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Short Communication

Experimental reproduction of proliferative enteropathy and the role of IFN-γ in protective immunity against *Lawsonia intracellularis* in mice

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Proliferative enteropathy was reproduced in IFN-γ receptor knockout (IFN-γ R$^-$) mice by experimental infection with *Lawsonia intracellularis* (*L. intracellularis*). The cecum and the colon of the infected mice were evidently enlarged 2 weeks post infection. The presence of *L. intracellularis* was identified in the stool and the cecum of the mice after infection. However, high levels of IFN-γ were detected in the sera of the infected mice 2 weeks PI. These data indicated that the IFN-γ produced in the infected mice should have been utilized by it's receptor to elicit protective immune responses against *L. intracellularis* infections.

Key words: IFN-γ R$^-$ mice, IFN-γ, *Lawsonia intracellularis*, proliferative enteropathy

Proliferative enteropathy (PE) is caused in pigs by infection with *L. intracellularis*, the obligate intracellular Gram-negative bacterium [6]. Typical clinical signs of PE in pigs are diarrhea and growth retardation which eventually lead to a high economic loss in pig industry worldwide [4,9]. The characteristic of PE lesions in naturally infected pigs with *L. intracellularis* is proliferation of immature epithelial cells in the ileum [5]. A more severe form of PE is described as proliferative hemorrhagic enteropathy (PHE) that develops congestion of mucosal blood vessels and severe necrosis of the intestinal epithelium [4]. Infections of *L. intracellularis* were further identified in a broad range of animals, including hamster, deer, ostrich, rabbits, rhesus macaques and mice [4,8]. However, the pathogenesis of *L. intracellularis* and the immune responses to *L. intracellularis* infection are not well understood. In this study, we reproduced PE lesions in IFN-γ R$^-$ mice by experimental infection with *L. intracellularis* and identified an important role of IFN-γ in protective immune responses against the infection of *L. intracellularis*.

L. intracellularis (KCTC 10686, Korea) was isolated from a pig that had clinical signs of an acute PHE. *L. intracellularis* was propagated in the McCoy cells for 7 days at 37°C in a mixed gas of 8.0% hydrogen, 8.8% carbon dioxide and 83.2% nitrogen. A total number of 1.5×10^7 *L. intracellularis* was used to orally infect IFN-γ R$^-$ mice G129RD (B&K Universal, UK). Disease signs of the infected mice were observed for 14 days and their feces were collected on days 0, 3, 5, 7, 9, 11, and 14 post infection (PI). The ceca of infected mice were obtained on day 14 PI. The identification of *L. intracellularis*-specific DNA in the feces and the ceca was performed by PCR as described elsewhere [3]. The following forward and backward primers were used to amplify 319-bp of the bacterial DNA: the forward primer, 5'-TAT GGC TGT CAA ACA CTC CG-3'; the backward primer, 5'-TGA AGG TAT TGG TAT TCT CC-3'. The thermal cycle conditions were 93°C for 30 sec, 45°C for 30 sec, and 72°C for 30 sec, 35 cycles. Blood samples were collected on days 0, 7, and 14 PI from the infected IFN-γ R$^-$ mice. Amount of IFN-γ in 50 μl serum was determined with ELISA kit according to the manufacturer's protocols (R&D systems, USA).

Infection of IFN-γ R$^-$ mice with *L. intracellularis* reproduced PE lesions in the cecum and the colon 14 days PI (Fig. 1). The infected mice excreted blood-containing stools and their intestinal walls of the cecum and the colon were considerably thickened and hyperemic (Fig. 1). However, the non-infected control mice neither excreted bloody stools nor produced PE lesions under the same conditions. The wild strain mice such as ICR, BALB/c, and C57BL/6 expressing IFN-γ receptor did not reproduce PE lesions in their intestines when they were inoculated with the same dose of *L. intracellularis* (data not shown). These results

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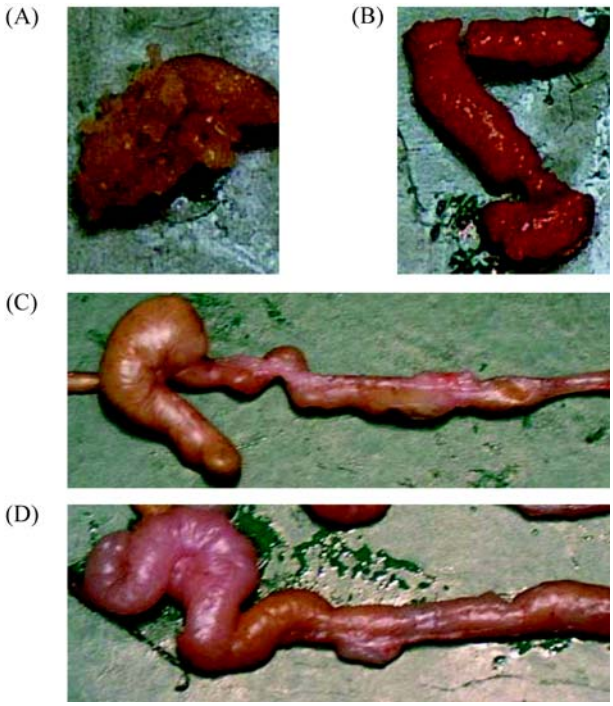

