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Cell-Mediated Immunity to Intestinal Infection

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Specified pathogen-free B6D2F1 mice were orally infected with various doses of *Listeria monocytogenes*. Oral inocula containing more than 2.5×10^8 live organisms consistently initiated infection in the Peyer's patches (PP) of the small intestine. At lower doses the incidence of infection was sporadic, with many mice showing no apparent infection in the PP. The PP appeared to be the only site of tissue invasion and *L. monocytogenes* survival in the intestinal tissues, as no organisms were recovered from mucosa dissected free of all visible PP. Within the PP, the bacteria multiplied and the infection then disseminated to the mesenteric lymph node (MLN), liver, and spleen. However, bacteria were almost completely eliminated from all tissues, both systemic and gut-associated by 6 days postinfection. Mice given a primary *L. monocytogenes* infection by the oral route were highly resistant to subsequent intravenous or oral challenge. Likewise, sublethal intravenous infection rendered mice highly resistant to subsequent oral infection. In addition, lymphocytes from the PP, MLN, and spleens of mice recovering from a primary oral infection were able to adoptively transfer immunity to normal recipients. Finally, after oral infection, mice did not display peripheral delayed hypersensitivity to *L. monocytogenes* antigens until the organisms had penetrated to the spleen.

The natural route of infection of invasive pathogenic enteric bacteria such as *Salmonella* spp. and *Yersinia enterocolitica* is from the gut lumen into the Peyer's patches (PP) of the distal ileum (3-6, 11). Within the PP, the organisms multiply rapidly and then disseminate into the mesenteric lymph nodes (MLN) and systemic tissues. For some unknown reason, even the penetration of very few organisms into the PP leads to systemic disease (5, 11) and as few as 10 virulent *S. enteritis* initially present in the PP are sufficient to result in the death of the murine host (5). Therefore, an understanding of the ways in which immunity in the PP, either specific or nonspecific, may be able to eliminate the initial small number of invading bacteria is of great importance in the development of immunotherapeutic regimes to protect against infectious organisms derived from the intestinal lumen.

Acquired immunity to the facultative intracellular parasite *Listeria monocytogenes* is exclusively cell mediated (16, 17, 20, 21). The mechanism of protection being via the generation of sensitized T lymphocytes which induce, at the site of infection, a population of activated macrophages with enhanced microbicidal properties (16, 17, 20-22). Therefore, by following the growth and destruction of *L. monocytogenes* in the PP and MLN, it should be possible to assess the role of cell-mediated immunity in these tissues. As parenteral injection of *L. monocytogenes* results in spleen and liver infection, but no PP infection (MacDonald and Carter, unpublished data), a model of oral listeriosis in mice was developed which resulted in reproducible *L. monocytogenes* infection in the gut-associated lymphoid tissues.

This paper concerns itself with a description of the model of oral listeriosis in mice and reports the kinetics of oral *L. monocytogenes* infection in normal and immunized animals. Our results clearly show that mice are susceptible to oral infection with *L. monocytogenes* and that after elimination of the primary infection, they become immune to subsequent intragastric or intravenous (i.v.) infection.

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MATERIALS AND METHODS

Animals. Mice used in these experiments were inbred C57BL/6 × DBA/2 Tru F_1 mice ($B6D2F_1$), of both sexes, age 5 to 8 weeks, obtained from the animal breeding facility at Trudeau Institute.

L. monocytogenes. *L. monocytogenes* EGD at an i.v. 50% lethal dose of 4×10^5 organisms in $B6D2F_1$ mice was used in this study. To prepare *L. monocytogenes* for oral infections, we injected i.v. several mice with a dose of 10 50% lethal doses from a stock frozen culture. On day 3, the mice were killed and their spleens were removed and homogenized in 5 ml of sterile saline. A portion of the homogenate was then added to 500 ml of Trypticase soy broth (BBL Micro-

biology Systems Cockeysville, Md.) and cultured at 37°C for 12 to 14 h. At the end of the culture period, the bacteria were concentrated 10-fold by centrifugation and suspended in 50 ml of cold Hanks balanced salt solution containing 5% heat-inactivated fetal calf serum. The resultant concentration of bacteria was usually between 10^9 and 5×10^9 viable organisms per ml. Mice were then given 0.2 ml of the bacterial suspension intragastrically by a feeding tube.

