Characteristics of the gastritis induced by *Listeria monocytogenes* in mice: microbiology, histopathology, and mRNA expression of inflammatory mediators with time course of infection

Jong-Hwan Park\textsuperscript{a,1}, Dong-Jae Kim\textsuperscript{a,1}, Yong-Ho Park\textsuperscript{b}, Seung-Hyeok Seok\textsuperscript{a}, Sun-A Cho\textsuperscript{a}, Min-Won Baek\textsuperscript{a}, Hui-Young Lee\textsuperscript{a}, Jae-Hak Park\textsuperscript{a,*}

\textsuperscript{a}Department of Laboratory Animal Medicine, College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University, San 56-1, Shinlim-dong, Kwanak-ku, Seoul 151-742, South Korea

\textsuperscript{b}Department of Veterinary Microbiology, College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University, Seoul, South Korea

Received 30 December 2003; received in revised form 18 May 2004; accepted 24 May 2004

Abstract

*Listeria monocytogenes* induces the suppurative gastritis in some mice strains. In this study, characteristics of the gastritis caused by *L. monocytogenes* infection in mice were examined with time course of infection. Mice were administered intragastrically with 1.8×10\textsuperscript{8} CFU of *L. monocytogenes*. Each three mice were sacrificed by cervical dislocation at 1, 3, 5, 7, 10, 14, 17, 21, and 28 days postinoculation (pi), respectively. Bacterial colonization in the stomachs reached the peak at 3 days pi, maintained over 4.3 log\textsubscript{10} CFU/g tissue until 14 days pi, and was cleared by 28 days pi. However, in the spleens and livers, the bacteria could not be detected after 7 days pi. The gastric lesions were the most prominent at between 3 and 7 days pi. The lesions consisted of marked neutrophilic infiltration, edema, vacuolar degeneration and necrosis of muscle cells and were more severe in the nonglandular region and fundus than in the pylorus, and were in submucosa, lamina muscularis, and serosa than in mucosa. mRNA expression of several cytokines (INF-\textgamma, IL-1\textbeta, IL-5, IL-6, IL-12, and TNF-\textalpha) and chemokines (KC, MCP-1) increased in the gastric tissue of infected mice at 1–7 days pi and slightly decreased at 14 days pi. These findings would be useful for studying the pathological mechanism of human febrile gastroenteritis due to *L. monocytogenes* infection.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: *Listeria monocytogenes*; Gastritis; Mouse; Stomach; Histopathology

1. Introduction

*Listeria monocytogenes*, an emerging pathogen since the late 1970s, is a facultative intracellular microorganism that causes ~2500 cases of serious illness and 500 deaths per year in the United States [1–4]. Risk factors for listeriosis include age (>65 years), pregnancy, human immunodeficiency virus infection, immunosuppressive therapy, diabetes, kidney disease, and cancer [5]. The clinical manifestations of listeriosis are variable, although sepsis, meningitis, and encephalitis are the most common features. Local inflammatory and/or pyogenic symptoms in different organs such as skin, eye, heart, bone, and peritoneum have also been reported [6,7]. Recently, mass outbreaks of febrile gastroenteritis have also been reported in healthy persons who ingested *L. monocytogenes*-contaminated cheese or delicatessen meat [8,9]. Symptoms included diarrhea, fever, headache, stomach cramps, and vomiting. However, pathological features or mechanisms about febrile gastroenteritis have not been clarified at all.

Several studies for murine listeriosis by intragastric inoculation have been reported [10–12], although most used a parenteral route of challenge. Marco et al. [10]...
revealed that no histological lesions were observed in the stomachs and intestines of mice orally inoculated with $1.3 \times 10^9$ CFU of *L. monocytogenes*, although the bacteria were detected in the stomachs by PAP reaction. Czuprynski et al. [12] also described no lesions in the ilea of both A/J and C57BL/6 mice inoculated with $10^8$ CFU of *L. monocytogenes*, but did not refer to gastric lesions in the mice. However, our previous study showed that intragastric inoculation of *L. monocytogenes* causes severe suppurative gastritis that is dependent on bacterial or mouse strains [13].

In the previous study, we could not determine the changes of histopathological lesions and bacterial colonization in mice stomachs with a time course of infection because all mice were necropsied only at 3 days after *L. monocytogenes* infection. In addition, gene expression of inflammatory mediators such as cytokines and chemokines in mice stomachs against *L. monocytogenes* infection has been unknown. Therefore, in the present study, we examined bacterial colonization, histopathological characteristics, and mRNA expression of inflammatory mediators, with a time course of infection, in mice stomachs infected with *L. monocytogenes* via an intragastric route.

