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Pathogenesis of *Listeria monocytogenes* for Gnotobiotic Rats

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Listeria monocytogenes colonized the gastrointestinal tract of adult germfree rats (10^{10} to 10^{11}/g, dry weight) within 24 h after oral exposure. Between 3 and 14 days after monoassociation, *L. monocytogenes* caused a self-limiting pseudomembranous colitis, bacteremia, and infection of the spleen and liver. Monoassociation of rats with *Listeria* for 8 weeks stimulated 32- and 4-fold increases in serum immunoglobulin A (IgA) and IgG, respectively, whereas serum IgM decreased 2-fold. The normal microbial flora was inhibitory to *Listeria* colonization, since *L. monocytogenes* was cleared from the gastrointestinal tract of formerly monoassociated rats within 20 days after conventionalization and did not colonize the gastrointestinal tract of conventional rats after intragastric instillation of 10^8 viable *L. monocytogenes*. *Listeria*-monoassociated rats delivered large litters of healthy pups whose gastrointestinal tracts were slowly colonized with *L. monocytogenes*. Between 3 and 60 days of age, *Listeria*-monoassociated rat pups exhibited eight- and fourfold increases in serum IgG and IgM, respectively; however, serum IgA was elevated (16-fold) only at 9 to 15 days of age. Adult *Listeria*-monoassociated rats had acquired cellular resistance to intravenous challenge with *L. monocytogenes*. Prolonged monoassociation of *L. monocytogenes* in rats attenuated its virulence for conventional rats.

Parenteral challenge of experimental animals with *Listeria monocytogenes* has been extensively studied as a laboratory model for acquired cellular resistance to a facultative intracellular pathogen (10, 11). The natural portal of entry for this widely distributed microorganism, although not yet ascertained, is likely to be the alimentary tract, although the intravenous (i.v.) or intraperitoneal route is most commonly used for experimental listeriosis (7). There have been few studies of the pathogenesis of *Listeria* after oral infection, and fewer still of the effect that alimentary tract colonization and infection with *Listeria* have on the development of acquired cellular resistance. Zachar and Savage (18) reported that *L. monocytogenes* was able to colonize the gastrointestinal (GI) tract of germfree (GF) mice, cross their gut mucosa, and cause a systemic infection which killed 28% of the *Listeria*-monoassociated (MA) mice. These investigators also reported that *L. monocytogenes* colonized the GI tract of, but did not kill, mice that were diassociated with a *Bacteroides* and a *Clostridium* species, whereas *Listeria* did not colonize the GI tract of specific-pathogen-free mice. In contrast, MacDonald and Carter (9) reported that intragastric inoculation of specific pathogen free mice with *L. monocytogenes* led to a *Listeria* infection of the ileal Peyer's patches that subsequently disseminated to the mesenteric lymph nodes, liver, and spleen. MacDonald and Carter (9) also demonstrated that oral immunization with live *L. monocytogenes* protected mice against a subsequent oral or i.v. challenge with *L. monocytogenes*.

Since rats are reportedly more resistant than mice to oral (8) or i.v. challenge with *L. monocytogenes*, we decided to investigate the effect of monoassociation with *L. monocytogenes* on gnotobiotic rats. The aims of this study were as follows: (i) to ascertain whether *L. monocytogenes* can colonize the GF rats' GI tract and disseminate to internal organs; (ii) to assess the role of the normal microbial flora in preventing *Listeria* colonization and infection in rats; (iii) to determine whether *Listeria* monoassociation will cause abortion in pregnant rats; and (iv) to determine whether *Listeria*-MA rats develop acquired cellular resistance to *L. monocytogenes*.

MATERIALS AND METHODS

L. monocytogenes. *L. monocytogenes* (serotype 1, from the culture collection of the Department of Medical Microbiology, University of Wisconsin Medical School, Madison) was passaged twice in rats, recovered from a spleen homogenate, and inoculated

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into 50 ml of brain heart infusion broth (BHI; Difco Laboratories, Detroit, Mich.) at 37°C for 18 h. One-milliliter portions were stored at −70°C.

Rats. Twenty-four female 60-day-old GF Sprague-Dawley rats were housed at the University of Wisconsin Gnotobiotic Laboratory in a sterile flexible plastic isolator and fed an autoclaved diet (5010C; Ralston Purina Co., St. Louis, Mo.). Fecal specimens were cultured weekly for fungi and for aerobic and anaerobic bacteria as described previously (2). At no time was any contaminant recovered.

Monoassociation of rats with *L. monocytogenes*. A frozen culture of *L. monocytogenes* was thawed, inoculated into 20 ml of BHI and incubated for 18 h at 37°C, and the culture (2×10^9 *L. monocytogenes*/ml) was introduced into the GF isolator according to methods described previously (2). Each rat's mouth was swabbed with the culture, and the remainder of the inoculum was poured into the rats' drinking water.