For i.v. injections, a stock frozen culture of *L. monocytogenes* (5×10^8/ml) was diluted to the appropriate concentration in sterile saline and injected via the left lateral tail vein in a volume of 0.2 ml.

Bacterial enumeration. The numbers of bacteria in the MLN, spleen, or liver were determined by plating serial 10-fold saline dilutions of organ homogenates on Trypticase soy agar (BBL). The lung was also routinely cultured to ensure that the oral injection of *L. monocytogenes* was injected entirely into the stomach. The results from any mice showing lung infection were discarded.

L. monocytogenes were enumerated in the PP and intestinal wall by homogenizing these tissues in sterile saline and plating serial 10-fold dilutions on phenylethyl alcohol (PEA) agar supplemented with glycine and lithium chloride (18). *L. monocytogenes* in the intestinal lumen were washed out by perfusing the small intestine with 10 ml of saline and plating the washouts on PEA agar. Preliminary studies showed that commercially available PEA agar was selective, but not as quantitative, as PEA agar made fresh from basic ingredients. Thus all samples were cultured on PEA agar containing 1.5 g of Trypticase peptone (BBL) per 100 ml, 0.5 g of phytone peptone (BBL) per 100 ml, 0.5 g of sodium chloride per 100 ml, 0.25% 2-phenylethanol (Sigma), and 1.5% agar Noble (Difco) prepared within 14 days of its use. In addition, to determine the best media for *L. monocytogenes* isolation and quantitation, we cultured the organism on PEA agar containing different concentrations of glycine and lithium chloride. In our system fresh PEA agar containing 0.05% lithium chloride and 1% glycine was selective and gave quantitative counts of *L. monocytogenes* which did not differ from those obtained when the same number of organisms were seeded on Trypicase soy agar. Also, it gave maximum intensity of the bluish-green sheen characteristic of *L. monocytogenes* colonies on this medium when observed under oblique white light.

Cell transfer. Mice were injected with *L. monocytogenes* by the oral or i.v. route. Six days later, the mice were given 5,000 U of penicillin intraperitoneally. Cells were then transferred on day 7. Spleen and MLN were disrupted by grinding fragments of the organs through a wire screen, clumps were allowed to settle out, and the cells in suspension were washed three times in Hanks balanced salt solution. A number of cells equal to one organ equivalent were then infused i.v. into normal recipients. PP cell suspensions were made by carefully dissecting the patches from the intestinal wall and teasing the tissues apart in Hanks balanced salt solution. The PP fragments were further broken up by passing them up and down several times in a 1-ml syringe. Any remaining clumps were then allowed to settle out. The cells in suspension were washed three times in Hanks balanced salt solution and infused into normal recipients. Immediately after cell transfer, all recipient mice and untreated controls were injected i.v. with 1×10^5 to 2×10^5 *L. monocytogenes*. Forty-eight hours later, the mice were killed and the numbers of viable *L. monocytogenes* in the spleen were established.

DTH to *L. monocytogenes*. Soluble *L. monocytogenes* antigens were kindly provided by R. J. North. Delayed footpad reactions were elicited by injecting 20 μg of the *L. monocytogenes* antigen in 0.04 ml of phosphate-buffered saline into a rear footpad (20). Delayed-type hypersensitivity (DTH) was measured as the difference at 24 h between the thickness of the foot receiving *L. monocytogenes* antigens and the contralateral untreated foot. All measurements were made with dial gauge calipers (Schnelltaster, H. C. Kroplin, GMBH, Hassen, West Germany) and are expressed in units of 0.1 mm.