Fig. 1. Reproduction of proliferative enteropathy lesions in IFN-γ R^- mice by infection with *L. intracellularis*. IFN-γ R^- mice were infected with *L. intracellularis* and their feces and intestines were examined 2 weeks after infection. (A) Normal stool of an uninfected control mouse, (B) blood-containing stool of a *L. intracellularis*-infected mouse, (C) normal intestine of an uninfected mouse, and (D) enlarged and hyperemic cecum and colon of *L. intracellularis*-infected mouse. Three mice were assigned for an infection group and for a control group, respectively. This experiment was repeated three times.

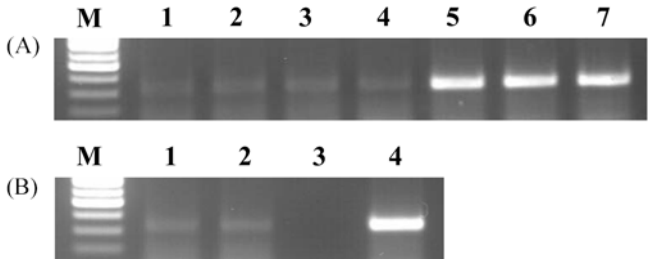

Fig. 2. Infection kinetics of *L. intracellularis* and identification of the bacteria in the cecum of IFN-γ R^- mice. (A) *L. intracellularis*-specific 319-bp DNA identified by PCR in the feces. Lanes: M, standard 100-bp DNA marker; 1, day 3; 2, day 5; 3, day 7; 4, day 9; 5, day 11; 6, day 14 PI; 7, a positive control. (B) *L. intracellularis*-specific 319-bp DNA identified in the ceca on day 14 PI. Lanes: 1 and 2, infected mice; 3, an uninfected mouse; 4, a positive control. Stools were collected from three infected mice at each day after infection.

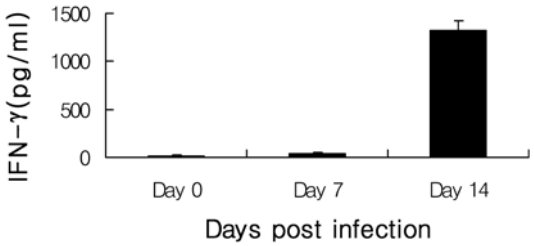

Fig. 3. Production of IFN-γ in IFN-γ R^- mice after infection with *L. intracellularis*. The amount of IFN-γ in sera of the infected mice was determined by ELISA on days 0, 7, and 14 PI.

implied that IFN-γ played an important role in the prevention of PE lesion development in the *L. intracellularis*-infected wild strain mice. It is known that PE lesions in pigs or hamsters are typically localized in their ilea [1,2,10]. However, PE lesions in the IFN-γ R^- mice were mainly observed in the cecum and the colon. This discrepancy may be attributable to the difference of host species. In order to examine the infection kinetics of *L. intracellularis* in IFN-γ R^- mice, PCR was employed to detect *L. intracellularis*-specific DNA in the feces of the infected mice. The bacterial shedding was identified in their feces from day 3 to day 14 PI (Fig. 2A). *L. intracellularis* was also detected in the cecum of the infected mice 14 days PI (Fig. 2B). These data indicated the successful colonization and proliferation of experimentally inoculated *L. intracellularis* in the large intestine of IFN-γ R^- mice. Our study more clearly demonstrated the time course of infection than a previous study in mice [8]. High production (1325.8 ± 93.9 pg/ml) of IFN-γ in the *L. intracellularis*-infected IFN-γ R^- mice was verified 14 days PI (Fig. 3). Since *L. intracellularis* is an obligate intracellular bacterium, it's replication in the intestinal epithelium may induce IFN-γ-mediated cellular immune responses against the pathogen like the case of other intracellular microorganisms [7]. However, the IFN-γ produced against *L. intracellularis* infection might not be utilized by IFN-γ receptor on the immune cells such as macrophages and T lymphocytes in the IFN-γ R^- mice. That might lead to unsuccessful clearance of *L. intracellularis* and to development of PE lesions in the large intestine. These results implied the importance of IFN-γ for the induction of a protective immunity against *L. intracellularis* infections.

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