Suppurative Gastritis in BALB/c Mice Infected with *Listeria monocytogenes* via the Intragastric Route


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Summary

Suppurative gastritis was demonstrated in BALB/c mice 3 days after intragastric inoculation with $10^9$ organisms of *Listeria monocytogenes* strain ATCC19113 (serotype 3). Also tested were four other strains of mice (C3H, C57BL/6, FVB and ICR) and three other strains of *L. monocytogenes* (HPB 3 [serotype 4b], HPB 410 [serotype 1/2a] and HPB 503 [serotype 1/2b]). After inoculation with ATCC19113 the numbers of bacteria found in the stomach wall were greater in C57BL/6 and ICR mice than in C3H and FVB mice; moreover, the gastritis produced in BALB/c and C57BL/6 mice was more severe than that produced in the other mouse strains. The gastritis produced in BALB/c mice with *L. monocytogenes* HPB 3, HPB 410 and HPB 503 was much more severe than that produced by ATCC19113. The inflammatory response occurred in the lamina muscularis and mucosa of the fundus. Massive necrosis of the gastric epithelium was observed, and there was oedema in a large part of the mucosal layer of the fundus. In addition, the submucosal layer was apparently expanded due to oedema, and in the cardia, the mucosal layer had become thin and flattened. Immunohistochemically, a polyclonal antibody against *Listeria* spp. produced labelling in areas of the gastric mucosa in which there was an inflammatory response and gastric epithelial necrosis.

**Keywords:** bacterial infection; gastritis; *Listeria monocytogenes*; mouse

Introduction

*Listeria monocytogenes* is a gram-positive, facultatively intracellular bacterium. It gives rise to disease in man and animals but is normally non-pathogenic for healthy human beings. However, in pregnant women, newborn infants, or debilitated, elderly or immunocompromised persons it may cause severe clinical disease including meningoencephalitis, septicaemia and abortion (Gray and Killinger, 1966; MacDonald and Carter, 1980; Ho et al., 1986; Bach and Davis, 1987; Marco et al., 1988; Marco et al., 1991). Experimental murine listeriosis has been widely used for studying host resistance, molecular pathogenesis, and disease due to intracellular pathogens. In most such studies parenteral routes of inoculation have been used rather than inoculation into the gastrointestinal tract (Mendier et al., 1991; Blanot et al., 1997; Flesch et al., 1998; Gattuso et al., 2000; Mannering et al., 2002). However, as human *L. monocytogenes* infection normally results from the ingestion of contaminated foods such as vegetables, milk and dairy products (Farber and Peterkin, 1991), it seems possible that animals infected by the intragastric route would provide a more suitable model of listeriosis.

In the limited number of reports of murine listeriosis produced by intragastric inoculation, the histopathological lesions consisted mainly of necroizing hepatitis, suppurative splenitis, and lymphadenitis, with no lesions in the central nervous system (CNS) or gastrointestinal tract (Marco et al., 1991).
1992; Czuprynski et al., 2002, 2003). Moreover, in man, gastrointestinal lesions have not been reported as a clinical feature of listeriosis. In preliminary experiments on murine listeriosis we observed suppurative gastritis after infection by the intragastric route, in contrast to the workers cited above. The aims of the present study were (1) to confirm our preliminary observation, (2) to describe the histopathology of the experimentally induced listeria gastroenteritis, (3) to investigate the influence of bacterial strain and laboratory mouse strain on infectivity and on the severity of the infection, and (4) to examine possible effects of preliminary intragastric administration of sodium bicarbonate.

Materials and Methods

Bacterial Inocula

*L. monocytogenes* strain ATCC 19113 (serotype 3) was obtained from the American Type Culture Collection (Manassas, VA, USA) and strains HPB 3 (4b), HPB 410 (1/2a), and HPB 503 (1/2b) were from Health Products and Food Branch, Health Canada (Ottawa, Canada). The bacteria were inoculated into Tryptic Soy (TS) broth (Difco, Sparks, MD, USA) and incubated overnight, with shaking, at 37°C. The bacterial cells were collected by centrifugation (3000 rpm [800 g] at room temperature [RT] for 15 min) and resuspended in sterile phosphate-buffered saline (PBS), pH 7.2, at a concentration of 10⁹ colony-forming units (CFU)/0.5 ml.

Animals

Female specific pathogen-free mice, aged 5–6 weeks, of five different strains (BALB/c, C3H, C57BL/6, FVB and ICR), were purchased from Daehan Biolink, Chung-Buk, Korea and used in accordance with the laboratory animal guidelines of Seoul National University. Food and water were provided *ad libitum* but removed from the cages 6 h before intragastric inoculation.

