecalis* ferments mannitol and reduces the indicator dye. Hence, *S. faecalis* colonies are deep gold and opaque, whereas *L. monocytogenes* colonies are colorless and translucent. To determine that NM medium permitted quantitative detection of *L. monocytogenes*, controls were run in which a known concentration of *L. monocytogenes* was mixed with either pure cultures of *S. faecalis* or suspensions of feces from SPF mice. In all cases, the number of *L. monocytogenes* colonies detected was in accord with the expected values.

Prereduced brain heart infusion broth was prepared as described in the Anaerobe Laboratory Manual (8). Media for characterization of the rough strain were prepared as described by Killinger (14).

Infection of mice. Mice were inoculated orally by gavage. Approximately 0.5 ml of a *L. monocytogenes* suspension was administered directly into the stomach through sterile polyethelene tubing. Germfree and diassociated mice were given about 100 bacteria. The inoculum size for SPF mice varied between 100 and 5 $\times 10^7$ bacteria. The specific dosage is stated with each experiment. The concentration of each *L. monocytogenes* inoculum was estimated by viable count.

Culturing procedures. Mice were killed by CO_2 asphyxiation. Stomach, ileum, cecum, and colon samples of determined wet mass were collected in 2 ml of ice-cold D broth in screw-cap tubes. A 4-ml portion of sterile glass beads was added. The suspension was stirred for 60 s at a fast setting on an adjustable Vortex-type mixer (Super Mixer, Matheson Scientific). Dilutions of the homogenized samples were made in D broth, and 0.1-ml duplicate samples were plated on tryptose or NM agar. Both media were incubated aerobically at 37°C; tryptose agar plates were incubated for 24 h, NM agar plates for 40 to 48 h.

Histological methods. Stomach, ileum, cecum, and colon samples were frozen with contents intact at −20°C in 2% methyl cellulose dissolved in 0.15 M saline (23). Sections of 4 μm were cut on a microtome-cryostat (International Equipment Co., Needham Heights, Mass.), fixed for 60 s in absolute methyl alcohol, stained with a modified tissue Gram stain (4),

TABLE 2. *NM agar*[a]

Ingredient[b]	Amt (g)
Tryptose (Difco)	10
Beef extract (Difco)	3
Sodium chloride (Mallinckrodt)	5
Mannitol (Fisher Scientific)	1
Bromothymol blue (Allied Chemical)	0.08
Nalidixic acid (Sigma)	0.04
Agar (Difco)	15
Distilled water	1,000 ml

[a] Modified from the selective medium described by Beerens and Tahon-Castel (2).

[b] All ingredients except nalidixic acid were mixed and autoclaved at 15 lb/in^2. for 15 min. Nalidixic acid was dissolved in 0.1 N NaOH, filter sterilized, and added to the cooled agar. Plates were poured 15 ml per 100-mm petri plate. Inoculated media were incubated aerobically at 37°C for a minimum of 40 h.

TABLE 1. *Characteristics of mice used*

Mouse	Strain	Sex	Source
Germfree	Crl:CD-1(ICR)GN	Male and female	Bred in our laboratory[a]
Germfree[b]	Hap:(ICR)GN	Male and female	Harlan Industries, Inc. Indianapolis, Ind.
SPF	Crl:COBS CD-1(ICR)BR	Male	Charles River, Wilmington, Mass.

[a] Parent breeding stock was obtained from Charles River, Wilmington, Mass.

[b] These mice were described by the supplier as germfree. During the investigation we discovered that the mice were delivered contaminated with strict anaerobes.

and examined with a Zeiss Universal Microscope. Both regular and phase optics were used.