Enumeration of *L. monocytogenes*. Within 1 h after removal from the isolator, rats were etherized and exsanguinated via cardiac puncture. One milliliter of citrated blood was collected, incubated with 9 ml of BHI at 37°C for 24 h, and then subcultured on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) to detect listeremia. The remainder of the blood was allowed to clot; the serum was harvested and stored at −70°C. Selected organs were placed in sterile glass homogenizers containing 9 ml of cold sterile peptone-saline (2 g of Tryptone [Difco] and 5 g of NaCl in 1 liter of deionized water) and homogenized. The homogenates were diluted in peptone-saline, plated on Trypticase-soy agar, and incubated at 37°C for 18 h. The bacterial counts were expressed as the $\log_{10}$ viable *L. monocytogenes* per gram of tissue.

Serum immunoglobulin levels. Serum levels of immunoglobulin M (IgM), IgG, and IgA were determined by the semiquantitative serial dilution Ouchterlony technique of Arnason et al. (1). Serum immunoglobulin titers are reported as the reciprocal of the highest twofold serum dilution giving a visible precipitin line against a constant dilution of the appropriate rat heavy-chain-specific antiserum (goat anti-rat γ-chain; Miles Laboratories, Inc., Elkhart, Ind.; goat anti-rat μ-chain and anti-rat α-chain, both courtesy of Kenneth Lee, University of Wisconsin, Madison).

Serum inhibition and serum agglutinins to *L. monocytogenes*. We have previously shown that rat serum kills *L. monocytogenes* in vitro (C. J. Czuprynski and E. Balish, manuscript in preparation). The ability of rat serum to inhibit the growth of *L. monocytogenes* was assessed by modification of a plaque assay described by Corbeil et al. (5). Briefly, 0.1 ml of a *Listeria* suspension in phosphate-buffered saline (PBS; 10^7/ml) was spread on a Trypticase-soy agar plate and allowed to dry. Twofold dilutions of rat serum in PBS were dropped (0.05 ml each) on the *Listeria*-inoculated plates and allowed to dry, and the plates were incubated at 37°C for 18 h. The titer of inhibition of *Listeria* growth was taken to be the highest serum dilution capable of visibly inhibiting *Listeria* growth as compared with a PBS control.

Serum agglutinins to *L. monocytogenes* were determined by serial twofold dilution of rat serum, in PBS, contained in the wells of a U-bottomed microtiter plate (Linbro, Hamden, Conn.). Each well then received an equal volume of formalinized *L. monocytogenes* suspended in PBS (5×10^9/ml). The plate was incubated at 37°C for 24 h, and the serum agglutinin titer was taken to be the highest serum dilution showing agglutination as compared with a PBS control.

Colonization of *Listeria*-MA rat pups. Two litters, consisting of 13 and 14 pups, respectively, were born on the same day to *Listeria*-MA females. Groups of three pups (never more than two from one litter) were killed at 3, 6, 9, 12, 15, 20, 25, 40, and 67 days of age, and the number of viable *L. monocytogenes* in the blood, spleen, liver, and different sections of the GI tract was determined.

Conventionalization of *Listeria*-MA rats. Eighteen 60-day-old rats (10 male and 8 female) were removed from the *Listeria*-MA isolator and housed in conventional animal quarters. Each rat's mouth was swabbed with a suspension of conventional (CONV) rat feces that was homogenized in peptone-saline. Groups of three rats were sacrificed within 1 h and at 1, 3, 5, 10, and 20 days after removal from the isolator (conventionalization). Organs were aseptically removed, homogenized, and plated on the following media: nalidixic acid-mannitol agar, a selective-differential medium for *L. monocytogenes* (18); Trypticase-soy agar to determine the total number of aerobic bacteria; and prereduced sheep blood agar in an anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) to determine the total number of anaerobic bacteria. Representative colonies were considered to be *L. monocytogenes* if they consisted of gram-positive, catalase-positive, pleomorphic rods that were motile at 22 but not 37°C and showed agglutination with *L. monocytogenes* serotype 1 antiserum (Difco).

Intragastric challenge of CONV rats. Twenty CONV rats were challenged with 10^8 viable *L. monocytogenes* via stomach gavage. Groups of four rats were sacrificed within 1 h and at 1, 3, 5, and 7 days after challenge. Organ homogenates were prepared, diluted, and then plated on nalidixic acid-mannitol agar to select for *L. monocytogenes*.

Immunization and challenge with *L. monocytogenes*. *L. monocytogenes* was grown in BHI for 18 h at 37°C and diluted to the appropriate *Listeria* concentration in peptone-saline. Twelve female 60-day-old CONV rats were injected i.v. with 10^4 *Listeria* in 0.5 ml of peptone-saline; 12 control rats received 0.5 ml of diluent. Seven days later, these two groups and 12 *Listeria*-MA rats were challenged i.v. with 10^5 *L. monocytogenes*. Four rats from each group were sacrificed at 1 h and at 2 and 4 days after challenge, and the number of *Listeria* in the blood, spleen, liver, and kidneys of each rat was enumerated.

