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Infection of Different Strains of Mice with *Lawsonia intracellularis* Derived from Rabbit or Porcine Proliferative Enteropathy

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Summary

This report describes intestinal lesions in five strains of mice infected orally with *Lawsonia intracellularis*-infected tissue homogenates from rabbits or pigs (RLI and PLI). BALB/cA, C3H/HeJ, C57BL/6J and ICR mice were susceptible to infection with RLI, whereas only C3H/HeJ, C57BL/6J and ICR strains were susceptible to PLI. In susceptible mice, crypt epithelial hyperplasia occurred in association with an inflammatory reaction, as in proliferative enteropathy (PE) in other species. The intestinal changes in the infected mice varied from mild to severe. Unlike rabbit or porcine PE, in which the changes are confined to the ileum, the lesions in mice were located in the caecum. Immunolabelling of *L. intracellularis* antigen was abundant in early infection when the epithelial hyperplasia was mild or absent. When the hyperplasia had become severe, however, immunolabelling was weak. For this reason, it is suggested that transitory infection of the epithelium induces epithelial hyperplasia. Genetic differences between mouse strains appeared to play an important role in the response to *L. intracellularis* infection. Moreover, the susceptibility of BALB/cA mice to RLI but not to PLI suggests that there are significant biological differences between *L. intracellularis* isolates from rabbit PE and porcine PE.

Keywords: bacterial infection; *Lawsonia intracellularis*; mouse strains; pig; proliferative enteropathy; rabbit

Introduction

Lawsonia intracellularis, an obligate intracellular gram-negative bacterium characterized by McOrist *et al.* (1995), is the confirmed aetiological agent of proliferative enteropathy (PE) in various animal species (Lawson and Gebhart, 2000), the most frequently affected of which are the pig and hamster. *L. intracellularis* infection in pigs (porcine proliferative enteropathy; PPE), a worldwide disease associated with diarrhoea and weight loss, is of considerable economic importance. Comparisons of 16S ribosomal RNA sequence fragments of isolates from the pig, hamster, deer and ostrich suggest that such isolates represent the same species (Cooper *et al.*, 1997). The ability of pig *L. intracellularis* to reproduce not only in pigs (McOrist *et al.*, 1993; Yeh *et al.*, 2006) but also in hamsters (Jasni *et al.*, 1994; Yeh *et al.*, 2006) suggests that these bacteria are not host-restricted; however, the bacteriological relationship (including pathogenicity in other species) between *L. intracellularis* isolates from different species remains unclear. Cross-infectivity studies have been made in mice inoculated experimentally with *L. intracellularis* derived from PPE (Collins *et al.*, 1999; Smith *et al.*, 2000; Go *et al.*, 2005); however, the ability of these isolates to produce PE lesions in different mouse strains varied. Both wild-type 129 mice and gamma interferon (IFN-γ) receptor knockout (IFN-γR$^-$) mice infected with pig *L. intracellularis* showed PE lesions (Smith *et al.*, 2000; Go *et al.*, 2005). C57BL/6, BALB/c (Collins *et al.*, 1999; Go *et al.*, 2005) and ICR (Go *et al.*, 2005) mouse strains were reported not to exhibit signs

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of infection; Smith *et al.* (2000), however, found that C57BL/6 mice showed PE similar to that produced in 129 wild-type mice. The basis for differences in susceptibility between mouse strains remains to be determined. An early report by Collins *et al.* (1999) suggested that wild-type mice were weakly susceptible to experimental infection with a PPE strain; however, there are no descriptions of the susceptibility of mice to such agents from other species.

The purposes of this report are (1) to describe experimental infections in mice of different strains by *L. intracellularis* derived from rabbit or pig PE, and (2) to discuss both the genetic basis of host–pathogen interactions and the biological differences between *L. intracellularis* isolates from the rabbit and the pig.

