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IL-22 suppresses the infection of porcine enteric coronaviruses and rotavirus by activating STAT3 signal pathway

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ABSTRACT

Interleukin-22 (IL-22), a member of the IL-10 superfamily, plays essential roles in fighting against mucosal microbial infection and maintaining mucosal barrier integrity within the intestine. However, little knowledge exists on the ability of porcine IL-22 (pIL-22) to fight against viral infection in the gut. In this study, we found that recombinant mature pIL-22 (mpIL-22) inhibited the infection of multiple diarrhea viruses, including alpha coronavirus, porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), and porcine rotavirus (PoRV), in the intestinal porcine epithelial cell line J2 (IPEC-J2) cells. mpIL-22 up-regulated the expression of the antimicrobial peptide beta-defensin (BD-2), cytokine IL-18 and IFN-λ. Furthermore, we found that mpIL-22 induced phosphorylation of STAT3 on Ser727 and Tyr705 in IPEC-J2 cells. Inhibition of STAT3 phosphorylation by S3I-201 abrogated the antiviral ability of mpIL-22 and the mpIL-22-induced expression of BD-2, IL-18, and IFN-λ. Together, mpIL-22 inhibited the infection of PoRV and enteric coronaviruses, and up-regulated the expression of antimicrobial genes in IPEC-J2, which were mediated by the activation of the STAT3 signal pathway. The significant antiviral activity of IL-22 to curtail multiple enteric diarrhea viruses *in vitro* suggests that pIL-22 could be a novel therapeutic against devastating viral diarrhea in piglets.

1. Introduction

Interleukin-22 (IL-22), an IL-10 family-related cytokine, is primarily secreted by T helper 17 cells, innate lymphoid cells (ILC), innate natural killer (INK) cells, and epithelial cells (Dudakov et al., 2015; Sonnenberg et al., 2011; Wolk et al., 2004). Unlike most other IL-10-related cytokines, IL-22 primarily targets nonhematopoietic epithelial cells and fibroblasts (Ouyang and Valdez, 2008; Sonnenberg et al., 2011; Wolk et al., 2004). The IL-22 receptor is composed of two heterodimeric subunits, IL-22R1 and a common IL-10R2 of the IL-10 family (Xie et al., 2000). While the IL-10R2, a shared receptor for IL-10, IL-26, IL-28, and IL-29, is expressed in various tissues, IL-22R1 is primarily expressed in mucosal epithelia such as the intestinal epithelial cells (IEC) (Eidenschenk et al., 2014; Ouyang and Valdez, 2008).

The growing evidence demonstrates that IL-22 is essential to host defense against invading pathogens and inflammatory response, especially in the mucosal tissue (Ouyang et al., 2011; Witte et al., 2010; Wolk et al., 2006). Initially, studies determined that IL-22 is essential for the host to control the infection of extracellular bacteria in the lung or gut (Eidenschenk et al., 2014; Ivanov et al., 2013; Zheng et al., 2008). Recent evidence indicates that IL-22 has a substantial role in host defense against mucosal viral infection, including human immunodeficiency virus (HIV-1), influenza virus, and rotavirus (Hernandez et al., 2015; Ivanov et al., 2013; Kim et al., 2012; Kumar et al., 2013). The mechanism of IL-22 protection against infection is elusive and includes the direct promotion of epithelial survival and regeneration and the maintenance of the tissue barrier, but IL-22 also induces epithelial-secreting antimicrobials such as defensins and antiviral cytokines such as IL-18 (Hernandez et al., 2015; Munoz et al., 2015; Ouyang and Valdez, 2008). Furthermore, IL-22 increases its protective role when combined with IL-18 or IFN-λ (Hernandez et al., 2015; Zhang

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et al., 2014). Given the critical role of IL-22 in epithelial regeneration, host defense, and pathology, IL-22 is an attractive target for clinical development in animals and humans. However, little knowledge exists regarding porcine IL-22 (pIL-22).