2. Results

2.1. Bacterial counts in stomachs, spleens, and livers

*L. monocytogenes* in mice stomachs could be detected from 1 day pi. Bacterial colonization reached the peak at 3 days postinoculation (pi) and lasted over 4.3 log10 CFU/g tissue until 14 days pi (Fig. 1A). The bacteria could be detected in only two (3.60 and 4.49 CFU/g tissue) of three infected mice at 17 days pi and one (4.70 CFU/g tissue) of those at 21 days pi. At 28 days pi, the bacteria were not detected in the stomachs of any mice. At 1 day pi, the bacteria could be detected in the spleen of one mouse (Fig. 1B). At 3 and 5 days pi, the bacteria were detected in the spleen and the liver of all mice, ranging from 2.60 to 4.96 log10 CFU/g tissue (Fig. 1B). After 7 days pi, the bacteria were not detected in the spleen and liver of any mice, except in the spleen of one mouse at 7 days pi. In this study, the limitation of the bacterial counts was 3.0 log10 CFU/g tissue in stomach and 2.0 log10 CFU/g tissue in spleen and liver.

2.2. Histopathological examinations

In the stomachs of the control mice, no or mild neutrophilic infiltration was observed in the submucosa of nonglandular region (NGR), fundus, or pylorus (Fig. 2A–C). However, degeneration or necrosis of the gastric epithelia was not observed in the control mice. At 1 day pi, neutrophilic infiltration slightly increased in the submucosa of NGR, and focal necrosis of the epithelia was seen in the fundic mucosa of one infected mouse. The lesions became more severe at 3 and 5 days pi (Fig. 4A and B). Marked infiltration of neutrophils was observed in the mucosa, submucosa, lamina muscularis, and serosa of NGR, fundus, and pylorus (Fig. 2D–F and Fig. 4A and B). Necrosis and degeneration of the gastric glands, edema, and congestion was seen in the lamina propria of the fundus and pylorus. In the submucosa of all parts of the stomachs, connective tissue was loose and expanded due to edema. Vacuolar degeneration and massive necrosis of muscle cells was observed in lamina muscularis. After 7 days pi, the gastric lesions in the pylorus decreased to a normal level, but lasted in the submucosa, lamina muscularis, and serosa of NGR, fundus, and pylorus with a slight decrease in severity (Fig. 2G and H). At this time, the inflammatory response included lymphocytes and macrophages as well as neutrophils. At 28 days pi, however, most of the inflammatory cells were macrophages and lymphocytes (Fig. 2I and J).

In the spleens and livers, no inflammatory responses were observed at 1 day pi. At 3 and 5 days pi, multifocal splenic and hepatic necrosis with moderate to marked neutrophilic infiltration was observed (Figs. 3A and B and 4C).
After 7 days pi in the spleens and 10 days pi in the livers, the lesions were alleviated and reached normal state at 10 days (Figs. 3C and 4C) and 28 days pi, respectively. Similarly with the lesions in the stomachs, before 7 days pi, inflammatory cells mostly consisted of neutrophils. However, since then, macrophages and lymphocytes infiltrated in the spleens and livers (Fig. 3D).

2.3. mRNA expression of inflammatory mediators in stomachs

mRNA expression of inflammatory mediators was assessed in the gastric samples of the mice killed at 0, 1, 3, 7, and 14 days pi (Fig. 5). Among the tested cytokines, INF-γ, IL-1β, IL-5, IL-6, IL-12, and TNF-α mRNA expression gradually increased in the stomachs of infected mice until 7 days pi and slightly decreased at 14 days pi (IL-6 mRNA was less expressed at 3 days pi than 1 day pi). IL-18 was constitutively expressed in the stomachs of both control and infected mice. Among the tested chemokines, mRNA expression of KC and MCP-1 increased in the stomachs of the infected mice at between 1 and 7 days pi and decreased at 14 days, which was similar to the pattern of cytokine gene expression. mRNA of MIP-2 was expressed in control mice with a slight difference of expression level, and more intensively in some of the mice killed at 1 and 7 days pi. However, its expression in the stomachs of mice killed at 3 and 14 days was not different from that in the control mice.
Fig. 3. Histopathological findings of the spleens and livers of mice killed at 3 (A and B) and 10 days (C and D). (A) Necrosis of a germinal center and (B) necrotic foci with infiltration of neutrophils and macrophages were observed in the spleen and liver of a mouse. (C) There was no inflammatory response and necrosis of germinal centers in the spleen. (D) Size of necrotic foci slightly decreased and inflammatory cells mostly consisted of lymphocytes and macrophages. HE staining, Bar=40 μm.