Results and statistics. All results, where applicable, are expressed as mean ± 1 standard error. Unless otherwise stated, there were five mice per group. Differences between means were estimated by Student's *t* test.

RESULTS

Pathogenicity of *L. monocytogenes* given by the oral route. Preliminary experiments indicated that after the oral injection of a large dose of *L. monocytogenes*, the only major site of *L. monocytogenes* infection in the intestine was the PP. A representative experiment showing the number of *L. monocytogenes* present at various sites 24 h after a single intragastric injection of 2.5×10^8 organisms is shown in Table 1. Low numbers of *L. monocytogenes* were detectable in the small intestinal contents, demonstrating a small residual population persisting after the

TABLE 1. *Recovery of L. monocytogenes from various sites in the small intestine*[a]

Animal no.	Log_{10} *L. monocytogenes*		
	Small intestinal contents[b]	Small intestinal wall[c]	PP
1	2.1	0	4.2
2	0	0	3.9
3	0	0	4.1
4	1.6	0	3.8
5	2.4	0	3.5
No. of mice showing infection	3/5	0/5	5/5

[a] At 24 h postinfection with 2.5×10^8 *L. monocytogenes* per os.

[b] The small intestine was removed by dissection, and the contents were washed out with 10 ml of saline and plated on PEA agar.

[c] The remaining small intestine after all visible PP had been removed by dissection.

large oral inoculum. On the other hand, no organisms were recoverable from homogenates of the small intestinal mucosal wall which had been carefully dissected free of all visible PP. This result is rather surprising in view of the fact that organisms were detected in the intestinal lumen. It is possible that our data are a true reflection of the inability of *L. monocytogenes* to colonize the mucus layer of the gut of normal mice, as was also shown recently by Zachar and Savage (35). However, real consideration must be given to the possibility that a combination of the use of PEA selective media and the release of bactericidal substances from inflammatory cells in the bowel wall during homogenization could sufficiently inhibit the growth of *L. monocytogenes* as to render the organisms undetectable.

Nevertheless, in all of the mice studied high numbers of *L. monocytogenes* were present in the PP. From this experiment it may be concluded that after oral infection, the primary site of *L. monocytogenes* tissue invasion and survival is probably the PP.

The minimum oral inoculum necessary to uniformly infect the PP of all mice was established in other experiments. Groups of mice were given graded doses of *L. monocytogenes* by the oral route, and 24 h later the PP were removed by dissection and the number of *L. monocytogenes* in these tissues was then estimated. The results shown in Table 2 demonstrate that at all doses greater than, and including, 2.5×10^8 *L. monocytogenes*, all mice were infected; however, at both lower doses (5×10^7 and 5×10^6), infection only occurred sporadically. Therefore, in all subsequent studies, mice were given between 2.5×10^8 and 2.5×10^9 *L. monocytogenes*.

Systemic spread of *L. monocytogenes* after oral infection. To establish whether the nidus of *L. monocytogenes* infection in the PP disseminated into other tissue, we orally injected a large group of mice with *L. monocytogenes* and at various intervals thereafter estimated the numbers of organisms in gut-associated and systemic tissues. These results are shown in Table 3. One day postinfection, all of the mice showed infection with *L. monocytogenes* in the PP; two mice had detectable *L. monocytogenes* in the MLN; and there was sporadic low-level infection in the livers and spleens. On days 2 and 3, however, nearly all of the mice had *L. monocytogenes* in the MLN, and by day 4 the organism was detectable in the livers and spleens of all mice. The number of *L. monocytogenes* in all tissues, both systemic and gut-associated, dropped dramatically on day 6, and by days 8 and 10, virtually no organisms could be cultured from any tissues.