Virulence of *L. monocytogenes* strains. A fresh fecal sample from a *Listeria*-MA rat and a portion of the stock *L. monocytogenes* strain used to originally monoassociate the isolator were cultured in 50 ml of BHI at 37°C for 18 h and diluted in peptone-saline. Each strain of *L. monocytogenes*, at a dose of 10^5, 10^6, or 10^7 viable *Listeria* per rat, was injected i.v. into 18 CONV rats (6 rats per group). The number of deaths that occurred within 7 days after i.v. challenge was recorded.

RESULTS

Effects of monoassociation with *L. monocytogenes* on total body and organ weight. Within 24 h after oral inoculation, *L. monocytogenes* colonized the rat and reached a population in the cecum of greater than 10^9 viable *Listeria* per g (dry weight), which then plateaued at approximately 10^{10} viable cells/g throughout the course of the experiment (Table 1). Although weight loss did occur during the 1st week of monoassociation (Table 1), no deaths occurred during the 8-week experiment, and the rats appeared to be healthy. The *Listeria*-MA rats also showed a transient reduction in cecal size, a phenomenon previously reported for rats monoassociated with some bacterial species (6). Splenomegaly, which peaked on day 7 with a twofold increase in spleen weight, was also observed in *Listeria*-MA rats.

Recovery of *L. monocytogenes* from blood, spleen, and liver. *L. monocytogenes* was first recovered from the spleen and liver on day 3 after monoassociation, peaked on day 5 (10^5 viable *Listeria*/g, wet weight), and then decreased 2 logs by day 7 and was no longer detectable by day 14. Listeremia paralleled *Listeria* counts in the spleen and liver (Fig. 1). The growth and clearance of *Listeria* from the spleen and liver of *Listeria*-MA rats was similar to the clearance of *Listeria* observed by others after i.v. injection of CONV rats with *L. monocytogenes* (11).

Serum immunoglobulins and anti-*Listeria* activity. Serum IgA, which was detectable in only two of four rats at 24 h after monoassociation with *Listeria*, showed a 32-fold increase in titer at 8 weeks, whereas there was an 8-fold increase in serum IgG and a 2-fold decrease in serum IgM during the same time period (Table 2). During the 8-week experiment, there was no significant change in either serum agglutinins to *L. monocytogenes* or serum inhibition of *Listeria* growth.

TABLE 1. *Effect of monoassociation with L. monocytogenes on total body and organ weight*

Day[a]	Viable listeria[b]	Wt (g, wet wt)[c]		
		Total body	Cecum	Spleen
1	9.43 ± 0.00	236 ± 9	19.71 ± 1.52	0.55 ± 0.04
3	9.73 ± 0.12	229 ± 4	8.25 ± 0.84	0.71 ± 0.03
5	9.89 ± 0.10	220 ± 4	6.34 ± 0.54	0.91 ± 0.03
7	10.01 ± 0.14	216 ± 9	10.22 ± 0.90	1.12 ± 0.10
14	10.67 ± 0.08	235 ± 10	9.92 ± 1.45	0.77 ± 0.05
8 wk	10.93 ± 0.10	307 ± 5	26.12 ± 0.33	0.63 ± 0.04

[a] Days (or weeks, as noted) after monoassociation with *Listeria*.
[b] Log_{10} viable *Listeria* per gram (wet weight) of cecal contents.
[c] Mean ± standard error of four rats.

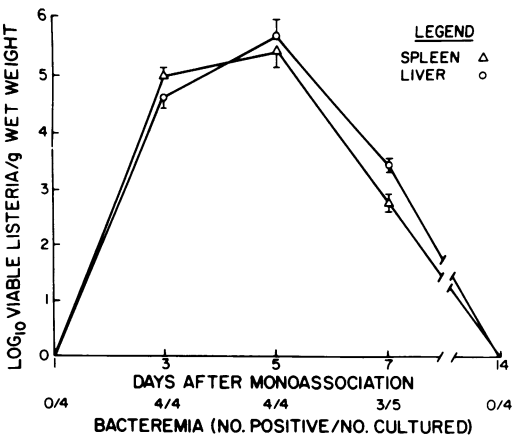

FIG. 1. *Recovery of L. monocytogenes from the blood, spleen, and liver of ex-GF rats monoassociated with L. monocytogenes.*

TABLE 2. *Effect of monoassociation with L. monocytogenes on serum IgA, IgG, and IgM*

Day	Mean reciprocal titer[a]		
	IgA	IgG	IgM
1	2[b]	16	64
3	ND[c]	16	64
5	ND	8	128
7	4[d]	16	128
14	8	64	64
8 wk	32	128	32

[a] Titers of sera from three to five rats. Mean reciprocal titers of IgA, IgG, and IgM for 60-day-old GF rats and 60-day-old CONV rats were (i) 0, 16, and 64 and (ii) 64, 256, and 16, respectively.
[b] Two of four rats had detectable IgA.
[c] ND, None detected.
[d] Four of five rats had detectable IgA.