Fig. 4. Histopathological severity of the stomachs, spleens, and livers of mice infected with $1.8 \times 10^8$ CFU of *L. monocytogenes* strain ATCC 51774. The severity was assessed by the degree of infiltration of inflammatory cells as 0–3 scales and the data was expressed as mean ± SD. (A) Mucosa of fundus and pylorus. (B) Submucosa and muscularis of NGR, fundus, and pylorus. (C) Spleens and livers.
3. Discussion

Gastric lesions in murine listeriosis have not been noteworthy because abortion or brain lesions including sepsis, meningitis, and encephalitis are the major symptoms in human listeriosis. Also most studies for murine listeriosis used the parenteral route of infection, which might not be able to induce lesions in the gastrointestinal tract. However, recent mass outbreaks of febrile gastroenteritis caused by \textit{L. monocytogenes} infection caused a great deal of concern in regards to gastrointestinal lesions in listeriosis. Several studies showed that maximum levels of \textit{L. monocytogenes} colonization were reached over 2–4 days pi in the liver and spleen of normal mice infected intragastrically. Thereafter, numbers declined, with bacteria being mostly cleared by 7 days pi. Similarly with this, in the present study, \textit{L. monocytogenes} could not be detected in the spleens and livers after 7 days pi. On the other hand, \textit{L. monocytogenes} colonization in the stomach reached the peak at 3 days pi and lasted until 21 days pi. These findings imply that \textit{L. monocytogenes} would colonize in the stomach longer than in the spleen or liver. Further study will have to clarify the reason of this organ-specific difference in the colonizing duration.

Marco et al. [10] showed that \textit{L. monocytogenes} could be cultured from fecal samples of mice infected intragastrically until 8 days pi at the end of experiment. In their study, an anti-\textit{L. monocytogenes} PAP reaction was positive in the stomachs until 5 days pi, but negative in the intestines of all infected mice. This means that the bacteria cultured from faeces might be those colonizing the stomachs rather than the intestines.

Marco et al. [10] also described no histopathological lesions in the stomachs, which is contrary to our results. They used Swiss CD1 mice and inoculated the bacteria without a sodium bicarbonate treatment. In a study conducted by Czuprynski et al. [14] an oral administration of sodium bicarbonate before bacterial inoculation enhanced the severity of an \textit{L. monocytogenes} EGD infection, although it did not enhance the virulence of \textit{L. monocytogenes} Scott A in another of their studies [11]. Therefore, a lack of sodium bicarbonate treatment can be a possible reason for the lack of gastric lesions in the Marco study [10]. Moreover, in our previous study [13], the degree
of the gastritis by *L. monocytogenes* infection was variable among several host strains; gastritis was more severe in BALB/c and C57BL/6 mice than in ICR, C3H, and FVB. The use of different host strains might also be the reason for a lack of gastric lesions in the study conducted by Marco et al. [10].

Neutrophils are the main inflammatory cell present in the CNS or the hepatic lesions of *L. monocytogenes*-infected mice [10,12,15]. In the present study, neutrophilic infiltration and necrosis was the early event of the lesions of the stomachs, spleens, and livers, which were most predominant at 3–7 days pi. However, after 7 days pi, chronic inflammation including infiltration of lymphocytes and macrophages occurred in the gastric lesions as well as hepatic lesions. In addition, the lesions in the spleens and livers started to alleviate from 7 and 10 days and reached normal state at 10 and 28 days, respectively. However, the gastric lesions, especially in submucosa and muscularis of NGR and fundus, lasted with a severity over 1.33 of mean score (Fig. 4B) until 28 days.

The gastric lesions were more severe in the NGR and fundus than in the pylorus, and were more severe in submucosa, lamina muscularis, and serosa than in the mucosa. These appearances are different from the gastric lesions by *Helicobacter* spp. infection. The mice models of the gastritis by several *Helicobacter* spp. [16–22] were established well. In the models, the gastric lesions were characterized by a chronic inflammation composed of lymphocytes, monocytes, and a plasma cell infiltration, the formation of lymphoid follicles, and the hyperplasia of gastric epithelia in antral mucosa.

