Comprehensive Study of the Intestinal Stage of Listerialiosis in a Rat Ligated Ileal Loop System

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Received 3 September 1997/Returned for modification 22 October 1997/Accepted 22 November 1997

The intestinal stage of listeriosis was studied in a rat ligated ileal loop system. Listeria monocytogenes translocated to deep organs with similar efficiencies after inoculation of loops with or without Peyer’s patches. Bacterial seeding of deep organs was demonstrated as early as 15 min after inoculation. It was dose dependent and nonspecific, as the ΔsinLΔB, the Δhly, and the ΔactA L. monocytogenes mutants and the nonpathogenic species, Listeria innocua, translocated similarly to wild-type L. monocytogenes strains. The levels of uptake of listeriae by Peyer’s patches and villous intestine were similar and low, 50 to 250 CFU per cm² of tissue. No listeria cells crossing the epithelial sheet of Peyer’s patches and villous intestine were observed by transmission electron microscopy. The lack of significant interaction of listeriae and the follicle-associated epithelium of Peyer’s patches was confirmed by scanning electron microscopy. The follicular tissue of Peyer’s patches was a preferential site of Listeria replication. With all doses tested, the rate of bacterial growth was 10 to 20 times higher in Peyer’s patches than in villous intestine. At early stages of Peyer’s patch infection, listeriae were observed inside mononuclear cells of the dome area. Listeriae then disseminated throughout the follicular tissue except for the germinal center. The virulence determinants hly and, to a lesser extent, actA, but not inlAB, were required for the completion of this process. This study suggests that Peyer’s patches are preferential sites for replication rather than for entry of L. monocytogenes, due to the presence of highly permissive mononuclear cells whose nature remains to be defined.

Listeria monocytogenes is a ubiquitous gram-positive bacillus that causes serious infections in humans and animals (16). Epidemiological investigations of both epidemic and sporadic cases have shown that human listeriosis is a food-borne illness (10). Infection of pregnant women may result in abortion, stillbirth, and neonatal meningitis or sepsis (15). Meningitis, meningoencephalitis, and bacteremia are the most common presentations in nonpregnant adults. Immunocompromised and elderly individuals are primarily affected. However, apparently healthy individuals also contract listeriosis.

The establishment of a systemic infection after ingestion of the organism is an essential step in the pathogenesis of listeriosis (13). This systemic infection appears to be initiated in the small intestines of laboratory animals (20, 21). However, the mechanisms involved in the translocation of L. monocytogenes across the intestinal mucosa are largely unknown. The ileal Peyer’s patches rather than the intestinal villi are the initial sites of Listeria invasion in mice (20, 21). This finding suggests that L. monocytogenes preferentially enters the host by crossing the follicle-associated epithelium (FAE) of the Peyer’s patches. The M cell could be the site of entry, as reported for other bacterial pathogens (33).

Several lines of evidence are consistent with an epithelial phase of invasion in listeriosis. Racz et al. found dividing listeriae in absorptive intestinal cells within 3 h of infection in an extensive electron microscopy study of guinea pigs (29). It has also been shown that L. monocytogenes enters cultured enterocyte-like cells (12, 22, 26) and initiates its cycle of intracellular infection inside these cells (32). Both apical and basolateral routes of entry into cultured enterocytes have been demonstrated (14, 18).

The intestinal step of Listeria infection has been studied experimentally by challenging rodents orally or intragastrically. Oral challenge is an approach that mimics the conditions of natural infection. It allows one to study important parameters interfering with the colonization of the gastrointestinal tract by L. monocytogenes, such as cellular immunity or indigenous bacterial flora (24, 38). However, the oral route has a number of limitations. Oral models are not very reproducible. Some authors have failed to achieve lethal infection by the oral route despite the use of high bacterial doses (ca. 10⁹ bacteria per animal) (20, 31, 38). Others have reported similar 50% lethal doses by the intragastric and intraperitoneal routes (27). The number of bacteria actually delivered to the intestinal tract is low and highly variable among individuals, due particularly to the bacterial activity of the gastric filter (20). It is not possible to assign a precise role to a given intestinal segment, especially in the translocation process, as the inoculated bacteria are distributed over the entire gastrointestinal tract. The ligated intestinal loop system overcomes these problems. It also makes quantification possible by adapting the standard gentamicin killing assay to the processing of tissue samples (25).

In this study, we used a ligated ileal loop system to examine the roles of intestinal villi and Peyer’s patches in the establishment of Listeria infection in the rat. We were particularly interested in determining whether the FAE was a preferential site for Listeria invasion and whether the infection of Peyer’s patches was an obligate step in the translocation process.