Intragastric Inoculation

Mice, hand-restricted without anaesthesia, were inoculated with an 18-gauge stainless steel ‘feeding needle’ (diameter 2.25 mm, length 50 mm) with a spherical tip (Fine Science Tools Inc., North Vancouver, BC, Canada). To minimize the risk of damage to the gastric mucosa, the inocula were deposited at the lower end of the oesophagus.

Experimental Design

All mice were killed by cervical dislocation at 3 days post-infection (dpi) for the assessment of *L. monocytogenes* counts and histopathological lesions in the stomach.

Preliminary experiment. An initial observation showed that suppurative gastritis occurred in each of five BALB/c mice given 100 μl of 10% sodium bicarbonate solution intragastrically (to neutralize gastric acid), followed 15 min later by intragastric inoculation with 0.5 ml of a suspension of strain ATCC19113 (10⁹ viable organisms).

This was followed by a preliminary experiment to confirm the initial observation and to assess the effect, if any, of the sodium bicarbonate. In this experiment five BALB/c mice (group Na) were given sodium bicarbonate followed by listeria suspension, as above. Five mice in a second group (group L) were similarly treated but without the administration of sodium bicarbonate. Five mice in a third group (controls) received 0.5 ml of sterile PBS intragastrically.

Effect of mouse strain. After sodium bicarbonate treatment (as above), C3H mice (*n* = 5), C57BL/6 mice (*n* = 6), FVB (*n* = 5) and ICR mice (*n* = 6) were each inoculated intragastrically with 0.5 ml of ATCC19113 suspension (10⁹ organisms).

Effect of *L. monocytogenes* strain. After sodium bicarbonate treatment (as above), BALB/c mice in three groups of five received 0.5 ml (10⁹ organisms) of strain HPB 3, HPB 410 or HPB 503.

Bacterial Count in Stomach Walls

The abdominal cavities of mice, killed 3 days after infection, were opened aseptically. The stomachs were then removed and opened along the greater curvature. After washing away the gastric contents with sterile PBS, a portion of each stomach was then weighed, homogenized and diluted in PBS, appropriate dilutions being plated on PALCAM (Merck, Darmstadt, German) agar. After incubation for 36–48 h at 37°C, colonies were counted and the number of bacteria per gram calculated.

Histopathological Examination

Gastric samples were fixed in 10% neutral formalin for 24 h, dehydrated in an alcohol-xylene series, and embedded in paraffin wax. From each block, Sections 2 μm thick were prepared and stained with haematoxylin and eosin (HE) for histopathological examination. The degree of gastritis was scored as follows: 0, no inflammatory response in the mucosa or submucosa; 1, mild infiltration of
neutrophils in the submucosa and lower mucosa but no gastric epithelial change; 2, moderate infiltration of neutrophils and macrophages in the mucosa and the submucosa, and focal necrosis of the gastric epithelium; 3, substantial infiltration by neutrophils and macrophages in the mucosa and submucosa, and multifocal necrosis of the gastric epithelium; 4, substantial infiltration by neutrophils and macrophages in the mucosa and submucosa, and massive necrosis of the greater part of the gastric epithelium; 5, substantial infiltration by neutrophils and macrophages in the mucosa, submucosa and muscle layer, massive necrosis of all parts of the gastric epithelium, and the presence of oedema, erosions and ulcers.

Immunohistochemistry

Sections (3 μm) of formalin-fixed, paraffin wax-embedded gastric tissue were placed on poly-L-lysine-coated slides and dried overnight at 55 °C. After dewaxing and rehydration with a xylene-alcohol series, the sections were incubated with H2O2 10% in methanol for 30 min to block endogenous peroxidase activity, and subsequently with normal goat serum for 1 h at 4 °C to block non-specific reactions. They were then incubated overnight at 4 °C with a rabbit polyclonal antibody against Listeria spp. (Virostat, Portland, Maine, USA), diluted 1 in 100 in PBS containing Tween 20, 0.05% (PBS-T). The sections were then given three 5-min washes before being incubated with biotinylated mouse anti-rabbit IgG antibody (Vector, Burlingame, CA, USA) at RT for 30 min. They were then treated with peroxidase-conjugated avidin–biotin complex (ABC) (Vector). Peroxidase activity was evaluated with 3, 3’-diaminobenzidine (Vector) as the chromogen. Finally, the sections were counterstained with Mayer’s haematoxylin for 30 sec and mounted.

Statistical Analysis

The significance of differences between the experimental and control groups was determined by Duncan’s Multiple Range Test (SAS ver. 8.1; SAS Institute Inc., Cary, NC, USA). Values of P < 0.05 were considered significant.