Host resistance to *L. monocytogenes* infection is controlled by cell-mediated immunity that is regulated by cytokines. Many studies demonstrated that endogenous cytokines such as INF-γ, TNF-α, IL-1, IL-6, IL-12, and IL-18 play important roles in host resistance against *L. monocytogenes* infection [23–29]. However, in the gastric tissue of *L. monocytogenes*-infected mice, the expression of these genes has not yet been confirmed. Therefore, in the present study, mRNA expression of the inflammatory mediator was assessed in the gastric tissue using RT-PCR. The results showed that mRNA expression of INF-γ, IL-1β, IL-5, IL-6, IL-12, and TNF-α among the cytokines and KC and MCP-1 among the chemokines was gradually increased in the stomachs of infected mice until 7 days and slightly decreased at 14 days. The degree of mRNA expressions was similar in pattern to that of histopathological lesions, which were the most severe at 3–7 days pi and after which gradually decreased.

IL-12 is a heterodimeric protein (p35/p40) produced by major antigen-presenting cells, including monocytes and macrophages, dendritic cells, B cells, and keratinocytes. In the dog and mouse, the p35 chain is constitutively induced, whereas the p40 chain is inducible following monocyte or macrophage activation. Therefore, in the present study, we used an IL-12p40 specific primer automatically designed by a primer selection program of BCM Search Launcher. IL-18 synergizes with IL-12 to induce IFN-γ production by natural killer and T helper (Th) 1 cell. It was reported that IL-18 appeared to be even more potent than either IL-12 or IFN-γ in the protection against *L. monocytogenes* [29]. However, in this study, IL-18 was constitutively expressed in the gastric mucosa of all mice regardless of *L. monocytogenes* infection. It is expected that IL-18 expression against *L. monocytogenes* infection would be organ-specific.

In mice, the best-known CXC chemokines are macrophage inflammatory protein-2 (MIP-2) (CXCL2) [30] and cytokine-induced neutrophil chemoattractant (KC) (CXCL1) [31,32], both of which are potent stimulators of neutrophil activation and tissue infiltration. MCP-1 is responsible for the recruitment of monocytes/macrophages and granulocytes from circulation to the inflamed mucosa [33]. Because marked neutrophilic infiltration was the early event in the gastric lesions induced by *L. monocytogenes* infection, it was expected that these chemokines would be increased at the early phase of infection. As expected, mRNA expression of these chemokines increased between 1 and 7 days pi, although MIP-2 expression in the stomachs of mice killed at 3 days pi was not different from that in the control mice.

In conclusion, in human listeriosis, massive outbreaks of febrile gastroenteritis have been reported recently, and its molecular or pathological features are unknown. In the present study, we demonstrated the characteristics (bacterial colonization, histopathological findings, mRNA expression of inflammatory mediators) of the gastritis induced by *L. monocytogenes* infection. These findings would be useful for studying the pathological mechanism of human febrile gastroenteritis due to *L. monocytogenes* infection.

4. Materials and methods

4.1. Bacterial culture

*L. monocytogenes* strain ATCC 51774 (serotype 1/2a) was obtained from the American Type Culture Collection (Manassas, VA, USA). The bacteria were inoculated into tryptic soy (TS) broth (Difco, Sparks, MD, USA) and incubated overnight with shaking at 37 °C. Hundred microliters of the culture was added to 10 ml of new TS broth and was incubated to an OD 0.5 at 600 nm. The culture was 10-fold diluted and plated on TS agar by overlay methods to count viable bacteria and to use as an inoculum.

4.2. Animals

Five- to six-week-old female specific-pathogen-free BALB/c mice were obtained from Seoul National
University Laboratory Animal Center (Seoul, South Korea). They were housed in polycarbonate cages in isolators under 22±2 °C, 50% humidity and fed a commercial pellet diet with water ad libitum. Food and water was removed from the cages 6 h prior to the inoculation of *L. monocytogenes* or killing.