MATERIALS AND METHODS

Bacterial strains and growth media. Listeria strains used in this study are listed in Table 1. Most experiments were performed with the wild-type L. monocytogenes.
Lymph nodes (MLN), liver, and spleen were aseptically removed and ground. For immunolabeling, sections were first incubated with 20% goat serum in PBS containing 1 mM CaCl$_2$ and 0.5 mM MgCl$_2$ for 30 min at room temperature. For detection of listeriae, sections were incubated sequentially with a rabbit antiserum to listerial O antigen 1/2 (J. Rocourt, Institut Pasteur, Paris, France), a-CD45R, 1:5; anti-ED1, 1:100) for 2 h at room temperature. Labeling was produced into biotin-streptavidin-conjugated goat anti-mouse immunoglobulin G and fluorescein isothiocyanate-conjugated streptavidin (Jackson ImmunoResearch Laboratories Inc.). All incubations were followed by three washes in PBS. Fluorescein isothiocyanate-phalloidin (Molecular Probes, Inc.) was used diluted 1:100 in PBS for f-actin staining. Slides were examined by fluorescence microscopy with a Leica DMRB microscope.

### RESULTS

**Quantitative study.** (i) *Listeria translocaion to deep organs.* Illeal loops with or without Peyer’s patches were inoculated with strain EGD (dose of ca. 10$^9$ CFU per loop) to determine whether Peyer’s patches were preferential sites for *L. monocytogenes* translocation. Spreading of the bacteria to MLN, liver, and spleen was studied over a 48-h period (Fig. 1). The inoculation of loops with and without Peyer’s patches gave similar bacterial counts at 3 h, indicating that the presence of Peyer’s patches was not essential to the invasion of the host through the gut. The growth curves between 3 and 48 h were also similar in each of the organs studied, suggesting that the patterns of infection resulting from either mode of inoculation were similar.

We inoculated ileal loops with and without Peyer’s patches with doses of ca. 10$^7$, 10$^8$, or 10$^9$ CFU per loop to examine whether the translocation of *L. monocytogenes* EGD was a dose-dependent process. The spread of bacteria to MLN, liver, and spleen was recorded at 3 and 24 h (Fig. 2). The degree of bacterial translocation was related to the amount of inoculum for both kinds of loops. The dose dependence was most apparent at 24 h. At this time point, however, the differences between doses of 10$^7$ and 10$^8$ bacteria were statistically significant, but those between doses of 10$^8$ and 10$^9$ bacteria were not.

Translocation was rapid irrespective of the presence or absence of Peyer’s patches. Bacteria were recovered from MLN, liver, and spleen in all challenged animals as early as 15 min after the bacteria were injected into the intestinal loops (dose of ca. 10$^8$ CFU per loop). The bacterial counts in each of these organs were similar for loops with and without Peyer’s patches (Table 2).

The nonpathogenic species *Listeria innocua* also crossed the intestinal barrier. The bacterial counts of *L. innocua* CLIP1162 in MLN, liver, and spleen at 3 h (dose of ca. 10$^7$ CFU per loop) were expressed as log$_{10}$ CFU per tissue. Results were considered statistically significant if $P$ values of <0.05 were expressed. Student’s $t$ test was used, and $P$ values of <0.05 were considered statistically significant.
were not significantly different from those of *L. monocytogenes* EGD (Table 3). However, CLIP1162 did not grow in these organs. There was a 300-fold difference in the bacterial counts of this strain and of EGD at 24 h (Table 3). This difference was statistically significant.

(ii) *Listeria* replication in Peyer’s patches and villous intestine. A procedure adapted from the gentamicin survival assay was used to study the growth of *L. monocytogenes* EGD and *L. innocua* CLIP1162 in Peyer’s patches and villous intestine over a 24-h period. There was no significant difference in the initial uptake of EGD by Peyer’s patches and villous intestine at any dose (Fig. 3). Similar, low numbers of gentamicin-protected bacteria were recovered from both intestinal tissues at 3 h. There was a dose-dependent effect. Bacterial counts at 3 h were not affected by lowering doses from 10⁹ to 10⁸ bacteria but decreased dramatically when the dose was lowered from 10⁸ to 10⁷ bacteria, which suggests that there are similar mechanisms of uptake by Peyer’s patches and villous intestine. *Listeriae* multiplied more readily in Peyer’s patches irrespective of the amount of inoculum used. At all doses, the number of bacteria at 24 h was 50 to 100 times greater in these organs than in villous intestine. The differences were statistically significant. Thus, *L. monocytogenes* invades villous intestine and Peyer’s patches with similar efficiencies but grows much more rapidly in Peyer’s patches. Bacterial counts 3 h after inoculation of ca. 10⁹ CFU per loop were similar for CLIP1162 (Table 3), suggesting similar mechanisms of invasion for *L. monocytogenes* and *L. innocua*. However, CLIP1162 did not grow in Peyer’s patches or villous intestine (Table 3).