Results

Preliminary Experiment

Counts of L. monocytogenes in gastric tissue ranged from 4.0 to 6.14 log10 CFU/g, regardless of sodium bicarbonate treatment. There was no significant difference in the mean (±SD) bacterial counts between groups L (5.26 ± 0.58 log10 CFU/g tissue) and Na (5.06 ± 0.87 log10 CFU/g tissue).

Histopathological examination revealed gastritis in all infected mice, regardless of sodium bicarbonate treatment. The mean gastritis score was slightly but not significantly higher in group Na (2.4 ± 0.55) than in group L (2.0 ± 0.82). Gastritis occurred in the mucosa and submucosa of the fundus, cardia and gastric body, but not in the pylorus. Neutrophils predominated in the infiltrate into the lamina propria, submucosa and muscularis layer of the gastric mucosa (Fig. 1A). Massive necrosis and degeneration of epithelial cells were observed in the fundic mucosa and lamina muscularis of the submucosa. Hyperplastic change and glandular formation of epithelial cells were also observed in the cardia of some infected mice (Fig. 1C). Immunohistochemical labelling was observed in the gastric mucosa in areas of inflammatory response and gastric epithelial necrosis; at high magnification, the labelling appeared to be mainly in the cytoplasm of neutrophils (Fig. 1D). No immunolabelling was observed in the gastric mucosa of the control mice.

Effect of Mouse Strain

L. monocytogenes was recovered from the gastric tissue of all the inoculated mice of strains ICR and C57BL/6 but from only some of the strain FVB and C3H mice (three out of five in each case). The bacterial count was significantly higher in ICR and C57BL/6 mice than in the other two mouse strains (Table 1). Despite the higher number of bacteria in the stomach wall of the ICR mice, the gastric lesions observed histopathologically were mild or absent, as also were the lesions in FVB and C3H mice. On the other hand, C57BL/6 mice showed obvious gastric lesions consisting of (1) inflammatory cell infiltration in the lamina propria, and (2) multifocal epithelial necrosis in the fundic mucosa (Fig. 1B). The gastritis score of the C57BL/6 mice was significantly higher than that of the FVB, C3H and ICR mice (Table 1).

Effect of L. monocytogenes Strain

The pathogenic effects of L. monocytogenes strains HPB 410 (serotype 1/2a) and HPB 503 (serotype 1/2b) in BALB/c mice were greater than those of strain HPB 3 (serotype 4b). This was reflected in loss of body weight, decreased food intake, and occurrence of clinical signs (ruffled fur, lethargy and tachypnoea from 2 dpi onwards), these effects
being associated more obviously with HPB 410 and HPB 503 infection than with HPB 3 infection.

Table 2 shows that the gastric bacterial counts were significantly higher in mice dosed with HPB 410 or HPB 503 than in those dosed with HPB 3 ($P < 0.05$). Moreover, the gastritis score was also higher in mice given HPB 410 or HPB 503 than in those given HPB 3, although the results with strain HPB 410 lacked statistical significance (Table 2).

The gastritis produced by strains HPB 3, HPB 410 and HPB 503 was much more severe than that seen earlier with ATCC19113. Thus, the inflammatory response was particularly severe in the lamina muscularis and the fundic mucosa. In most parts of the latter, severe epithelial necrosis and oedema were observed (Fig. 2A). In addition, the submucosa was strikingly oedematous, and in the cardia the mucosal layer was thin and flattened. These

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Number of inoculated mice</th>
<th>Number yielding listeria from gastric tissue</th>
<th>Log$_{10}$ CFU/g of gastric tissue (mean ± SD)</th>
<th>Gastritis score</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H</td>
<td>5</td>
<td>3</td>
<td>$2.90 ± 0.13$ *</td>
<td>$0.4 ± 0.55$ *</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>6</td>
<td>6</td>
<td>$4.58 ± 0.22$ †</td>
<td>$2.17 ± 0.75$ †</td>
</tr>
<tr>
<td>FVB</td>
<td>5</td>
<td>3</td>
<td>$3.68 ± 0.22$ *</td>
<td>$0.80 ± 0.10$ *</td>
</tr>
<tr>
<td>ICR</td>
<td>6</td>
<td>6</td>
<td>$4.82 ± 1.68$ †</td>
<td>$1.17 ± 0.75$ *</td>
</tr>
</tbody>
</table>

* † Means with the same symbol are not significantly different ($P > 0.05$).
three strains, unlike ATCC19113, also produced lesions (focal epithelial necrosis, neutrophilic infiltration and oedema) in the pylorus, albeit less strikingly than in the fundus (Fig. 2B).