RESULTS

Characterization of rough strain. Strain R behaved identically to strain S in all biochemical, physiological, and immunological tests used in characterizing them. Cells of both strains were gram positive, were motile when grown at 25°C but nonmotile when grown at 37°C, agglutinated to the same titer (1:1,024) in antiserum; fermented the same carbohydrates (arabinose, dextrin, esculin, glucose, lactose, maltose, salicin, sucrose, and trehalose) and failed to ferment the same ones (dulcitol, inositol, lactose, and maltose), did not liquefy gelatin, produced no H_2S or indol, and did not reduce nitrate. The cells of strain R were filamentous, however, and formed rough colonies with serrated edges, whereas cells of strain S were coccobacillary and formed smooth colonies with entire edges. The 50% lethal dose was not determined for either strain. Nevertheless, strain R is probably much less virulent than strain S (Table 3).

Course of listeric infection in mice. SPF mice, inoculated by gavage with doses of *L. monocytogenes* S as high as 5×10^7 bacteria, never showed any obvious signs of disease. By contrast, germfree mice inoculated by gavage with only about 100 of the strain S bacteria, all developed diarrhea and mottled fur and lost weight by day 3 after inoculation. About 30% of the animals died between days 5 and 8 after the pathogen was given (Table 3). At autopsy, *L. monocytogenes* S was isolated from the liver, spleen, and GI tract of all dead animals. The livers and spleens were studded with foci. By day 9, the surviving mice appeared healthy even though they still harbored large *L. monocytogenes* populations in their GI tracts.

TABLE 3. *Mortality in germfree and SPF mice infected with L. monocytogenes S or R*

Mouse	Strain	No. of animals inoculated[a]	No. of animals dead[b]	Dead/inoculated (%)
SPF	S	27	0	0
Germfree	S	75	21	28
Germfree	R	22	1	4

[a] Total numbers of mice infected orally in several experiments. See text for numbers of bacteria in infecting doses.

[b] See text for explanation.

With one exception, germfree mice inoculated with *L. monocytogenes* R never showed obvious signs of disease. One mouse did die 15 days after inoculation. Samples of the GI tract, spleen, and liver of the animal were cultured. *L. monocytogenes* S made up about 90% of the recovered bacteria from all tissues. We speculate, therefore, that strain R "reverted" early in the colonization and that the mouse died due to infection by a smooth strain. Reversion of the rough strain to smooth was observed in all mice associated with *L. monocytogenes* R for at least 3 weeks. In such mice, however, the smooth strain made up only about 10% of the population. All *L. monocytogenes* R inocula were plated on tryptose agar as a check for *L. monocytogenes* S. No *L. monocytogenes* S colonies were ever detected in the inocula.

Colonization of the GI tracts of germfree mice by L. monocytogenes S and R. *L. monocytogenes* S colonized the entire GI tract of germfree mice (Table 4). The highest population levels of the strain were found in the cecum and colon (10^8 to 10^9 bacteria per g of tissue). As assessed by culture of feces and colons, strain R also colonized the tracts of germfree animals (Table 5). Population equilibrium of both strains in the animals was established within 24 h of inoculation and did not change for the duration of a given experiment (Table 5). Associated mice were kept for 3 to 33 weeks.

Microscopic examination of Gram-stained tissue sections revealed *L. monocytogenes* in the lumen of the stomach, ileum, cecum, and colon.

TABLE 4. *Population level of L. monocytogenes S in the GI tracts of monoassociated ex-germfree mice*

Mouse	Sex	No. of mice/group	Duration of association (weeks)	Median (range) of $\log_{10}$ *L. monocytogenes* S[a]			
				Stomach	Ileum	Cecum	Colon
Crl:CD-1(ICR)GN	Male	2	8	7 (7–8)	7 (7–8)	9 (9)	9 (9)
Crl:CD-1(ICR)GN	Male	10	10	8 (6–8)	6 (5–8)	9 (8–9)	8 (8–9)
Crl:CD-1(ICR)GN	Male[b]	3	32	7 (7)	6 (5–6)	8 (8)	8 (8)
Hap:(ICR)GN[c]	Male	2	33	7 (7)	5 (5–6)	9 (9)	8 (8)
Hap:(ICR)GN[c]	Female	2	33	7 (6–8)	6 (6)	9 (9)	8 (8)

[a] Figures calculated per gram (wet weight) of tissue.