Histopathological and electron microscope study. (i) Villous intestine (Fig. 4). At early stages (<3 h) of infection with EGD (dose of ca. 10⁹ CFU per loop), myriad bacteria were seen in the intestinal lumen, apparently embedded in the mucus layer. There were also many clusters of 10 to 20 bacteria stuck to mucus released by goblet cells. Numerous bacteria were present in the lumen, but very few were seen associated with the epithelial cell surface. A moderate inflammatory reaction was observed, with leukocytes and erythrocytes in the intestinal lumen. This reaction was probably related to the burden of intraluminal listeriae, as it occurred even in areas where the

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<th>Organ</th>
<th>Log₁₀ (SD) CFU/organ</th>
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<tr>
<td><strong>Loops with Peyer’s patch</strong></td>
<td><strong>Loops without Peyer’s patch</strong></td>
</tr>
<tr>
<td>MLN</td>
<td>3.9 (0.9)</td>
</tr>
<tr>
<td>Liver</td>
<td>4.7 (1.1)</td>
</tr>
<tr>
<td>Spleen</td>
<td>4.4 (1.6)</td>
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epithelial layer was free of bacteria. No listeriae were observed crossing the epithelial barrier by TEM, although sections from different tissue samples collected from five animals were examined. Challenges with high bacterial concentrations (ca. $10^{10}$ CFU per loop) were no more successful for TEM analysis. SEM was not performed. At 24 h, small foci of infection were found in 1 of 100 to 200 intestinal villi. These foci were located in the chorion, at the tips of villi. The overlying epithelium never contained bacteria. A typical lesion was populated by a few inflammatory cells, exclusively consisting of mononuclear cells, and contained no more than 10 to 20 bacteria. Local inflammatory reaction was moderate.

(ii) Peyer’s patches (Fig. 5). Histopathological analysis was consistent with the quantitative data obtained by counting gentamicin-protected bacteria. At early stages of infection (≤3 h), a few bacteria were observed associated with the FAE. The lack of significant interaction of listeriae with the FAE was also demonstrated by SEM (data not shown). Gram staining produced very few pictures of bacteria crossing the epithelial layer (one or two pictures per follicle and per section). However, as we used normal and not germ-free animals, it is possible that the organisms seen crossing the FAE were not listeriae. We did not find any bacteria with the same morphology as listeriae in any tissue or organ (Table 4).

Role of virulence factors. The roles of inlAB, hly, and actA in the intestinal step of Listeria infection were analyzed by studying deletion mutants for these genes in the ligated loop system. These mutants translocated to MLN, liver, and spleen as efficiently as their parental strains, as expected from the results obtained with L. innocua CLIP1162 (Fig. 6). Only the hly mutant did not grow within these organs (Fig. 6). There were no significant differences in early bacterial counts in Peyer’s patches and villous intestine between the actA and hly mutants and their parental strain (Fig. 6). However, the ability to grow in both Peyer’s patches and villous intestine of the hly mutant and, to a lesser degree, the actA mutant, was affected (Fig. 6). The inlAB mutant, BUG949, appeared 5- to 10-fold less invasive than its parent, but the difference did not reach a significant P value. Coinfection experiments were carried out to compare BUG949 and its parent, BUG600, in more detail. Intestinal loops were inoculated with a 1:1 mixture (ca. $10^6$ CFU per loop) of BUG949(pAT18) and BUG600(pAT28) or of BUG949(pAT28) and BUG600(pAT18). Bacterial counts in tissue samples (Peyer’s patches, villous intestine, MLN, liver, and spleen) were determined for each strain, using erythromycin and spectinomycin as selective markers. Contrasting with previous results with separate animals, there was no difference between the inlAB mutant and its parent at any time point and in any tissue or organ (Table 4).