Histopathology. Monoassociation with *L. monocytogenes* caused extensive histopathological changes in the rats. The stomach and small intestine were normal, although stimulation of ileal Peyer's patch development was observed by day 5 after oral inoculation. The most pronounced pathological changes were observed in the colon. Infiltration of mononuclear cells into the colonic mucosa and submucosa, which in some sections was so intense it obscured the normal colonic architecture, was initially observed on day 3 (Fig. 2). Inflammation continued to increase, causing ulceration of the colonic mucosa and pseudomembrane formation (Fig. 3) on days 5 through 7 after monoassociation. Repair of colonic lesions was evident by day 14, and it continued until, at 8 weeks after monoas-

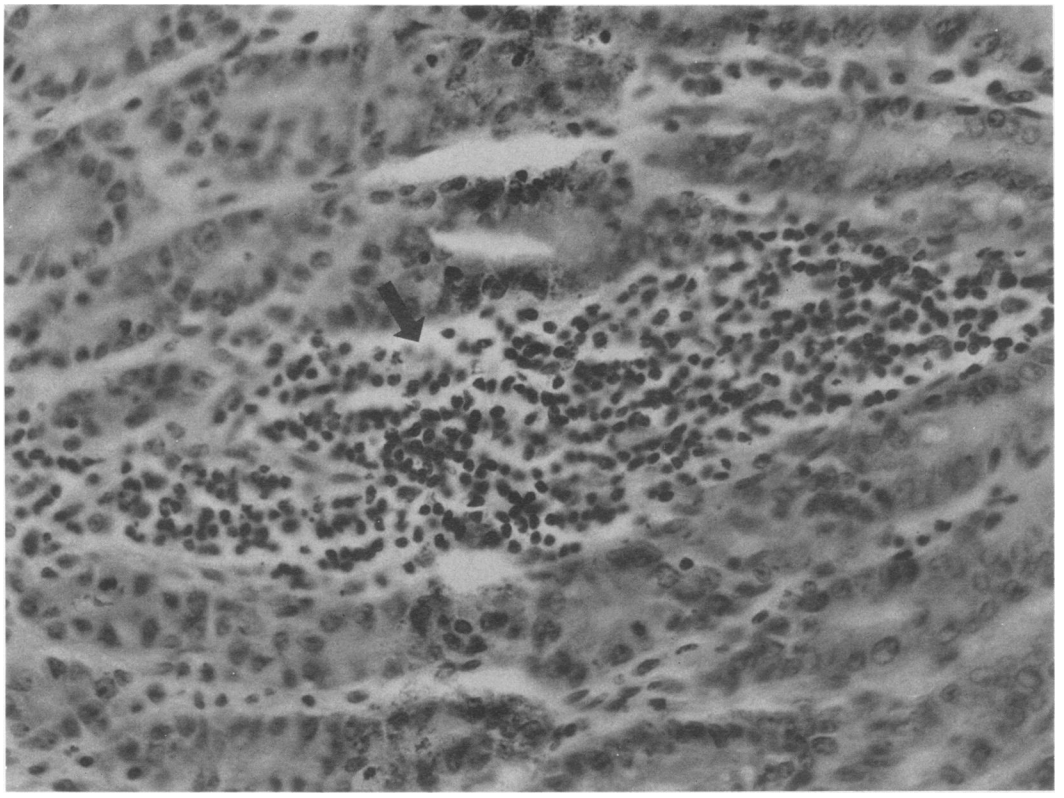

FIG. 2. *Infiltration of mononuclear cells (arrow) into the colonic mucosa of a rat 3 days after monoassociation with L. monocytogenes. Hematoxylin-eosin staining, ×225.*

sociation, the colon appeared normal. Mononuclear cell infiltration, compression of hepatic plates, and hemosiderin deposition in the liver were observed on days 5 and 7 after monoassociation (Fig. 4).

Reproduction and neonatal colonization. *L. monocytogenes* can cause septic abortion in both humans and animals, including rats (7, 8). In contrast, during 8 months of monoassociation *Listeria*-MA rats regularly delivered large litters of healthy young. Contrary to the rapid (24 h) gut colonization observed for adult GF rats, gut colonization with *L. monocytogenes* occurred slowly in young rat pups (3 to 6 days of age) and then gradually increased until, by 20 days of age, large numbers of *L. monocytogenes* were present in the stomach (10^6/g, dry weight), ileum (10^8/g, dry weight), and cecum (10^{10}/g, dry weight) (Table 3). *L. monocytogenes* was intermittently recovered from the spleen and liver of these rat pups, which was in sharp contrast to the dramatic spleen and liver infection seen when adult rats were monoassociated with *L. monocytogenes* (Table 3). Between 3 and 60 days of age the rat pups showed an eightfold increase in serum IgG, a fourfold increase in serum IgM, and a transient increase in both serum IgA and serum agglutinins to *L. monocytogenes* (Table 4).