**Discussion**

Gastric lesions have not previously been reported as a feature of human listeriosis, or of murine listeriosis induced by intragastric inoculation, although Marco *et al.* (1992) reported that *L. monocytogenes* could colonize the gastric mucosa of mice for up to 5 dpi. The present study showed, however, that intragastric inoculation with *L. monocytogenes* could induce suppurative gastritis in mice. This apparent discrepancy may have been due to the use of different strains of bacteria or mice, both of which were shown by the present study to be capable of influencing the gastric lesions. Marco *et al.* (1992) used *L. monocytogenes* strain SLCC 2371/NCTC 7973 and Swiss CD1 mice to induce listeriosis by intragastric inoculation. Difference between methods of intragastric inoculation may also have played a part; unfortunately, however, Marco *et al.* (1992) did not give details of the technique used. Possibly gastric lesions occurred but were ignored in the earlier study. Czuprynski *et al.* (2002), who also infected mice intragastrically, described histopathological lesions in the brain, spleen, liver and ileum, but not in the stomach.

Gastric lesions induced by *L. monocytogenes* infection included massive necrosis of epithelial cells and infiltration of neutrophils in the lamina propria and submucosa of the cardia and fundus, as well as hyperplastic change and glandular formation in the mucosa of the cardia. These lesions differed from those associated with *H. pylori* infection. The gastric lesions induced by *L. monocytogenes* occurred mainly in the cardiac and fundic mucosa, whereas those associated with *H. pylori* occur mainly in the antral and pyloric mucosa. Long-term infection with *H. pylori* is reported to induce chronic and atrophic gastritis and a high-grade lymphoma in mice (Lee *et al*., 1997; Wang *et al*., 2000); in the present study, however, *L. monocytogenes* induced severe suppurative gastritis at 3 dpi.

Neutralization of gastric acid by the administration of sodium bicarbonate did not affect the gastric bacterial counts or gastritis score resulting from subsequent intragastric inoculation with

![Fig. 2A,B. (A) Substantial necrosis of gastric epithelium and associated neutrophilic infiltration present in much of the fundic mucosal layer of a mouse infected with *L. monocytogenes* HPB 503. (B) Marked neutrophilic infiltration and oedema present in the pyloric mucosa of a mouse infected with *L. monocytogenes* HPB 410. HE. × 40.](image-url)
L. monocytogenes. Czuprynski et al. (2002) showed that prior administration of sodium bicarbonate did not enhance the pathogenic effect of L. monocytogenes strain Scott A for intragastrically inoculated mice; nonetheless, it did so in mice infected with L. monocytogenes EGD (Czuprynski and Faith, 2002). These authors concluded that the effect of sodium bicarbonate administration was dependent on the bacterial strain and the infecting dose.

The gastric lesions induced in the present study were influenced by both the mouse strain and the bacterial strain. Previous studies demonstrated that genetic regulation of innate resistance to listeriosis in mice inoculated intravenously or intraperitoneally is controlled chiefly by the Hc locus on chromosome 2 (Cheers and McKenzie, 1978; Gervais et al., 1984; Czuprynski et al., 1985). Mouse strains that possess the resistant allele at the Hc locus are far more resistant to intravenous or intraperitoneal inoculation with L. monocytogenes than are mouse strains with the susceptible allele. C57BL/6 and A/J strains of mice are the prototype resistant and susceptible strains, respectively, for inoculation with L. monocytogenes by these routes (Gervais et al., 1984; Czuprynski et al., 1985). Czuprynski et al. (2003) also showed that C57BL/6 mice were resistant to L. monocytogenes infection by intragastric inoculation. However, in the present study, gastritis was more severe in C57BL/6 mice than in FVB, C3H, or ICR mice, although high numbers of bacteria were recovered from the stomachs of the ICR mice. The C57BL/6 strain is well established as being suitable for providing an animal model of gastritis caused by Helicobacter spp. (Lee et al., 1997; Wang et al., 2000; Court et al., 2002; Walker et al., 2002) or Acinetobacter 1worffi (Zavros et al., 2002a). Therefore, it is feasible that gastritis resulting from intragastric inoculation with L. monocytogenes might be more readily induced in the C57BL/6 strain than in other strains. In this study, strains of serotypes 4b, 1/2a and 1/2b (which are responsible for most human outbreaks of listeriosis) induced more severe gastritis and bacterial colonization than did a serotype 3 strain. This accorded with a study by Barbour et al. (2001).

This study confirmed that severe supplicative gastritis could be induced in mice by intragastric inoculation with L. monocytogenes; this gastritis was influenced by both the mouse strain and the bacterial strain. Unlike the human stomach, the mouse stomach has a gastric acidic range of approximately 3–5 mEq, which provides an environment suitable for colonization by non-H. pylori organisms (Zavros et al., 2002b). Gastrointestinal lesions have not been reported so far in human listeriosis. Clarification of the molecular pathogenesis of the gastritis produced by L. monocytogenes in mice is required.

Acknowledgments

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