These results are consistent with the notion that after an oral injection of *L. monocytogenes*, there is penetration and growth in the PP; the

TABLE 2. *Recovery of L. monocytogenes from the PP of mice given different oral doses*[a]

Animal no.	$\log_{10}$ *L. monocytogenes*				
	2.5×10^9	5×10^3	2.5×10^8	5×10^7	5×10^6
1	4.4	2.3	4.1	0	0
2	4.3	4.1	4.3	3.8	0
3	2.2	3.8	2.0	0	0
4	4.3	4.7	3.9	0	0
5	4.5	3.8	4.0	4.1	0
No. of mice showing infection	5/5	5/5	5/5	2/5	0/5

[a] For bacterial enumeration, PP were dissected from the mice 24 h after oral infection.

TABLE 3. *Systemic spread of L. monocytogenes after oral infection*[a]

Day postinfection	Animal no.	$\log_{10}$ bacteria/organ			
		PP	MLN	Spleen	Liver
1	1	4.1	3.6	0	3.4
	2	4.3	1.7	2.4	0
	3	2.0	0	0	2.3
	4	3.9	0	0	0
	5	4.0	0	0	0
2	1	3.6	3.6	4.3	3.3
	2	5.7	0	0	0
	3	5.2	3.5	0	0
	4	3.7	4.0	4.0	3.8
	5	3.9	4.7	3.4	3.3
3	1	3.6	3.8	4.0	2.9
	2	3.2	5.1	2.8	4.0
	3	2.5	4.0	0	0
	4	3.1	4.5	2.0	3.4
	5	3.2	3.6	0	0
4	1	2.9	0	4.2	2.7
	2	2.9	2.9	4.4	3.5
	3	3.0	0	4.3	3.0
	4	1.9	3.7	4.1	3.3
	5	2.6	3.7	4.0	3.2
6	1	1.9	3.4	3.5	3.1
	2	2.8	0	0	0
	3	3.0	0	1.6	0
	4	2.2	0	0	0
	5	2.0	3.2	0	2.9
8	1	0	0	2.1	0
	2	0	0	0	0
	3	0	0	2.6	0
	4	0	0	0	0
	5	0	0	0	0
10	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0

[a] A total of 2.5×10^8 *L. monocytogenes* per os.

organisms then pass to the MLN, probably via the lymph, where they again multiply and eventually disseminate into the deep organs. Infection, however, was never fatal, as even 6×10^9 *L. monocytogenes* per os failed to cause any deaths (data not shown).

Growth of secondary *L. monocytogenes* infection in mice immunized by the oral route. Experiments were carried out to establish whether mice given a primary oral infection were subsequently immune to oral or i.v. challenge. Figure 1 shows the *L. monocytogenes* growth curve after i.v. infection in the livers and spleens of control mice, and of mice who had previously received a single oral infection. In immunized mice, there was virtually no *L. monocytogenes* growth in the liver and spleen over the 3-day period, whereas in control mice the organisms multiplied rapidly reaching levels greater than 10^7 in the spleen and 10^8 in the liver.

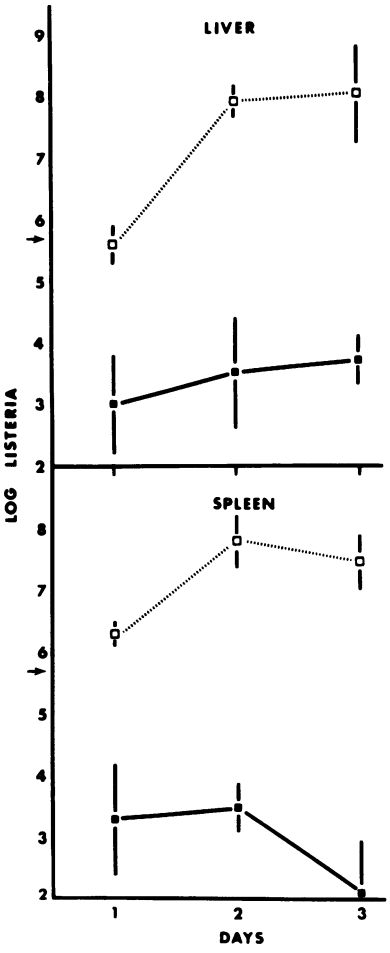

FIG. 1. *Growth of L. monocytogenes in the spleens and livers of control mice (broken line) and mice recovering from a primary oral infection with 10^9 L. monocytogenes (solid line). Mice were challenged with 4.5×10^5 L. monocytogenes i.v. 7 days after the primary infection. Means ± 1 standard error of five mice per group.*