[b] These animals became contaminated with *Bacillus* species during week 5 after the beginning of the experiment.

[c] Contaminated with anaerobic bacteria of undefined types throughout experiment.

TABLE 5. *Population level of L. monocytogenes in feces or colons of monoassociated ex-germfree mice*

Mice	Sex	No. of mice/ group	Colonial form of strain	Sample	*L. monocytogenes* population level[a]						
					Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Crl:CD-1(ICR)GN	Male	7[b]	S	Feces	8 (8–9)	9 (7–9)	9 (9)	8 (8)	8 (8–9)	8 (8)	8 (8–9)
Crl:CD-1(ICR)GN	Female	3	R	Feces	8 (7–9)	8 (8–9)	8 (8)	8 (8–9)	8 (8–9)	8 (8–9)	8 (8–9)
Crl:CD-1(ICR)GN	Male	4	R	Colon	8 (5–9)	ND	ND	ND	ND	ND	ND
Crl:CD-1(ICR)GN	Male	4	R	Colon	ND	ND	ND	7 (7–8)	ND	ND	ND
Crl:CD-1(ICR)GN	Male	5	R	Colon	ND	ND	ND	ND	ND	ND	8 (8)
Hap:(ICR)GN[c]	Female	8[d]	S	Feces	8 (6–8)	8 (8)	8 (8)	8 (7–9)	8 (7–9)	8 (8–9)	8 (7–9)
Hap:(ICR)GN[c]	Male	8[b]	S	Feces	8 (7–8)	8 (7–9)	8 (8–9)	8 (8–9)	8 (7–10)	9 (8–9)	9 (8–9)

[a] Median (range) of $\log_{10}$ *L. monocytogenes* per gram (wet weight) of feces or tissue. ND, Not done.
[b] Number of mice at the start of the experiment; 1 mouse died due to listeric infection by day 7.
[c] Contaminated with anaerobic bacteria of undefined types throughout experiment.
[d] Number of mice at the start of the experiment; two mice died due to listeric infection by day 7.

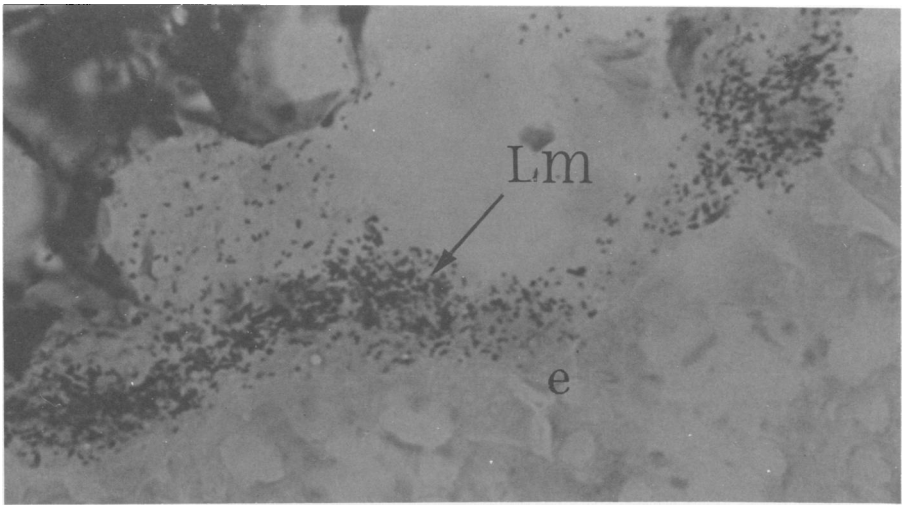

FIG. 1. *L. monocytogenes S (Lm) in a layer near the epithelial surface (e) in the proximal colon of a monoassociated ex-germfree mouse. Magnification;* × *848.*

