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Enhanced protection against infection with transmissible gastroenteritis virus in piglets by oral co-administration of live attenuated *Salmonella enterica* serovar Typhimurium expressing swine interferon-α and interleukin-18

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ABSTRACT

The enhanced effect of cytokine combinations has been assessed empirically, based on their immunobiological mechanisms. However, far less is known of the enhanced protection of practical cytokine combinations against viral infection in the livestock industry, due to cost and production issues associated with mass administration. This study demonstrates the enhanced protection of oral co-administration of swine interferon-α (swIFN-α) and interleukin-18 (swIL-18) against infection with transmissible gastroenteritis virus (TGEV) in piglets using attenuated *Salmonella enterica* serovar Typhimurium as carrier of cytokine proteins. A single oral co-administration of *S. enterica* serovar Typhimurium expressing swIFN-α and swIL-18 induced enhanced alleviation of the severity of diarrhea caused by TGEV infection, compared to piglets administered *S. enterica* serovar Typhimurium expressing swIFN-α or swIL-18 alone. This enhancement was further observed by the reduction of TGEV shedding and replication, and the expression of IFN-stimulated gene products in the intestinal tract. The results suggest that the combined administration of the swIFN-α and swIL-18 cytokines using attenuated *S. enterica* serovar Typhimurium as an oral carrier provides enhanced protection against intestinal tract infection with TGEV.

1. Introduction

Many neonatal cows and pigs are more susceptible to intestinal and respiratory infection by various pathogens such as viruses and bacteria, due to suboptimal immune functions. Furthermore, the worldwide trend toward a reduced reliance on in-feed antibiotics has increased the pressure to develop alternative strategies for the management of infectious diseases in the livestock industry. To overcome this reliance for controlling infectious diseases, there has been greater emphasis on the use of cytokines such as interleukin (IL)-2 [1], IL-12 [2], IL-18 [3], and interferon-alpha (IFN-α) [4] to provide effective long-term protective immunity to industrial animals. Extensive research into the biological effects of cytokines has revealed their important role in host defense and

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inflammatory responses as a regulatory link between innate immunity and the development of antigen-specific acquired immune responses [5].

The potential effectiveness of cytokine combinations has been addressed empirically, based upon mechanisms determining the nature of innate and acquired immunity [6–8]. Likewise, the enhanced effects of cytokine combinations for immunomodulation or to test antiviral activity of cytokines has been described in several infectious diseases of livestock animals such as foot-and-mouth disease (FMD) [9,10], porcine reproductive and respiratory syndrome (PRRS) [11], and pseudorabies [12]. IFN-α, which is a type I IFN, is a cytokine that is produced in response to viral and microbial infection. The outstanding characteristic of IFN-α is its ability to nonspecifically inhibit viral growth by inducing the expression of numerous cellular genes through the interaction with specific type I receptor complexes and triggering of the Janus-activated kinases (JAKs)-signal transducer and activators of transcription (STAT) 1/2 pathway [13]. Accordingly, attempts to control viral infection by antiviral activity of swine IFN-α (swIFN-α) have been assessed in infection models of pseudorabies virus (PrV) [14], vesicular stomatitis virus (VSV) [15], influenza virus [15], transmissible gastroenteritis virus (TGEV) [16], porcine reproductive and respiratory syndrome virus (PRRSV) [17], and foot-and-mouth disease virus (FMDV) [18]. IL-18, originally known as IFN-γ-inducing factor, is synthesized as a 24-kDa precursor protein, which is then enzymatically cleaved to an 18-kDa mature IL-18 protein [19]. Mature IL-18 can act on T helper 1 (Th1) cells, nonpolarized T cells, natural killer (NK) cells, B cells, and dendritic cells (DCs) to produce IFN-γ in the presence of IL-12, through specific IL-18R complexes and triggering of MyD88-IRAK-TRAF [19]. In addition to its potent induction of IFN-γ, IL-18 also plays an important role in viral infection [20,21]. In particular, virus-infected macrophage-derived IL-18 and type I IFN (IFN-αβ) produced by the same cells synergistically induces rapid IFN-γ production, leading to a possible induction of Th1 immune responses [21]. Despite the great values of the use of cytokines including IFN-α and IL-18 in livestock, hurdles for the practical use of cytokines, alone or in combination, in the livestock industry include cost, labor, time, and protein stability for the mass administration. To overcome these limitations, it is essential to develop an effective delivery system for the mass administration of cytokines.

To this end, our previous study reported 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 [22]. Furthermore, cytokines produced by *S. enterica* serovar Typhimurium may be able to provide immunomodulatory functions to both mucosal and systemic sites, because orally administered *S. enterica* serovar Typhimurium can colonize the secondary lymphoid and nonlymphoid tissues including the lymph node, spleen, and liver, as well as the gut-associated lymphoid tissues (Peyer's patch) [23]. Based on the mechanisms of immunobiological activities induced by type I IFN and IL-18, it is assumed that the combined administration of *S. enterica* serovar Typhimurium expressing swIFN-α and swIL-18 may provide more enhanced protection against viral infection. To test this hypothesis, we presently investigated the efficacy of oral co-administration of *S. enterica* serovar Typhimurium expressing swIFN-α and swIL-18 in piglets using TGEV infection. TGEV, which belongs to *Coronaviridae* Family, causes serious intestinal infectious diseases of swine. This infection is especially severe in newborn pigs less than two weeks old, resulting in severe economic losses on affected farm [24]. Here the single oral co-administration of *S. enterica* serovar Typhimurium expressing swIFN-α and swIL-18 induced enhanced alleviation of TGEV-induced clinical signs in piglets. Therefore, a useful value of attenuated *S. enterica* serovar Typhimurium for combined administration of swIFN-α and swIL-18 contributing to confer effective protection against viral infection was discussed herein.

2. Materials and methods

2.1. Animals and ethics statement

TGEV-seronegative crossbreed F1 (Large white-Landrace × Duroc) piglets were obtained from a local breeding farm 5 days after birth. They were housed separately in specialized cages that were maintained in sterile stainless steel isolators (four piglets/isolator) and fed with commercial sterile milk and water *ad libitum*. 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. Cells and viruses

TGEV KT-2 strain [25] was a kind gift from the National Veterinary Research and Quarantine Service of the Republic of Korea and used for the piglet challenge. Swine testicle (ST) cells were used to propagate TGEV. ST cultures were infected with TGEV KT-2 at a multiplicity of infection (MOI) of 0.01, and were incubated in a humidified CO_2 incubator for 1 h at 37 °C. After adsorption of virus, the inoculum was removed, and 10 ml of Dulbecco's Modified Eagle's Medium (DMEM, Gibco BRL/Invitrogen, Carlsbad, CA) supplemented with 2% fetal bovine serum (FBS; Gibco BRL/Invitrogen), penicillin (100 U/ml) and streptomycin (100 U/ml) was added. Approximately 48–72 h post-infection, cultures of the host cells showing an 80–90% cytopathic effect were harvested. The virus stocks were clarified by centrifugation at 800 × *g* for 10 min to remove cell debris, titrated using cytopathic effect assay, and then stored in aliquots at −80 °C until needed.

2.3. Bacterial strains, plasmid, media and growth conditions

Escherichia coli χ6212 ($F^-\lambda^-\phi80\Delta$(*lacZYA-argF) endA1 recA1 hsdR17 deoR thi-1 glnV44 gyrA96 relA1* Δ*asdA4*) [26] was used as the host strain for construction of the Asd+