The histopathology of Peyer’s patches at 24 h was consistent with quantitative data. The inlAB mutant gave lesions that were indistinguishable from those produced by its parental strain (data not shown). The infectious foci were infrequent and smaller with the actA mutant, suggesting that this mutant did not disseminate in the follicular tissue (Fig. 7). No foci of infection were observed in the specimens obtained from loops inoculated with the hly mutant (data not shown).

DISCUSSION

The sequence of events leading to the invasion of the host by L. monocytogenes via the intestine was examined in a rat li-
gated ileal loop system. We used an in vivo adaptation of the gentamicin survival assays developed for studying bacterial invasion of cell monolayers. Bacteria were kept inside ligated loops for 1 h to allow bacterial invasion. The loop were then deligated, washed, and treated in situ with gentamicin to eliminate the extracellular bacteria present in the gut. These experimental conditions were aimed at preventing nonspecific phenomena that might have interfered with *Listeria* invasion. *L. monocytogenes* produces several factors that are highly toxic to cells and are potent proinflammatory agents (28). Therefore, the presence of large numbers of listeriae inside the intestinal lumen for long periods, a situation which is probably rare in cases of human listeriosis (see below), may result in profound epithelial damage. Epithelial lesions may, in turn, artificially promote *Listeria* invasion by rendering the basolateral surface of intestinal cells accessible to bacteria. It has been shown that entry of *L. monocytogenes* into polarized monolayers of Caco-2 intestinal cells is greatly increased by disrupting intercellular junctions (14). In experimental shigellosis, the invasion of the intestinal mucosa by shigellae is promoted by the inflammatory reaction triggered by the few organisms that cross the intestinal barrier via M cells (25). The recruitment of neutrophils in situ makes bacterial invasion possible by destroying the cohesion of the epithelial barrier and rendering the basolateral surface of intestinal cells accessible to intraluminal shigellae. These findings are highly relevant as shigellosis is essentially an acute inflammatory bowel disease. In contrast, listeriosis is mainly a systemic infection transmitted by the intestinal route rather than an intestinal disease per se.

We found no evidence of a preferential involvement of the FAE, and more specifically of M cells, in the passage of *L. monocytogenes* across the epithelial barrier. The presence of

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**FIG. 4.** Histological and immunohistological analysis of villous intestine 1 h after inoculation with EGD. (a) Immunofluorescence labeling of listeriae. Bacteria are abundant in the intestinal lumen, apparently embedded in mucus; note that very few bacteria are close to the epithelial cell surface. (b) Gram-Weigert staining. Aggregates of bacteria are stuck to mucus released by goblet cells; no bacteria are seen associated with the epithelial cell surface. Bars = 10 μm.
Peyer’s patches in ligated loops did not affect the rate of Listeria translocation to deep organs. The levels of uptake of listeriae by Peyer’s patches and villous intestine were similar and low. Gentamicin-protected listeriae in either organ 3 h after inoculation with $10^9$ CFU amounted to ca. 50 to 250 CFU per cm$^2$ of tissue. Much higher values were recently reported for Shigella flexneri, which initially invades the intestinal mucosa via M cells in experimental shigellosis (25, 37). Counts of gentamicin-protected shigellae in Peyer’s patches reached ca. 15,000 CFU/cm$^2$ of tissue 2 h after the inoculation of rabbit intestinal loops with ca. $5 \times 10^9$ CFU (25). TEM analysis of the early stage of Listeria infection was consistent with the very low numbers of listeriae recovered from tissues after gentamicin treatment. Despite the examination of many grids prepared from both villous intestine and Peyer’s patches, we did not find any organisms resembling listeriae inside absorptive epithelial cells or M cells.

The passage of L. monocytogenes across the epithelial sheet does not seem to require any of the virulence factors involved in infection of epithelial cells in vitro. Mutants with deletion of

FIG. 5. Histological, immunohistological, and TEM analysis of Peyer’s patch infection with EGD. (a and b) Gram-Weigert staining. (a) Six-hour infection (a few bacteria are seen in the dome area); (b) 24-h infection (many bacteria can be seen in the follicular tissue, note the major inflammatory reaction consisting of both mononuclear cells and neutrophils); bars = 5 μm. (c and d) Twenty-four-hour infection, double-fluorescence labeling of listeriae (c) and the macrophage-related antigen ED1 (d). A very large number of bacteria infect the follicular tissue except the germinal center; bars = 20 μm. (e and f) Twenty-four-hour infection, TEM of listeriae located within a mononuclear cell (e) and listeriae inside a neutrophil (f); bars = 1 μm.
inLAB, hly, and actA, the main virulence genes so far identified in L. monocytogenes, did not have reduced translocation. L. monocytogenes and the nonpathogenic related bacterial species L. innocua disseminated to deep organs with similar efficiencies. Consistent with these findings, L. monocytogenes was recovered from MLN, liver, and spleen as early as 15 min after inoculation. Thus, the translocation process may occur without previous intraepithelial replication. Our results are in full agreement with recent studies in mice that failed to detect Listeria-specific antigens within intestinal epithelial cells after oral challenge (21) but are in apparent contradiction to a previous electron microscopy study of guinea pigs by Racz et al., showing replicating listeriae inside absorptive intestinal cells (29). This discrepancy may be due to differences in experimental procedures: Racz et al. used starved, opium-treated guinea pigs; enteric lesions were not observed in nonpretreated control animals.