Mice which had recovered from a primary oral infection were also resistant to a second oral infection (Table 4). There were no dramatic differences between control and immunized mice at day 1; however, on days 2 and 3, only small numbers of *L. monocytogenes* were recoverable from immunized mice, whereas there was extensive growth of *L. monocytogenes* in the tissues of the control mice.

Growth of a secondary *L. monocytogenes* infection in mice immunized by the i.v. route. It is apparent from the above results that primary oral *L. monocytogenes* infection renders mice highly resistant to subsequent oral or i.v. infection. It was decided, therefore, to investigate whether prior i.v. infection, which renders mice highly immune to i.v. challenge (16, 17, 20), also resulted in mice being resistant to oral infection. Table 5 shows the results of such an experiment. Some *L. monocytogenes* were recovered from the PP and MLN of immunized mice on day 1, although the level of infection was not as uniform, nor as high, as in control mice. However, on days 2 and 3, virtually no *L. monocytogenes* were detected in the PP or MLN of immunized mice, whereas there was extensive *L. monocytogenes* growth in the tissues of the control mice.

Eight days after the i.v. immunizing dose of 10^4 *L. monocytogenes* and penicillin treatment, four of five mice retained relatively large numbers of *L. monocytogenes* in the spleen. This result contrasts with the results of other workers who have demonstrated almost complete elimination of bacteria from the tissues by 5 to 6 days postinfection (16, 20, 21). Possible explanations for this unexpected finding may be higher i.v. immunizing doses of bacteria used in this study or differences in the rate of elimination of residual bacteria between different strains of mice.

Transfer of anti-*L. monocytogenes* immunity. Protective immunity to *L. monocytogenes* can be adoptively transferred by lymphocytes (16, 17, 20). Therefore, cells were isolated from mice recovering from a primary oral *L. monocytogenes* infection and infused into normal recipients in an attempt to demonstrate lymphocytes capable of mediating anti-*L. monocytogenes* immunity in the gut-associated lymphoid tissues. These mice and untreated control mice were then immediately challenged with 1.5×10^5 *L. monocytogenes* i.v. Protection was measured as a decrease in the numbers of *L.*

TABLE 4. *Growth of an oral L. monocytogenes infection in the tissues of mice immunized by a primary oral infection*[a]

Day after oral exposure	Animal no.	Log_{10} *L. monocytogenes*/organ					
		PP		MLN		Spleen	
		Immune[b]	Control[c]	Immune	Control	Immune	Control
1	1	3.3	3.7	0	0	4.0	2.5
	2	0	4.6	0	3.5	0	2.7
	3	0	1.6	1.7	3.5	0	2.0
	4	2.0	1.9	1.7	2.0	0	0
	5	2.0	3.5	1.7	3.5	3.2	0
	6	0	3.6	0	0	0	0
	7	4.0	0	1.7	0	0	0
	8	1.3		1.7		0	
	9	2.2				3.8	
	No. infected[d]	6/9	6/7	5/8	4/7	3/9	3/7
2	1	0	4.2	0	4.6	0	2.8
	2	2.4	4.2	0	4.8	0	2.7
	3		4.0	0	4.9	0	3.9
	4		4.4	0	4.7	0	4.6
	5		3.3	0	4.2	2.5	3.8
	6	1.3	3.1	0	4.4	2.7	3.1
	7			0	4.0	0	0
	8		3.7	0	4.3	0	3.5
	9		3.4	0	3.5	0	4.0
	No. infected	2/9	8/9	0/9	9/9	2/9	8/9
3	1	0	3.9	0	4.3	0	3.7
	2	0	3.8	0	4.4	0	3.3
	3	0	3.2	0	4.0	0	2.2
	4	2.0	1.3	0	5.1	1.7	3.2
	5	2.0	3.4	0	4.8	0	4.6
	6	0	2.1	0	4.6	0	2.8
	7	0	0	0			
	8	0		0	5.3	3.2	4.1
	9	0	3.5	0	4.6	0	4.3
	No. infected	2/9	7/8	0/9	8/8	2/9	8/8