Inhibition of *L. monocytogenes* by the rat gut flora. Within 24 h after conventionalization, the formerly *Listeria*-MA rats had acquired a large gut population (10^9 to 10^{10}/g, dry weight) of both aerobic and anaerobic bacteria. Concomitant with the acquisition of a conventional flora was a decrease in the number of *L. monocytogenes* recovered from the stomach, ileum, and cecum (Table 5). Twenty days after conventionalization, no *L. monocytogenes* was recovered from these organs even after cold enrichment of tissue homogenates at 4°C for 7 days. As a further indication of the inhibition of *L. monocytogenes* by the rat gut flora, viable *L. monocytogenes* could not be recovered from the GI tract of CONV rats that had received intragastric instillation of 10^8 viable *Listeria* 24 h earlier (data not shown).

Development of acquired cellular resistance to *L. monocytogenes*. During the first 48 h after i.v. injection of 10^5 viable *Listeria*, the

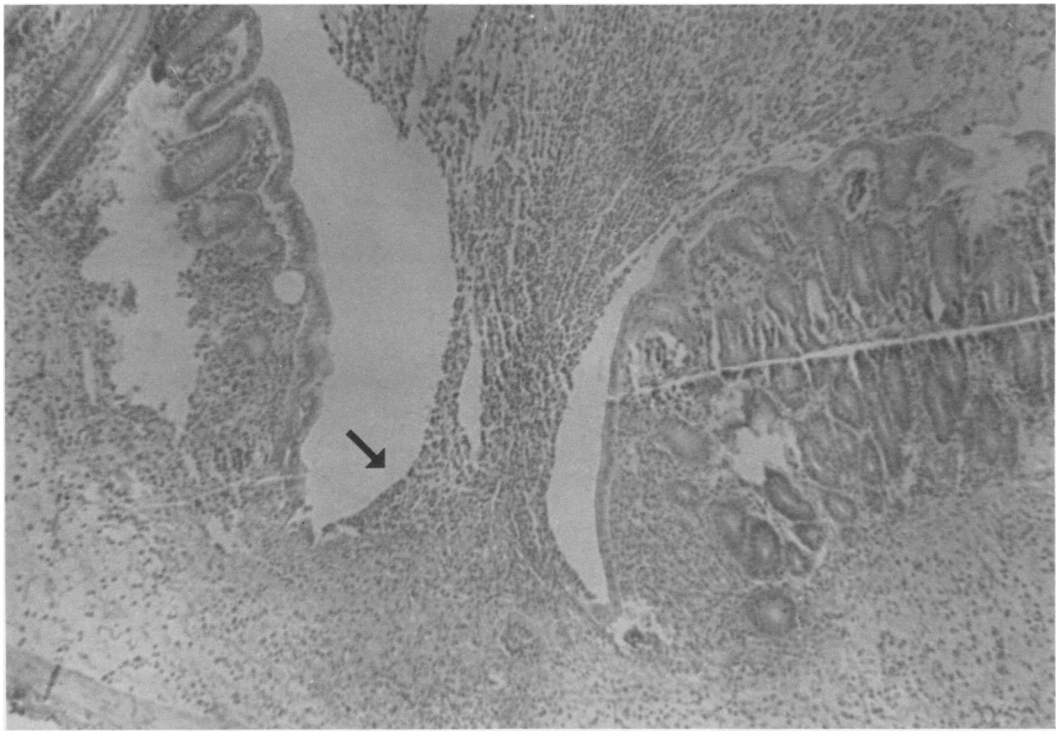

FIG. 3. *Pseudomembrane (arrow) adherent to a colonic ulcer in a rat 5 days after monoassociation with L. monocytogenes. Hematoxylin-eosin staining, ×30.*

number of *L. monocytogenes* in the spleen, liver, and kidneys decreased in both *Listeria*-MA and immune CONV rats, whereas in control CONV rats *Listeria* increased in number in these organs during the same time period (Fig. 5). *L. monocytogenes* was not recovered from the blood of MA or immune CONV rats; however, 2 and 4 days after i.v. injection, *Listeria* was recovered from blood cultures from four of four and two of four control CONV rats, respectively. The rapid clearance of *Listeria* from the blood, spleen, liver, and kidneys of *Listeria*-MA rats suggests that they had developed acquired cellular resistance to *L. monocytogenes*, although enhancement of nonspecific resistance mechanisms is also a possibility.

