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Oral administration of *Salmonella enterica* serovar Typhimurium expressing swine interleukin-18 induces Th1-biased protective immunity against inactivated vaccine of pseudorabies virus

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ABSTRACT

Enhancing and/or modulating innate and adaptive immunity by cytokines appears to be greatly useful to provide effective protective immunity against infectious diseases. However, an effective delivery system for mass administration in livestock industry is needed because of limitations such as cost, labor, time, and protein stability. Here the immunomodulatory functions of swine interleukine-18 (swIL-18), known as IFN-γ-inducing factor (IGIF), were evaluated in a vaccination model of pseudorabies virus (PrV) using attenuated *Salmonella enterica* serovar Typhimurium as the oral delivery system. The oral administration of *S. enterica* serovar Typhimurium expressing swIL-18 prior to vaccination with inactivated PrV vaccine induced enhanced levels of serum PrV-specific IgG and its IgG2 isotype, compared to administration of *S. enterica* serovar Typhimurium harboring the empty vector. Furthermore, *S. enterica* serovar Typhimurium expressing swIL-18 mounted Th1-biased cellular immune responses against PrV antigen, as evaluated by the production of IFN-γ and IL-4 from peripheral blood mononuclear cells of piglets. Subsequently, Th1-biased immunity induced by *S. enterica* serovar Typhimurium expressing swIL-18 showed rapid response and rendered piglets displayed more alleviated clinical signs following the virulent PrV challenge. Also, this alleviation of clinical signs was further confirmed by the reduction of nasal excretion of PrV after challenge. The present study demonstrates the extended use of immunomodulatory functions of swIL-18 orally delivered by attenuated *S. enterica* serovar Typhimurium.

1. Introduction

Pseudorabies virus (PrV) is an alpha herpesvirus of swine and is strongly related to the prototype alpha herpesvirus of human, herpes simplex virus type-1 (HSV-1) (Van et al., 2011). PrV causes fatal swine disease known as Aujeszky's disease that is characterized by neurological symptoms and death in young piglets, and respiratory and reproductive disorders in older pigs (Kluge et al., 1999; Hahn et al., 2010). Thus, the most significant economic losses of this disease are associated with the acute phase of the infection, in which there is a high abortion rate and neonatal mortality (Kluge et al., 1999; Hahn et al., 2010). Attempts to control PrV in swine and to reduce its associated economic losses have been classically made by active immunization with modified live or inactivated

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vaccines. Although modified live vaccine can minimize both the clinical symptoms and viral shedding during the acute phase of a PrV infection (Vilnis et al., 1998; Pomorska-Mol et al., 2010), they still hold some disadvantages such as the risk of reversion to virulence (Vilnis et al., 1998; Zuckermann, 2000) and the risk of interference with efficient antigen presentation (Zuckermann, 2000). In contrast, inactivated PrV vaccine is safe with no risk of reversion to virulence, but the high mortality of vaccinated animals from the disease highlights the need for considerable improvement in the immunogenicity of inactivated PrV vaccine (Lipowski, 2006).

Hence, several attempts to augment the immunogenicity of vaccine have been made. These include (i) co-administration of immunomodulatory molecules, defined as molecular adjuvants such as cytokines, co-stimulatory molecules or chemokines (Han et al., 2009; Kaburaki et al., 2010), (ii) enhanced presentation using dendritic cell (DC)-directed antigen, and (iii) amplification of DC recruitment at the site of antigen presentation (Westermann et al., 2004; Eo et al., 2001). In particular, among these strategies, there has been considerable interest in cytokines and the role that they appear to play in modulating adaptive immune responses (Heegaard et al., 2010). Furthermore, the potential effectiveness of cytokine combinations has been addressed empirically, based on mechanisms determining the nature of innate and adaptive immunity (Gherardi et al., 2003; Larkin et al., 2003; Bartee et al., 2009). The combined administration of two or more cytokines may produce effects that are additive or synergistic (Steinke and Borish, 2006). Therefore, the enhanced effects of cytokines for immunomodulation have been described in several infectious diseases of livestock animals such as foot-and-mouth disease (FMD) (Moraes et al., 2007), porcine reproductive and respiratory syndrome (PRRS) (Xue et al., 2004), and PrV (Han et al., 2009). Interleukin-18 (IL-18), originally known as a potent IFN-γ-inducing factor (IGIF), is synthesized as a 24-kDa precursor protein, which is then enzymatically cleaved to an 18-kDa mature IL-18 protein (Nakanishi et al., 2001). IL-18 is able to exhibit pleiotropic immunomodulatory activity and induce a Th1-type response, particularly in collaboration with IL-12 (Nakanishi et al., 2001). Mature IL-18 can act on Th1 cells, B cells, and DCs to produce IFN-γ in the presence of IL-12, through specific IL-18R complexes and triggering of MyD88-IRAK-TRAF (Nakanishi et al., 2001). Despite immunomodulatory functions of IL-18 cytokine in the control of infectious diseases in livestock, hurdles of the practical use of IL-18 in the livestock industry include cost, labor, time, and protein stability for mass administration.