Most bacteria were seen in the cecum and colon. We also noted that *L. monocytogenes* marginated in the proximal colon (Fig. 1), i.e., the density of the bacteria along the mucous layer was much higher that that in the lumen. In some areas, the bacteria could be seen in intimate association with the epithelium (Fig. 2). Margination was seen repeatedly in monoassociated animals. It became apparent, however, only during the second week of colonization. Both smooth and rough strains marginated. Margination was also seen in unstained sections examined with phase optics. To determine what proportion of the population is present in the lumen versus at the periphery, we cultured two samples from the proximal colons from animals monoassociated with strain S: one sample was left with the contents intact and the other was perfused with cold phosphate-buffered saline (0.02 M, pH 7.3) until the contents were flushed out. The population levels in the rinsed colon were generally 10-fold lower than those in the intact colon (Table 6).

Colonization of the GI tract of SPF mice by L. monocytogenes S. *L. monocytogenes* S did not colonize the GI tract of SPF mice when the animals in groups of five or six for each dosage were given orally 1×10^2, 1×10^3, or 5×10^4 of the bacteria. The organism was isolated, however, from the tract of 1 mouse in a group of 5 inoculated with 5×10^7 bacteria. The cold enrichment technique of Gray et al. (7) was applied to all initially negative tissue samples. The samples were stored at 4°C and screened 1 to 3 months later. *L. monocytogenes* was not detected in any of the samples.

Interference of indigenous anaerobic bacteria with L. monocytogenes. Our inability to demonstrate that *L. monocytogenes* could colonize the GI tract of SPF mice led us to

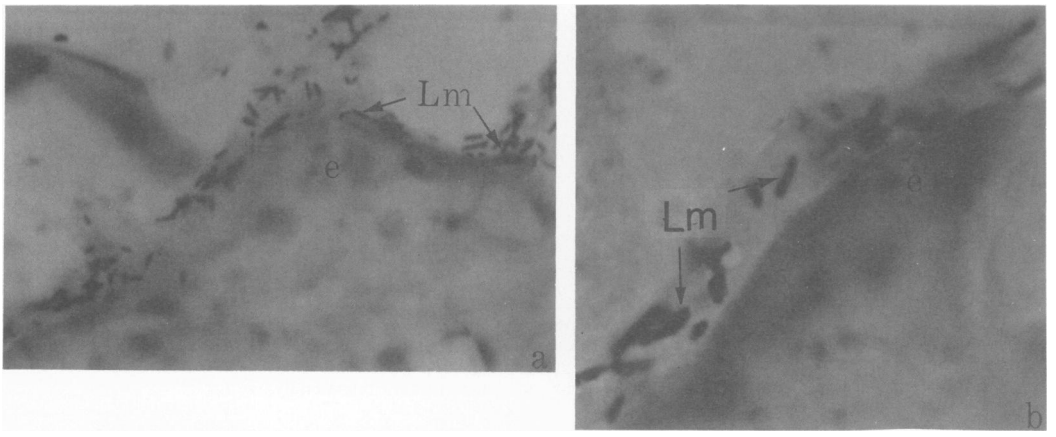

FIG. 2. *L. monocytogenes S (Lm) in intimate association with the colonic epithelium (e) in a monoassociated ex-germfree mouse. (a.) Magnification; × 1,440. (b.) Magnification; × 3,600.*

TABLE 6. *Population level of L. monocytogenes S and R in the proximal colons of monoassociated ex-germfree mice*

Strain	No. of mice[a]/ group	Duration of association (days)	Population level[b]	
			Intact colon	Colon with contents removed[c]
S	9	20	9 (8–9)	7 (7–8)
S	8	20	9 (9)	8 (7–8)
R	9	20	8 (8–9)	7 (7–8)
R	4	22	8 (8)	7 (7–8)

[a] Male, Crl:CD-1(ICR)GN.