Attenuation of *L. monocytogenes* by gut passage. The constant maintenance of *L. monocytogenes* in the rat gut for 8 months caused an attenuation of its virulence for rats. Intravenous injection of CONV rats with 10^7 viable stock *L. monocytogenes* caused 100% mortality, whereas no rats died when injected with 10^7 organisms of the strain recovered from *Listeria*-MA rats. Both *Listeria* strains were identical in terms of Gram stain, cell and colony morphology, hemolysis on sheep blood agar, biochemical reactions (except that the MA strain was a weak sucrose fermentor, whereas the stock strain did not ferment sucrose), motility, and serology (both serotype 1).

DISCUSSION

L. monocytogenes rapidly colonizes and multiplies in the GI tract of adult GF rats (up to 10^{11}/g, dry weight), causing a pseudomembranous colitis, bacteremia, and subsequent infection of the spleen and liver. This study, together with the data of Zachar and Savage (18) and MacDonald and Carter (9) on orally challenged mice and the results of Osebold and Inouye (13, 14) on rabbits and sheep, supports the hypothesis that the GI tract can be a portal of entry for *L. monocytogenes*.

Listeria-MA rats develop acquired cellular resistance to *L. monocytogenes* as indicated by the clearance of *L. monocytogenes* that had disseminated to the spleen and liver after the monoassociation of GF rats and by the clearance of *L. monocytogenes* after the i.v. injection of *Listeria* into MA rats. The acquired cellular resistance to *L. monocytogenes* appears to effectively restrict *Listeria* to the rat gut. Cell-mediated immunity was shown by Owens and Berg

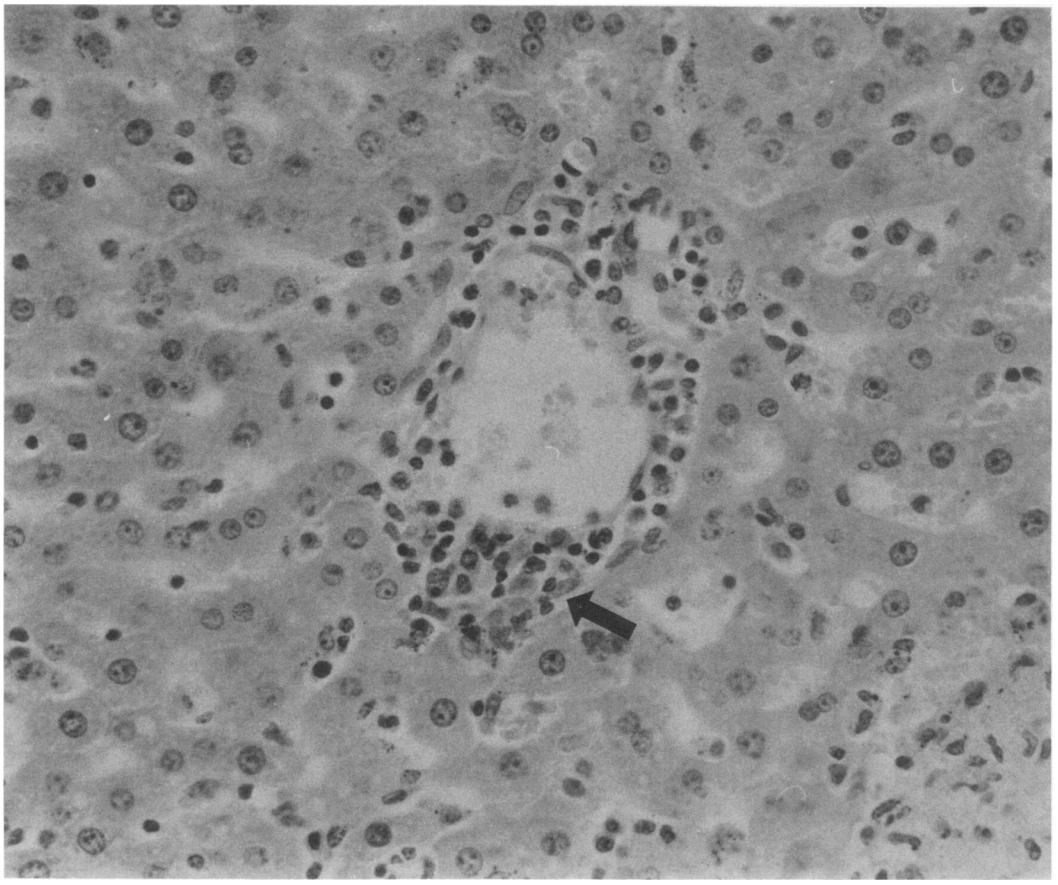

FIG. 4. *Mononuclear cell infiltration (arrow) surrounding a central vein in the liver of a rat 5 days after monoassociation with L. monocytogenes. Hematoxylin-eosin staining, ×250.*

TABLE 3. *GI colonization and translocation of L. monocytogenes in rat pups born to Listeria-MA mothers*