Materials and Methods

Mice

Female animals ($n = 26$), aged 3–4 weeks, of each of five strains were used. The strains were A/J (SLC, Shizuoka, Japan) and BALB/cA, C3H/HeJ, C57BL/6J and ICR (Clea, Tokyo, Japan). The animals were housed in plastic cages and acclimatized for 1 week before use.

Bacterial Strains and Inocula

Two strains of *L. intracellularis* were used, one (PLI) from mucosal lesions in a pig with PE slaughtered in an abattoir in the south of Hokkaido, and the other (RLI) from a wild rabbit with PE found in a park in the northeastern region of Honshu.

Because of the difficulty of culturing *L. intracellularis in vitro* (Lawson *et al.*, 1993; Guedes and Gebhart, 2003a; personal observations) homogenized intestinal tissue (10%) was used to provide inocula (see below), each mouse receiving 1 ml orally by means of a feeding tube (SF-FT0380; Termo, Tokyo, Japan).

Preparation of Inocula

Before preparing inocula from infected or uninfected (control) pig or rabbit intestine, the presence or absence of disease was confirmed by the use of polymerase chain reaction (PCR) and immunohistochemical methods on intestinal mucosa samples. Briefly, the PCR procedure after DNA extraction was routinely carried out with a species-specific primer pair (sense: 5′TTACAGGTGAAGTTATTGGG3′ and anti-sense: 5′CTTTCTCATGTCCCATAACG3′) designed by Jones *et al.* (1993). Positive intestinal samples gave a primer-specific band consistent with the 279 base-pair *L. intracellularis*-specific PCR product; negative control samples showed no specific PCR products. Immunohistochemical examination by methods described below confirmed the PCR results for each sample. Inocula were prepared from tissue samples according to Lawson *et al.* (1993), the ileal mucosa being scraped and homogenized in 100 ml of ice-cold Ringer's solutions (115 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 10 mM glucose, 5 mM Tris, pH 7.5) containing fetal bovine serum 5%. To standardize the dose of *L. intracellularis*, homogenates were diluted in 0.1 M phosphate-buffered saline (PBS) and exposed for 1 h at 37°C to rabbit antiserum containing antibodies to synthetic peptides derived from the sequence of amino-acid residues from 41 to 56 of the *Lawsonia* surface antigen (LsaA) protein (McCluskey *et al.*, 2002; Watarai *et al.*, 2004). Samples were then incubated with FITC-labelled anti-rabbit IgG (Cappel, Solon, OH, USA) for 1 h at 37°C, and *L. intracellularis* numbers were counted under a fluorescence microscope. Approximately 4.6×10^6 *L. intracellularis* were administrated orally to each mouse.

Experimental Procedure

From each of the five mouse strains, 10 animals were inoculated with PLI, 10 with RLI, three with normal pig intestinal homogenate (controls) and three with normal rabbit intestinal homogenate (controls). Two mice from each of the 10 infected groups were humanely killed for examination at 4, 7, 14, 21 and 28 days post-inoculation (dpi); and one from each of the 10 control groups was killed on each of three occasions (7, 14 and 21 dpi). The experiment was conducted in accordance with the guidelines of the University Animal Care and Use Committee.

Histology and Immunohistochemistry (IHC)

Samples collected from intestinal tissue (duodenum, terminal ileum, caecum and proximal colon) were fixed in 10% neutral buffered formalin and embedded in paraffin wax, and sections (4 μm) were stained with haematoxylin and eosin (HE) or Warthin–Starry silver.

Immunohistochemistry was performed with rabbit antiserum against LsaA as the primary antibody, and secondary antibody conjugated with horseradish peroxidase-labelled polymer (Envision+ kit; Dako, Burlingame, CA, USA). Pretreatment procedures for sections included dewaxing followed by incubation in 0.1% trypsin for 20 min at 37°C. Endogenous peroxidase activity was subsequently blocked by incubation in 3% H_2O_2 for 5 min at room temperature. The sections were exposed to primary antibody (1 in 1000) for 1 h at room temperature, and then incubated with the secondary antibody for 30 min at room