[a] Mice were immunized by a single oral injection of 5×10^8 *L. monocytogenes*. Seven days later, along with untreated controls, they were given an oral injection of 2×10^9 *L. monocytogenes*.

[b] Mice receiving an oral infection 7 days after a primary oral exposure.

[c] Mice receiving a primary oral exposure.

[d] Different numbers of animals per group are shown because some results were discarded due to the inoculum being injected into the lung rather than the stomach.

monocytogenes recoverable at 48 h from the spleen of mice receiving lymphocytes, compared to the numbers of *L. monocytogenes* in control spleens. The results (Table 6) indicate that partial protection was achieved with cells from the PP, MLN, and spleens of mice recovering from a primary oral infection. The greatest level of protection conferred to normal recipients was by spleen cells from orally immunized mice. Cells from the PP and MLN also transferred some protection, although not of the same magnitude as spleen cells. This difference was probably related to the low numbers of PP and MLN cells which were transferred.

Development of DTH to *L. monocytogenes* after oral injection. After immunization of mice with *L. monocytogenes* by the i.v. route, a DTH reaction can be elicited in a rear footpad by an injection of a small amount of *L. monocytogenes* antigen (20). The development of DTH was therefore studied in mice given *L. monocytogenes* by the oral route. Figure 2 shows the results, in which it can be seen that significant DTH reaction was not elicited until day 6 postinfection.

DISCUSSION

There have been several reports in the literature suggesting that the natural route of infection of *L. monocytogenes* for humans and animals occurs via the gastrointestinal tract (1, 2, 9, 19, 23, 24, 31). Unfortunately, although the evi-

TABLE 5. *Growth of an oral L. monocytogenes infection in the tissues of mice immunized by the i.v. route*[a]

Day after oral exposure	Animal no.	Log_{10} *L. monocytogenes*/organ					
		PP		MLN		Spleen[d]	
		Immune[b]	Control[c]	Immune	Control	Immune	Control
1	1	0	3.0	0	4.2	3.7	2.4
	2	2.9	3.6	0	4.1	3.7	0
	3	3.0	4.1	2.7	3.9	0	0
	4	2.2	4.2	2.4	3.4	4.0	0
	5	2.9	4.3	2.1	3.5	3.9	2.1
2	1	1.7	3.0	0	4.3	3.2	2.6
	2	0	3.9	0	4.7	3.6	3.3
	3	0	2.9	1.6	4.7	3.2	4.9
	4	0	3.9	0	4.4	3.9	3.1
	5	0	3.4	0	4.5	3.5	3.2
3	1	0	2.4	0	4.4	3.1	2.8
	2	0	2.2	0	4.8	3.2	3.8
	3	0	3.2	0	4.8	2.5	3.6
	4	0	2.2	0	4.3	3.8	4.7
	5	0	1.3	0	5.0	0	4.7

[a] Mice were immunized with 10^4 *L. monocytogenes* i.v. on day 0. On day 6 they received 5,000 U of penicillin intraperitoneally, and on day 7 they were given 2×10^9 *L. monocytogenes* per os. Cultures of the PP and MLN of i.v.-immunized mice were uniformly negative for *L. monocytogenes*, all of the bacteria being located in the liver and spleen.

[b] Mice receiving an oral infection 7 days after a primary i.v. injection.

[c] Mice receiving a primary oral infection.

[d] Residual *L. monocytogenes* infection from the primary immunizing dose.