Age (days)	Viable listeria/g (dry wt)[a]				
	Stomach	Ileum	Cecum	Spleen	Liver
3	NT[b]	NT	3.04 ± 0.25[c]	NT	NT
6	4.71 ± 0.33	NT	5.49 ± 0.68[d]	NT	NT
9	6.60 ± 0.00	NT	7.84 ± 0.51[d]	1.64 ± 1.64	1.65 ± 1.65
12	6.39 ± 0.00	NT	8.16 ± 0.14[d]	ND[f]	ND
15	4.23 ± 0.21	NT	9.05 ± 0.20[d]	1.35 ± 1.35	1.22 ± 1.22
20	5.85 ± 0.50	7.76 ± 0.38	10.06 ± 0.21[e]	ND	ND
25	8.85 ± 0.26	8.54 ± 0.17	10.35 ± 0.07[e]	3.65 ± 1.98	3.33 ± 1.74
40	9.36 ± 0.27	8.04 ± 0.21	9.97 ± 0.08	0.85 ± 0.85	3.35 ± 1.72
67	8.65 ± 0.27	7.60 ± 0.27	9.63 ± 0.06	ND	ND

[a] Mean ± standard error $\log_{10}$ viable *Listeria* of three rats.
[b] NT, Not tested.
[c] Viable cells in entire GI tract.
[d] Viable cells in cecum and in large and small bowels.
[e] Viable cells in cecum and colon.
[f] ND, None detectable.

(15) and MacDonald and Carter (9) to limit the translocation of bacteria from the GI tract in mice. The role of cell-mediated immunity in protection against parenteral *Listeria* challenge is clearly established (10, 11). In the natural pathogenesis of listeriosis, however, other defense mechanisms may be of equal or greater importance. *Listeria*-MA rats had elevated lev-

TABLE 4. *Immunoglobulins and agglutinins to L. monocytogenes in sera from Listeria-MA rat pups*

Age (days)	Reciprocal titer of pooled sera[a]			
	IgM	IgG	IgA	Agglutinins
3	16	16	1	256
6	16	32	2	NT[b]
9	64	128	64	1,024
12	128	128	64	1,024
15	64	128	64	1,024
25	16	128	16	256
40	2	16	8	32
60	64	128	8	64

[a] Titers from three rat pups. Reciprocal titers of IgM, IgG, IgA, and *Listeria* agglutinins in pooled sera from 60-day-old GF and CONV rats were (i) 64, 16, 0, and 0 and (ii) 16, 256, 64, and 4, respectively.

[b] Not tested.

TABLE 5. *Effect of conventionalization on the persistence of L. monocytogenes in the rat GI tract*

Day post-conventionalization	Viable listeria/g (dry wt)[a]		
	Stomach	Ileum	Cecum
0	9.88 ± 0.14	8.88 ± 0.00	10.88 ± 0.04
1[b]	<3.00	6.53 ± 0.21	9.60 ± 0.08
3	1.60 ± 1.60	1.81 ± 1.81	5.41 ± 0.04
5	1.47 ± 1.47	1.47 ± 1.47	ND[c]
10	1.06 ± 1.06	2.22 ± 1.12	ND
20	ND	ND	ND

[a] Mean ± standard error $\log_{10}$ viable *Listeria* of four rats.

[b] Within 24 h of conventionalization, the stomach, ileum, and cecum contained 10^9 to 10^{11} aerobic and anaerobic bacteria per gram (dry weight) of tissue and contents.

[c] ND, None detected.

els of serum IgA, and presumably also secretory IgA, which could inhibit adherence of *L. monocytogenes* to the gut epithelium and confine *Listeria* to the intestinal lumen. The importance of the gut normal flora in host defense against *L. monocytogenes* is demonstrated by the rapid elimination of *L. monocytogenes* from the GI tract during the conventionalization of *Listeria*-MA rats and by the inability of *L. monocytogenes* to persist in the GI tract of CONV rats inoculated intragastrically with *L. monocytogenes*. The relatively low incidence of human listeriosis might reflect the normal flora's inhibition of ingested *L. monocytogenes*. The normal flora may be as important in preventing human listeriosis, particularly neonatal meningitis, as it is in preventing infant botulism, where the absence of inhibitory bacterial species from the gut flora of human infants and suckling CONV or adult GF mice allows the intestinal growth of and toxin production by *Clostridium botulinum* (12).