Our results suggest that L. monocytogenes uses nonspecific mechanisms to cross the epithelial sheet. Virtually all oxygen-tolerant bacterial species are able to translocate from the gastrointestinal tract to MLN and other extraintestinal sites in various animal systems (4). Intestinal bacterial growth is the major mechanism promoting bacterial translocation (34). E. coli continuously seeds the MLN as long as it maintains a population of at least 10⁸ CFU per g of cecum in monoaassociated gnotobiotic mice (5). The number of E. coli translocating to the MLN decreases when E. coli populations are reduced in the cecum and small intestine, by introducing an antagonistic indigenous microflora (5). Not all bacterial species translocate at the same efficiency. Enterobacteriaceae and Pseudomonas aeruginosa translocate 5 to 10 times more efficiently than gram-positive organisms such as Staphylococcus epidermidis and Lactobacillus brevis (4, 34). This is consistent with the low degree of Listeria translocation that we detected despite the use of high bacterial doses and the large reduction in the number of listeriae translocating to deep organs when inocula were reduced to less than 10⁸ bacteria per loop. The mechanisms of translocation promoted by bacterial overgrowth are unknown. Both intracellular and paracellular passages of microorganisms have been suggested (4).

The ability of L. monocytogenes to replicate within Peyer’s patches was a major finding of this study. The rate of bacterial growth during the first 24 h was 10 to 20 times higher in Peyer’s patches than in villous intestine regardless of dose. Histology was consistent with the results of bacterial counts. Infection of Peyer’s patches was detected throughout the experiments and consisted of multiple, progressively confluent infectious foci, whereas infectious foci of the villous intestine were infrequent and small. At early stages of infection, listeriae were found exclusively within mononuclear cells in both tissues. This finding suggests that a subpopulation of mononuclear cells in Peyer’s patches, and more precisely in the subepithelial dome, is

![Figure 6. Role of Listeria virulence factors. Loops were inoculated with the mutants and their parental strains at a dose of ca. 10⁹ CFU/loop, and the numbers of bacteria in Peyer’s patches, villous intestine, MLN, liver, and spleen were determined at 3 and 24 h postinoculation. The mean and standard deviation of log₁₀ CFU per organ are shown (mean of four rats for each point).](http://iai.asm.org/)

### TABLE 4. Coinfection experiments with BUG600 and BUG949 derivatives expressing differential selective markers

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<th>Organ or tissue</th>
<th>Log₁₀ (SD) CFU/sample at 3 h</th>
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<tr>
<td></td>
<td>BUG600 Spec⁺</td>
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<tr>
<td>MLN</td>
<td>3.6 (1.5)</td>
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<tr>
<td>Liver</td>
<td>3.6 (0.8)</td>
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<tr>
<td>Spleen</td>
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<tr>
<td>PP</td>
<td>2.4 (1.0)</td>
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<td>VI</td>
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* Abbreviations: Ery, erythromycin; Spec, spectinomycin; PP, Peyer’s patch; VI, villous intestine.

* Peyer’s patch or pieces of villous intestine of similar size. Similar results were obtained in coinfection experiments using BUG600 Ery⁺ and BUG949 Spec⁺.
highly permissive to *L. monocytogenes*. Both resident macrophages and dendritic cells are plausible candidates. There is a population of long-living, major histocompatibility complex class II-negative, sialoadhesin-positive cells expressing macrophage markers in the subepithelial dome of Peyer’s patches in rodents (7). These cells have a deactivated phenotype, and their microbicidal activity is thus likely to be low.