TABLE 6. *Transfer of anti-L. monocytogenes immunity into normal recipients by PP, MLN, and spleen cells*[a]

Transfer of	Log_{10} *L. monocytogenes* in the spleen		Difference (log_{10})	*P*
	Recipients	Controls		
PP cells[b]	6.3 ± 0.3	7.2 ± 0.1	0.9	0.001
MLN cells[c]	5.8 ± 0.2	6.7 ± 0.1	0.9	0.05
Spleen cells[c]	4.0 ± 0.1	7.0 ± 0.3	3.0	0.001

[a] Mice were immunized by a single oral dose of 2×10^9 *L. monocytogenes*, and cells were transferred 7 days postinfection into groups of five normal recipients. These mice and untreated controls were then immediately injected with 1.5×10^5 *L. monocytogenes* i.v. The numbers of *L. monocytogenes* in the spleens were determined 48 h later.

[b] Recipient mice received 1.5×10^7 cells from immunized donors.

[c] Recipient mice received one organ equivalent from donor mice (MLN, 3.0×10^7 cells; spleen, 1.8×10^8 cells).

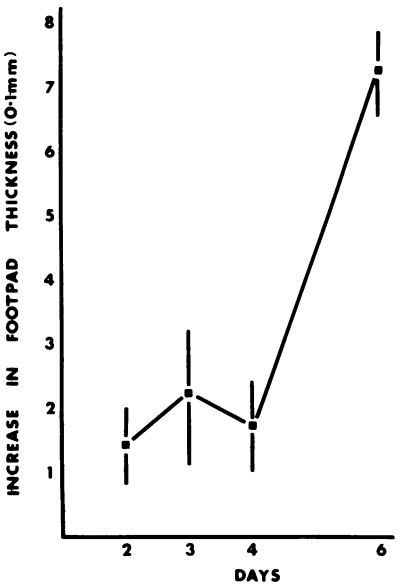

FIG. 2. *Development of DTH to L. monocytogenes antigens after a single oral injection of 2.5×10^8 L. monocytogenes. Means ± 1 standard error of five mice per group.*

dence is compelling, it remains circumstantial. Experimental studies in mice have shown that it is possible to produce systemic infection and even death by giving large doses of *L. monocytogenes* orally (12). In addition, it is possible to systemically infect rabbits (10, 23) and sheep (24) by intragastric administration of *L. monocytogenes*. The demonstration of *L. monocytogenes* in the mesenteric nodes of sheep and rabbits infected intragastrically (23, 24) is some evidence that infection is derived from the intestine. However, this may also reflect a generalized bacteremia following dissemination of the organisms from other foci of infection. Unfortunately, no studies have been carried out to trace the exact route of infection of *L. monocytogenes* from the gut to the systemic tissues and also to determine the immunological sequelae of oral infection.

In the present study, we investigated the events which occur after a single intragastric injection of *L. monocytogenes* in mice. The first important findings were that infection of the PP could occur in a few mice at relatively low doses of *L. monocytogenes*, but at higher oral doses of *L. monocytogenes* ($>2.5 \times 10^8$), all of the mice became infected. Moreover, the route of tissue invasiveness and survival was the PP and not the intestinal villi. The apparent predilection of pathogenic enteric bacteria to penetrate into, and multiply within, the PP is not well understood. After an intragastric injection with certain