Porcine diarrhea caused by viruses is one of the major problems affecting suckling piglets and causes substantial economic loss in the pork industry. The most common pathogens of porcine viral diarrhea are porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), and porcine rotavirus (PoRV) (Wang et al., 2016; Zhang et al., 2013; Zhao et al., 2016). PEDV and TGEV are alpha coronaviruses (Brian and Baric, 2005), and PoRV is a member of the genus *Rotavirus* within the *Reoviridae* family (Taniguchi and Komoto, 2012). All three diarrhea viruses primarily infect villous epithelial cells throughout the small intestine and cause serious injury of IEC, including severe diffuse atrophic enteritis and superficial villous enterocyte swelling (Wang et al., 2016). The manifestations of the infection of the three viruses are indistinguishable and characterized by vomiting, anorexia, watery diarrhea, dehydration, and weight loss, with a high morbidity and mortality in suckling piglets (Wang et al., 2016; Zhang et al., 2013). The co-infection of two or three viruses of PEDV, TGEV, and PoRV in the field is frequent and makes the development of therapeutic and prophylactic strategies to protect suckling pigs against diarrhea complications (Zhang et al., 2013; Zhao et al., 2016).

In this study, we found that the recombinant mature porcine IL-22 (mpIL-22) prepared using a prokaryotic expression system broadly inhibited the infection of alpha coronaviruses, PEDV and TGEV, and PoRV in intestinal epithelial cells. The antiviral activity of mpIL-22 was through the activation of STAT3 signaling and the up-regulated expression of antimicrobials and antiviral cytokines.

2. Material and methods

2.1. Cells and viruses

The IPEC-J2 cell line was kindly provided by Dr. Anthony Blikslager (North Carolina State University, Raleigh, NC). The cells were grown in Dulbecco's modified Eagle's medium (DMEM): nutrient mixture F-12 (Ham) (1:1) with Gluta AMAX_-I (DMEM = F12) (Gibco), supplemented with 5% fetal bovine serum (FBS), 5 μg/ml insulin-transferring-selenium supplements (Life Technologies), 5 ng/ml epidermal growth factor (Life Technologies), and 1% penicillin-streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C. Cell culture media were changed every 2 days, and the cells were passaged every 4–5 days by trypsinization with 0.25% trypsin-EDTA. African green monkey kidney cells (Vero E6) were grown and maintained in DMEM supplemented with 10% heat-inactivated FBS and penicillin-streptomycin and incubated at 37 °C with 5% CO_2.

The Vero cell-adapted PEDV CV777 strain, kindly provided by Maurice Pensaert at Ghent University (Merelbeke, Belgium), was propagated as previously described (Hofmann and Wyler, 1988; Sun et al., 2015). Briefly, Vero cells were inoculated with the virus at a multiplicity of infection (MOI) of 1 and cultured in serum-free DMEM for 72 h at 37 °C with 5% CO_2. The progeny virions were filtered and titrated using the $TCID_{50}$ method. TGEV Hua isolate H87 was derived from the virulent strain H16 by serial passage in PK-15 cells in our laboratory (Wang et al., 2010). The PoRV OSU strain was propagated in MA104 cells, and titers were determined by a plaque assay as previously described (Feng et al., 2009).

2.2. Phylogenetic analysis

An alignment of pIL-22 sequence with other mammalian IL-22 molecules (human, mouse, bovine, sheep, goat) was carried out using the Clustal W program. The amino acid (aa) sequence homologies among the species were analyzed using the MegAlign program of DNAstar (DNAstar Inc., Madison, Wis.). The phylogenetic tree based on the nucleotide sequence of the IL-22 genes was constructed by the neighbor-joining method, using DNAstar MegAlign Version 8.1.2. by Clustal W. The sequence data for phylogenetic analysis were taken from the GenBank nucleotide sequence database with the following accession numbers: pig IL-22, XM_001926156; human IL-22, NM_020525; mouse IL-22, NM_016971; bovine IL-22, EF560596; sheep IL-22, HE617662 and goat IL-22, HM542482.