[b] Median (range) of $\log_{10}$ *L. monocytogenes* per gram (wet weight) of colon.

[c] The lumen of the colon was perfused with 2 to 3 ml of cold phosphate-buffered saline (0.02 M, pH 7.3) to flush out the contents.

postulate that the indigenous microbial flora inhibits *L. monocytogenes* from colonizing the tract. We tested this hypothesis by using germ-free mice colonized with two known members of the normal flora, *Bacteroides* 116-2 and *Clostridium* 109-2, as a simplified model ecosystem. *Clostridium* 109-2 was chosen because it colonizes the mucous layer and the lumen of the colon and cecum, thus occupying space where *L. monocytogenes* marginates in monoassociated animals. *Bacteroides* is a necessary precursor in the colonization of the germ-free GI tract by *Clostridium* 109-2 (29). *Bacteroides* 116-2 colonized the lumen of the GI tract and is rarely found along the epithelium (29).

Three groups of mice were used. Group 1 was inoculated with *L. monocytogenes* S and served as a control. Group 2 was initially colonized by *Bacteroides* 116-2, followed 1 week later by *Clostridium* 109-2. Two weeks after the *Clostridium* inoculation, the mice were given *L. monocytogenes* S. Group 3 was initially colonized with *L. monocytogenes* S. Two weeks later the mice were colonized with *Bacteroides* 116-2, followed 1 week later by *Clostridium* 109-2. Portions of the GI tracts of two mice were examined histologically before a new organism was introduced to ensure that the bacterial type given earlier had colonized the tract appropriately. All triassociated mice were killed 2 weeks after the last bacterial type was given. All control mice were killed and sampled 3 weeks after they were given *L. monocytogenes* S.

L. monocytogenes S was successful in colonizing the GI tracts of mice previously colonized by *Bacteroides* 116-2 and *Clostridium* 109-2 (Table 7). Population levels of *L. monocytogenes* in the triassociated mice did not vary greatly from those of monoassociated controls. The sequence of the colonization also did not appear to affect the population levels of the pathogen as assessed by culture techniques.

By contrast, the pathogen was influenced in its ability to marginate on the colonic epithelium (Table 7). As expected from the results of other experiments, *L. monocytogenes* marginated well in animals in which only it was present. However, margination was seen in only two of eight mice in group 2 and was markedly reduced in those animals. The organism did not marginate in four of the animals in group 3 and did so poorly in the others. In animals in both groups 2 and 3, *Clostridium* 109-2 had colonized the mucous layer on the epithelium of the proximal colon. Likewise, *Bacteroides* 116-2 not only colonized the lumen but also marginated along the epithelium. Thus, these anaerobes may have prevented *L. monocytogenes* from marginating on the epithelium by colonizing the area, even though they were not able to prevent the organism from colonizing the lumen of the tract.

TABLE 7. *Population levels and margination of L. monocytogenes S in proximal colons of associated ex-germfree mice*

Group	Bacteria given[a]	No. of mice[b]/ group	*L. monocytogenes*/g of colon[c]	*Margination*[d]
1	*L. monocytogenes*	8	9 (9)	8 (4+)
2	*Bacteroides* 116-2, *Clostridium* 109-2, *L. monocytogenes*	8	8 (8)	2 (2+)
3	*L. monocytogenes, Bacteroides* 116-2, *Clostridium* 109-2	9	8 (8)	4 (1+)

[a] Listed in order given.

[b] Male, Crl:CD-1(ICR)GN.

[c] Median (range) $\log_{10}$ *L. monocytogenes* per gram (wet weight) of colon.

[d] Number of mice showing margination, defined as a layer of bacteria on or in mucus covering colonic epithelium in Gram-stained frozen histological sections examined by ordinary light microscopy at 250 diameters magnification (Fig. 1). The entire epithelial surface in each of three sections per animal were screened. (4+), 75 to 100% of microscopic fields showed margination; (2+), 25% of fields showed margination; (1+), 10 to 30% of fields showed margination.