Cells of the dendritic lineage may also be key target cells in Peyer’s patches. A population of dendritic cells that form a dense layer just beneath the dome epithelium has recently been identified in murine Peyer’s patches (19). These cells are thus well positioned for interaction with listeriae passing into Peyer’s patches. The ability of this population to internalize bacteria was not evaluated, but other studies have demonstrated that cells of the dendritic lineage may naturally have, or acquire, phagocytotic functions (30). A recent study has shown that *L. monocytogenes* invades mouse dendritic cells in vitro and develops a complete infection cycle within these cells, with escape from the phagocytosis vacuole and cell-to-cell spread (17). Invasion of dendritic cells could result in either cell death or persistent infection. Dendritic cells may thus be a reservoir for *L. monocytogenes*. Infected dendritic cells may also contribute to the spread of *L. monocytogenes* into the host as a result of their status as antigen-presenting cells. Indeed, dendritic cells home to intestinal and extraintestinal lymphoid tissues to induce a primary T-cell response after contact with antigens in the periphery (35).

These data suggest two possible pathways for *Listeria* translocation. Passage across the epithelial sheet is common to both pathways and does not require any *Listeria*-specific virulence factor. This is an inefficient process directly related to amount of bacteria in the gut. After crossing the epithelial sheet, listeriae either spread via the lymph and blood to distant tissues in a few minutes or are taken up by mononuclear cells in the subepithelial region. The number of listeriae inside mononuclear cells seems to depend on killing activity of the cell population encountered by these organisms. In the chorion of intestinal villi, nonpermissive cells may be involved, resulting mostly in abortive infection. In Peyer’s patches, listeriae may invade weakly bacteriocidal mononuclear cells, either resident macrophages or dendritic cells, and replicate almost without restriction. *Listeria* virulence factors involved in intracellular growth and cell-to-cell spread are necessary for this step. Listeriae may subsequently enter lymph or blood vessels and gain access to other intestinal and extraintestinal tissues. It is unknown whether bacteria are transported by mononuclear cells or travel extracellularly (3).

A large outbreak of gastroenteritis due to *L. monocytogenes* in chocolate milk has recently been reported (8). The most common symptoms in the affected people were diarrhea and fever. No infection of extraintestinal sites was observed. The chocolate milk implicated in the outbreak contained very high levels of listeriae, and the median dose ingested was estimated to be as high as $2.9 \times 10^{11}$ CFU per person. This may explain why infection presented as a gastrointestinal illness in this outbreak whereas gastrointestinal symptoms are not typical in most cases of listeriosis. These data are consistent with our results, as we found that gross intestinal lesions developed only after inoculation with very large doses of listeriae ($\geq 10^9$ CFU per loop) in our system. The mechanisms by which *L. monocytogenes* causes diarrhea in humans are unknown. The association of fever with diarrhea and the demonstration of high

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**FIG. 7.** Histology and immunohistology of Peyer’s patches 24 h after infection with the actA mutant. (a) Immunofluorescence labeling of bacteria. The foci of infection are less numerous and smaller than with EGD; they are restricted to the dome area. (b) Gram-Weigert staining. Discrete foci of bacterial replication inside mononuclear cells can be seen (arrows). Bars = 20 μm.
levels of serum antibody to listeriolysin O in infected individuals suggest an invasive intestinal process (8). This is also consistent with our experimental results.

Peyer’s patches are usually regarded as the “Achilles heel” of the mucosal barrier because of the capacity of M cells to actively take up particles, including viruses, bacteria, and protozoan organisms (33). Little attention has been paid to the lymphoid tissue, which is another area of weakness in the gut. This tissue contains cell populations that may be used by a large variety of pathogenic organisms to establish local or general infections. For example, some viruses that cause systemic diseases, such as reovirus, cross the epithelial barrier through M cells, replicate in M-cell-associated mononuclear cells, and then enter the host circulatory system (2). Infected B cells transport mouse mammary tumor virus to the mammary gland (23). Human immunodeficiency virus type 1 may initially encounter its CD4+ target cells in lymphoid tissue present in the rectal mucosa (1). L. monocytogenes and its virulence factors may exploit mononuclear cells, macrophages, or dendritic cells, which are dedicated to antigen processing and presentation but not to bacterial killing. This possibility is currently being studied by our group.

ACKNOWLEDGMENTS

We thank M. Leborgne and G. Pivert for technical assistance, G. Milon for helpful discussions, P. Cossart and T. Chakraborty for the gift of strains, and R. Fournier for help with the manuscript. B.P. received financial support from the Institut National de la Santé et de la Recherche Médicale. This work was supported by the University Paris V and the CEE (grant BMH4CT96 0659/RA03813).

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