2.3. Clone and prokaryotic expression of pIL-22

To clone pIL-22, total cellular mRNA was isolated from IPEC-J2 cells, and 1 μg total mRNA was used to prepare cDNA using a PrimeScript™ II 1st strand cDNA synthesis kit (Takara, Dalian, China). Primers used for pIL-22 are summarized in Table 1. Amplification was performed using PrimeSTAR HS DNA polymerase (Takara, Dalian, China) under the following conditions: 1 cycle of 94 °C for 5 min, 35 cycles of 98 °C for 10 s, 60 °C for 15 s, 72 °C for 1 min and 1 cycle of 72 °C for 10 min. The cDNA fragment encoding the predicted mpIL-22 was cloned into a pET-30 vector containing a 6-histidine tag at the N-terminus by *Eco*RI and *Sal*I, resulting in the plasmid pET-30a-IL-22, which was transformed into BL21 (DE3). The recombinant mpIL-22 was purified by a Ni–NTA column according to the manufacturer's protocol.

2.4. Stimulation of IPEC-J2 by mpIL-22 and in vitro virus infection

IPEC-J2 cells were initially plated into 6-wells for 2 days to grow a confluent monolayer. The cells were stimulated in the presence of DMEM F12 (as control) or a range of concentrations (4, 40 and 400 ng/ml) of mpIL-22 protein for 24 h. For the kinetics experiment, IPEC-J2 cells were stimulated with 40 ng/ml mpIL-22 and then harvested at the indicated times post treatment. The cells were washed and harvested for total cellular RNA extraction and quantification.

Following mpIL-22 stimulation for 24 h, IPEC-J2 cells were washed with PBS twice and inoculated with the Group A PoRV OSU strain, PEDV CV777 strain, or TGEV H87 strain at an MOI of 1. For S3I-201 inhibition experiment, IPEC-J2 cells were stimulated with 40 ng/ml mpIL-22 for 24 h, followed by incubation with 20 μM S3I-

Table 1
Real-time Q-PCR primers used for quantification of virus genomes and host cell gene expression.

Target	Sequence
IL-18	Forward: 5′-TCT ACT CTC TCC TGT AAG AAC-3′
	Reverse: 5′-CTT ATC ATG TCC AGG AAC-3′
IFN-λ	Forward: 5′-CCACGTCGAACTTCAGGCTT-3′
	Reverse: 5′-ATGTGCAAGTCTCCACTGGT-3′
BD-2	Forward: 5′-CCAGAGGTCCGACCACTA-3′
	Reverse: 5′-GGTCCCTTCAATCCTGTT-3′
GAPDH	Forward: 5′-CCTTCCGTGTCCCTACTGCCAAC-3′
	Reverse: 5′-GACGCCTGCTTCACCACCTTCT-3′
β-actin	Forward: 5′-GTGCGGGACATCAAGGAGAAG-3′
	Reverse: 5′-CGTAGCTCTTCATCCAGGGAG-3′
Survivin	Forward: 5′-CCGATTTGGCTCAATGTTTC-3′
	Reverse: 5′-GACAGAAAGGAAAGCACAACC-3′
PEDV-ORF3	Forward: 5′-GCACTTATTGGCAGGCTTTGT-3′
	Reverse: 5′-CCATTGAGAAAAGAAAGTGTCGTAG-3′
TGEV-S	Forward: 5′-GCTTGATGAATTGAGTGCTGATG-3′
	Reverse: 5′-CCTAACCTCGGCTTGTCTGG-3′
PoRV-VP6	Forward: 5′-CAACTGCACCACAAACTGAAAGA-3′
	Reverse: 5′-CTCGGTAATAAAAGGCAGCAGAA-3′