Salmonella spp., bacteria adhere to the villi and have been visualized penetrating the villus epithelium (32-34). However, there seems to be little or no growth of the organism in the lamina propria (5). The reason for this is not clear, but may be related to the fact that the lamina propria of the villi contain many macrophages (30), which may phagocytose and eliminate the invading bacteria. In contrast, when only a few bacteria penetrate into the PP, there are rapid growth of the organisms and subsequent systemic dissemination (5). In fact, specialized absorptive cells in the PP epithelium may be disadvantageous to the host, as pathogenic bacteria may utilize this route to enter the tissues (26, 27). However, the ability of bacteria to penetrate the intestinal epithelium seems not in itself to be responsible for the infection in the PP; for bacteria may cross the epithelium overlying the villi (32) and yet do not give rise to local mucosal infection (5). These results would appear to indicate that in the PP, cells capable of mediating nonspecific antibacterial immunity (polymorphs, macrophages) are either not present or are functionally inactive. The former proposition is untenable by virtue of the fact that by histology, macrophages are present in the PP (28). However, nonfunctional, adherent cells (macrophages?) have been documented in murine PP by other investigators (13). Although this defect was reported in the induction of cell-mediated allograft reactions and humoral antibody synthesis in vitro, it may be that there is a generalized defect in PP macrophages such that they are also incapable of killing invading pathogenic bacteria. This view, however, must be reconciled with the observation that feeding particulate material produces particle-filled macrophages in PP (15), indicating that there is no defect in phagocytosis.

There is also little doubt from the data presented in this paper that *L. monocytogenes* can produce systemic bacteremia when given orally. There is the first multiplication of the organism in the PP and dissemination, presumably via the efferent lymphatics to the MLN. Finally, there is also systemic involvement. Interestingly, by the time the infection is uniformly established in the spleen, there is already a reduction in the numbers of organisms in the PP and MLN. It is very well established that acquired immunity to *L. monocytogenes* is cell mediated (16, 17, 20, 21). Therefore, the elimination of *L. monocytogenes* from the PP and MLN of the orally infected mice indicates that a cell-mediated anti-*L. monocytogenes* immune response occurs in these sites. The actual effector cell in anti-*L. monocytogenes* immunity is the macrophage (17, 22), and an influx of these cells into the PP coincident with *L. monocytogenes* elimination has also been demonstrated (MacDonald and Carter, in preparation).

After a primary oral *L. monocytogenes* injection, mice were also highly resistant to subsequent oral or i.v. injection. Similarly, mice given a primary i.v. infection were highly resistant to subsequent oral infection. The time at which a secondary infection was given (7 days after the primary infection) is close to the time of maximum production of mediator T cells in the spleen of i.v.-infected mice (20). There is also considerable nonspecific macrophage activation at this time (22). The rapid elimination of the bacteria in the PP of orally immunized mice receiving a second oral challenge is therefore probably due to a combination of enhanced initial kill by activated macrophages remaining in the PP and also due to the activity of mediator T cells still present in the tissues and circulation which can mobilize more macrophages into the PP and MLN. As might be expected, cell suspensions prepared from the PP, MLN, and spleens of orally infected mice were all capable of transferring some degree of anti-*L. monocytogenes* resistance to normal recipients, indicating the presence of activated anti-*Listeria* T cells in these sites. Interestingly, the capacity of orally infected mice to demonstrate peripheral DTH reactivity to *L. monocytogenes* antigens was not apparent until 6 days postinfection. On day 4 when bacteria were being eliminated from the PP and MLN, there was little, if any, peripheral DTH reactivity. DTH reactivity appeared, however, soon after extensive splenic involvement. The reason for this observation is unclear, but may be related to the different recirculating pathways of cells from the gut-associated tissues and spleen cells (29). Alternatively, the disparity between the emergence of DTH and protective immunity may be explained on the basis that these two phenomena, although related, can occur independently of one another. Data to support this have been presented by Osebold et al. (25), who demonstrated that mice displaying undetectable DTH to *L. monocytogenes* were nevertheless immune in terms of their ability to resist subsequent infection. However, this latter viewpoint must be reconciled with the recent observation that the T cells which mediate DTH and CMI to *L. monocytogenes* are of the same Ly phenotype (14).

Finally, the results presented in this paper substantiate the concept that the induction of systemic cell-mediated immunity, whether it be by gastrointestinal or systemic immunization, can adequately protect mice from subsequent infection derived from the gut (8). Extreme difficulty has been found in attempting to system-

ically vaccinate mice against oral infection with various species of *Salmonella* (7), but it is probable that by using the correct vaccination regime to induce activated T cells, systemic immunization can prevent infection derived from the gastrointestinal tract.

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