The association of *L. monocytogenes* with

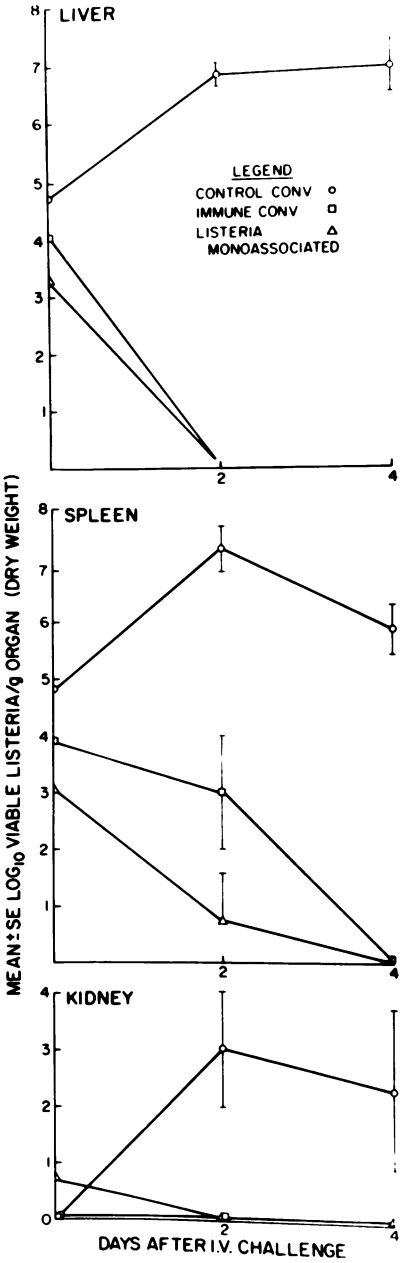

FIG. 5. *Recovery of L. monocytogenes from the liver, spleen, and kidney of control CONV, immune CONV, and Listeria-MA rats after i.v. challenge with 10^5 viable L. monocytogenes.*

pseudomembranous colitis has not been reported previously. The mechanism for the induction of this colitis is unknown. *L. monocytogenes* apparently does not produce an enterotoxin, since we observed no cytopathic effects when a PBS extract of *Listeria*-MA rat cecal contents was added to tissue culture cells. We

also did not observe any fluid accumulation after *L. monocytogenes* was injected into rabbit ileal loops according to the method of Sedlock and Diebel (17). *L. monocytogenes* does have invasive properties, since it is a facultative intracellular parasite and can penetrate epithelial cells (16). If the colitis results from *Listeria* invasion of the colonic mucosa, however, then the initial lesions would be expected to appear in the mucosal epithelium near the intestinal lumen. This was not the case, since the first pathological change noted was an intense mononuclear cell infiltration of the colonic mucosa and submucosa. *L. monocytogenes* has been reported to have a cell wall component that is mitogenic for B lymphocytes (4) and a lipid component which stimulates the monocytosis for which it is named (7). In the presence of the enormous gut burden of *L. monocytogenes*, perhaps lymphocyte and monocyte stimulation results in a hypersensitivity reaction that causes the colitis in *Listeria*-MA rats. *Listeria*-MA rats might be a valuable model for non-enterotoxin-induced colitis. *L. monocytogenes* is not considered to be an enteric pathogen; however, it is possible that in some cases of human colitis *Listeria* has been overlooked as an etiological agent since it is not a bacterial species that is commonly sought from human fecal specimens. Recent outbreaks of nosocomial listeriosis in which an association between GI distress and onset of listerial meningitis or septicemia was noted suggest that GI infection with *Listeria* may precede systemic infection (3).

L. monocytogenes is reported to cause abortion in both humans and animals (7). *Listeria*-MA rats, however, are unimpaired in their ability to breed regularly and deliver large litters (12 to 16 pups) of healthy young. This finding suggests that the pregnant MA rats had preexisting immunity to *L. monocytogenes* which protected both the mother and the developing fetuses.

Listeria-MA infant rats developed normally despite the tremendous number of *L. monocytogenes* present in their environment. Because of their age, it is assumed that these pups are immunoincompetent. Since the incidence of listeriosis is increased in immunosuppressed humans and animals, it is surprising that these MA rat pups were not more susceptible to the *L. monocytogenes* in their environment. Perhaps the pups' slow gut colonization with *L. monocytogenes* was important in their resistance to *Listeria*. There are several possible explanations for the slow colonization. The mothers' colostrum might contain a humoral or cellular component that transfers protection against *L. monocytogenes* to the rat pups. Alternatively, the infant rat gut may be an inhospitable environment for *L. monocytogenes* because of nutritional or physiological factors. A similar phenomenon has been observed in the GF mouse model for infant botulism, in which *C. botulinum* cannot colonize the intestinal tract of GF suckling mice that are less than 4 days old (12). The decreased virulence of *L. monocytogenes* after prolonged monoassociation could also explain the infant rats' resistance to *L. monocytogenes*, although it is not clear at what point in the study the attenuation of *Listeria* occurred.

Prolonged passage of *L. monocytogenes* in the MA rat gut (8 months) decreased its virulence for CONV rats. The attenuation of *Listeria* could have been caused by intestinal secretions (i.e., bile salts, protease, and IgA) or by the induction of a phenotypic change in response to the nutritional environment in the rat gut. Despite the attenuation of virulence, there was virtually no difference between the MA and stock *L. monocytogenes* strains as determined by standard biochemical and serological tests for identification of *L. monocytogenes*. The attenuation of pathogenic bacteria by passage in the GI tract, as a means of producing suitable vaccine strains, remains an intriguing possibility.

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