To overcome these limitations, our previous study demonstrated that attenuated aspartate β-semialdehyde dehydrogenase (Asd)-negative *Salmonella enterica* serovar Typhimurium devoid of antibiotic resistance genes is an effective delivery system for the mass administration of cytokines without the need for antibiotic selection (Kim et al., 2010; Lee et al., 2011). The present study extends the immunomodulatory effect of swine IL-18 (swIL-18) produced by *S. enterica* serovar Typhimurium on vaccination with inactivated PrV vaccine. The oral administration of *S. enterica* serovar Typhimurium expressing swIL-18 prior to PrV vaccination produced Th1-biased immune responses against inactivated PrV vaccine, thereby alleviating clinical signs caused by the virulent PrV challenge. Furthermore, the administration of *S. enterica* serovar Typhimurium expressing swIL-18 induced the reduction of nasal excretion of PrV after viral challenge. Therefore, our results provide extended insights into the value of *S. enterica* serovar Typhimurium expressing swIL-18 to modulate immune responses induced by parenteral injection of inactivated PrV vaccine.

2. Materials and methods

2.1. Animals and ethics statement

Seronegative crossbreed F1 (Large white-Landrace × Duroc) piglets (3–4 weeks old) were obtained from a local breeding farm and housed separately in stainless steel cages (2–3 piglets/cage). Piglets were reared with formulated commercial feed and water provided *ad libitum* throughout the whole experimental period. All experimental procedures and animal management procedures were undertaken in accordance with the requirements of the Animal Care and Ethics Committees of Chonbuk National University. The animal facility of the Chonbuk National University is fully accredited by the National Association of Laboratory Animal Care.

2.2. Viruses and cells

The wild-type PrV YS strain and thymidine kinase-deleted PrV were generously supplied by the National Veterinary Research and Quarantine Service in the Republic of Korea. The viruses were propagated in the porcine kidney cell line, PK-15, using DMEM supplemented with 2.5% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 U/ml). PK-15 cultures were infected with PrV at a multiplicity of infection of 0.01, and then incubated in a humidified CO_2 incubator for 1 h at 37 °C. After absorption, the inoculum was removed and 10 ml of a maintenance medium containing 2.5% fetal bovine serum was added. Approximately 48–72 h after infection, a culture of host cells that showed an 80–90% cytopathic effect was harvested. The virus stocks were then concentrated by centrifugation at 50,000 × *g*, titrated using a plaque assay and stored in aliquots at −80 °C until needed.

2.3. Attenuated S. enterica serovar Typhimuirum expressing swIL-18

Attenuated *S. enterica* serovar Typhimurium expressing swIL-18 was constructed as described elsewhere (Lee et al., 2011). Attenuated *S. enterica* serovar Typhimurium χ8501 (*hisG Δcrp-28 ΔasdA16*), which was kindly provided by Dr. H.Y. Kang (Pusan National University, Korea) (Kang et al., 2002), was used as host for the delivery of swIL-18 and grown at 37 °C in Lennox broth, Luria-Bertani (LB) broth, or on LB agar. Diaminopimelic acid (DAP; Sigma–Aldrich, St. Louis, MO) was added (50 μg/ml) to induce the growth of Asd-negative bacteria (Nakayama et al., 1988). Phosphate-buffered saline (PBS, pH 7.4) containing