DISCUSSION

Germfree mice developed disseminated focal necrosis after being given *L. monocytogenes* orally. However, only about 30% of them died from the infection. The survivors showed no obvious signs of disease but harbored a large bacterial population in their GI tracts. Thus, in addition to producing disseminated lesions, *L. monocytogenes* colonizes the GI tract of germfree mice. At 24 h after the mice were infected with about 100 of the organisms, the bacterial population level reached 10^5 to 10^7 bacteria per gram of tissue in the stomach and ileum and 10^8 to 10^9 bacteria per gram of tissue in the cecum and colon. Thus, *L. monocytogenes*, presumably a nonindigenous organism, colonized the GI tract of germfree mice to levels similar to those reached by indigenous bacteria (25). Histological examination revealed that within 2 weeks after inoculation, the organism had marginated along the mucous layer of the proximal colon. This margination suggests a possible interaction of *L. monocytogenes* with the mucous layer.

In contrast, SPF mice remained healthy after being given up to 5×10^7 bacteria. *L. monocytogenes* was isolated from the GI tract of only 1 mouse out of a total of 27 inoculated. We believe that interference by the indigenous microflora is involved in preventing the establishment of *L. monocytogenes* in the GI tract of SPF mice. Indigenous microbes are suspected to interfere with pathogens by direct and indirect mechanisms (20). Direct interference is mediated by some activity of the indigenous microflora which may affect the pathogen. Production of antimicrobial substances and competition for nutrients or attachment sites may be examples of direct interference (20). *L. monocytogenes* has been shown to be sensitive to *Streptococcus faecium* enterocin in vitro (15). In our model system, *Clostridium* 109-2 and possibly also *Bacteroides* 116-2 displaced *L. monocytogenes* from the mucous layer. Indirect interference involves some influence exerted by the indigenous flora on the host, which in turn may affect the interaction of the host and pathogen. For example, the rate of peristalsis is slower in germfree mice than in conventional mice (1). Thus, *L. monocytogenes* would be propelled faster through the bowel of SPF mice than those of germfree animals.

If indeed the indigenous microflora prevents the establishment of *L. monocytogenes* in the GI tract, then how does the microorganism persist in a population of presumably healthy animals in nature? We suggest one possible model that is consistent with our data and, we believe, is consistent with the literature. We propose that *L. monocytogenes* colonizes the GI tract during disruption of the balance within the microbial ecosystem. Depending on the degree and duration of the imbalance and the susceptibility of the animal, colonization by the pathogen, especially of intestinal surfaces, may result in a carrier state or an active infection.

Imbalance in the GI microbial ecosystem can be caused by outside environmental factors. For example, starvation (28) and antimicrobial drugs (19, 22, 24) alter the composition of the microbial communities, especially those on epithelial surfaces of murine GI tracts. Guinea pigs fed food containing *L. monocytogenes* after prolonged periods of starvation developed listeriosis, whereas control nonstarved animals did not (26). Similarly, in preliminary experiments in our laboratory, SPF mice have been deprived of food and bedding for 48 h and then inoculated with about 5×10^7 *L. monocytogenes* S. A control nonstarved group was inoculated simultaneously with the same dosage. The mice were killed 1 week later. *L. monocytogenes* was isolated from the GI tract of all starved animals and only one of the control mice.

Starvation undoubtedly has influences on mechanisms of host resistance other than just those mediating microbial interference in the GI tract. Thus, such findings are at best suggestive that interference from indigenous microbes in-

fluences colonization by *L. monocytogenes* in SPF animals. Nevertheless, such findings, taken together with our results reported herein on the behavior of the pathogen in monoassociated gnotobiotic mice strongly support a role for microbial interference as a limiting factor in colonization by *L. monocytogenes* of the GI tract.

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