4.3. Experimental designs

Three untreated mice were sacrificed at 0 day pi and provided as controls. Twenty-seven mice were handily restricted without anesthesia, and each were initially inoculated intragastrically with 100 μl of 10% sodium bicarbonate (w/v in sterile water) to neutralize the gastric acid using an 18 gauge stainless steel feeding needle (2.25 mm diameter and 50 mm length) with a round tip (Fine Science Tools Inc., North Vancouver, BC, Canada). Fifteen minutes later, the mice were inoculated intragastrically with 0.25 ml of the bacterial inoculum (![](https://doi.org/10.1016/S0026-6527(04)00198-8)). Each of the three mice was sacrificed by cervical dislocation at 1, 3, 5, 7, 10, 14, 17, 21, and 28 days pi, respectively. Bacterial counts, histopathological lesions, and mRNA expression of inflammatory mediators in the stomachs were then assessed.

4.4. Bacterial counts in the stomach, spleen, and liver

After being sacrificed, the abdominal cavities of the mice were aseptically opened, and the spleens, stomachs, and livers were removed. The stomachs were opened along the greater curvature and the gastric content was gently washed with sterile phosphate buffered saline (PBS; pH 7.2). A fraction of the stomach (junction of NGR and fundus), spleen, and liver was homogenized in PBS for bacterial counts. Another fraction was frozen at −70 °C for RNA extraction. The remaining fraction was fixed in 10% buffered formalin for histopathological examinations. Tenfold dilutions of the homogenates were plated on PALCAM (Merck, Darmstadt, Germany) agar. After incubation for 36–48 h at 37 °C, the numbers of bacteria per gram were calculated.

4.5. Histopathological findings

Formalin-fixed tissues were 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). Histopathological severity was assessed as 0–3 scales and the results were expressed as mean ± SD.

4.6. RNA isolation

RNA was isolated from the gastric samples that had been stored at −70 °C by using TRIZol® Reagent (Invitrogen, Carlsbad, CA, USA). Each gastric sample was electrically homogenized (Ultraturrax T8, IKA, Heidelberg, Germany) in 5 ml round-bottom glass tubes in 500 μl of TRIZol on ice. Each homogenate was transferred to a 1.5 ml Eppendorf tube, vortexed, and incubated at room temperature (RT) for 5 min. Two hundred microliters of chloroform was added and the tubes were vortexed for 15 s and placed on ice for 10 min. Phases were separated by centrifugation for 20 min at 12,000 rpm in a refrigerated microcentrifuge. The upper aqueous phase was transferred to a new 1.5 ml tube, and RNA was precipitated by the addition of 0.5 ml of isopropanol. The tubes were allowed to stand at −20 °C for at least 12 h. The precipitate was pelleted by centrifugation for 15 min at 12,000 rpm at 4 °C, and the pellets were washed with 1 ml of 70% ethanol. After the removal of the supernatant, the pellets were dried at RT for 20 min before being resuspended in 100 μl diethyl pyrocarbonate-treated water. The RNA concentration was determined by the absorbance at 260 nm. RNA was stored at −80 °C until use.

4.7. Reverse transcription-PCR (RT-PCR)

cDNA was reverse transcribed from 1 μg of total RNA using a GeneAmp RNA PCR Core kit (Applied Biosystems, Foster City, CA, USA). PCR was performed using i-Taq DNA polymerase (iNtRON, Sungnam, Kyungki-Do, South Korea) and specific primer sets for cytokines and chemokines. The primers for IFN-γ [34], IL-1β [31], IL-18 [34], TNF-α [35], KC [36], MCP-1 [36], and β-actin [35] were used as reported previously. The primers for the other genes were designed by the primer selection program of BCM Search Launcher (Baylor College of Medicine, Human Genome Sequencing Center, Houston, TX, USA) as follows (5′–3′); IL-5: F, TGTTAATCCCTTTGTGTTACCC; R, GCCCTTTAAGTTCAGTTACGC; IL-6: F, CATGGTCTCCTGGAAATCTGTT; R, AACTGATATGCTTAGGGCATACGCAC. IL-12p40: F, CAAATTACTCCGGAATTT; R, TTGCATTGGACTTCGGTA; MIP-2: F, CTGGTTGGCCATGTTA; R, GGCTTCCTCCTTCCAGGTCAGT. PCR conditions were as follows: 3 min of denaturation at 95 °C; followed by 27–32 cycles consisting of 30 s of denaturation at 94 °C, 30 s of annealing at 50–60 °C, and 1 min of extension at 72 °C; and 7 min of final extension at 72 °C after amplification. The PCR product was detected by the electrophoresis of 9 μl of the reaction solution in a 1.5% agarose gel, followed by staining with ethidium bromide. The bands of the PCR product were visualized on a UV transilluminator.

Acknowledgements

This work was supported by the Brain Korea 21 Project.
References


