ABSTRACT

Interferons (IFNs) have been known as antiviral genes and they are classified by type 1, type 2, and type 3 IFN. The type 1 IFN consists of IFNα, IFNβ, IFNτ, and IFNω whereas the type 2 IFN consists of only IFNγ, which is a key cytokine driving T helper cell type 1 immunity. IFNλ belongs to the type 3 IFN, which is also known as IL-28 and IL-29 possessing antiviral activities. Type 1 IFN is produced by viral infection whereas type 2 IFN is induced by mitogenic or antigenic T-cell stimuli. The IFNτ of bovine was first discovered in an ungulate ruminant recognition hormone. IFNτ belongs to the type 1 IFN with the common feature of type 1 IFN such as antiviral activity. IFNs have been mostly studied for basic research and clinical usages therefore there was no effort to investigate IFNs in industrial animals. Here we cloned porcine IFNα8 from peripheral blood mononuclear cells of Korean domestic pig (Sus scrofa domestica). The newly cloned porcine IFNα8 amino acid sequence from Korean domestic pig shares 98.4% identity with the known porcine IFNα8 in databank. The recombinant porcine IFNα8 showed potent antiviral activity and protected bovine Madin-Darby bovine kidney (MDBK) cells from the cytopathic effect of vesicular stomatitis virus, but it failed to protect human Wistar Institute Susan Hayflick (WISH) cells and canine Madin-Darby canine kidney epithelial-like (MDCK) cells. The present study demonstrates species specific antiviral activity of porcine IFNα8.

Keywords: Porcine IFNα8; Mx dynamin-like GTPase-1; 2′, 5′-oligoadenylate synthetase-1; Recombinant protein; Antiviral assay

INTRODUCTION

Interferon (IFN) subclasses exist in vertebrates that have antiviral properties and cell-mediated immunity varying with cell types, animal species, and viruses. The type 1 IFN constitutes a large multigene family that includes IFNα, IFNβ, IFNτ, and IFNω. It is known that these 4 members of type 1 IFNs activate common cell surface receptors, IFNaR1 signal transducing chain and IFNaR2 ligand binding chain, respectively (1-3). Unlike vitamin and some hormone, cytokines need cell surface receptors to activate downstream signaling in
Cloning of Sus scrofa domestica IFNa8 from Korean Domestic Pig

**Abbreviations**

*E. coli, Escherichia coli; IFN, interferon; IPTG, isopropyl β-D-1-thiogalactopyranoside; IRF, interferon regulatory factor; ISG, interferon-stimulated gene; LPS, Lipopolysaccharide; MDBK, Madin-Darby bovine kidney epithelial; MDCK, Madin-Darby canine kidney epithelial-like; OAS, oligoadenylate synthetase; JAK, Janus kinase; STAT, signal transducers and activators of transcription; VSV, vesicular stomatitis virus; WISH, Wistar Institute Susan Hayflick.*

**Author Contributions**

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order to induce immune responses against different types of pathogen such as virus, bacteria, fungus, and parasite (4-7).

IFNy is only type 2 IFN that binds to distinct receptors and locates a separate chromosomal locus. Initially, IFNy was considered as a molecule produced from CD4+ T helper cell type 1 lymphocytes, CD8+ cytotoxic lymphocytes, and natural killer (NK) cells exclusively (8-10). IFNy from professional antigen presenting cells (APCs) such as monocyte, macrophage and dendritic cells acting locally has an important function in cell self-activation and activation of nearby cells (11,12). T lymphocytes are the major source of IFNy in the acquired immunity although IFNy is produced by NK cells. Possibly professional APCs are likely to be a part of function in early host defense against infections (11,13).

IFNαs are classified as type 3 IFNs (known as IL-28 and IL-29) and they are cytokines with IFN-like activities. Several types of viruses induce IFNα1 and IFNα2/3 in similar patterns. The IFNαs were characterized as interferon-stimulated genes (ISGs) unlike direct induction of IFNα/β by viral infection, thus identifying type 3 IFNs. In vitro assays revealed that IFNαs have detectable antiviral function against encephalomyocarditis virus, but they have limited activities against herpes simplex virus type 2 unlike potent inhibition of both viruses by IFNα (14).

The cytosolic part of receptor is associated with Janus kinase (JAK) activating after type 1 IFN binding to IFNaR1/2 that subsequently phosphorylates downstream signaling molecule named signal transducers and activators of transcription (STAT). The dimerization of STAT interacts with interferon regulatory factor (IRF)-9 to form a trimeric interferon-stimulated gene factor-3 (ISGF-3) complex translocating into the nucleus where it binds an interferon-stimulated regulatory element (ISRE) resulting in the induction of IFN-1 gene (15). The induction of IRF-1 drives the expression of IRF-2, which interacts with other regulatory elements to control the expression of type 1 IFN responsive genes (16,17).

IFNγ also belongs to type 1 IFN that present only in ruminants. Among type 1 IFNs, IFNγ amino acid sequence exhibits the highest homology with IFNo, but also shares a limited identity with IFNα/β. The amino acid sequence of IFNγ shares 50% and 25% identity with IFNα/β, respectively (18). IFNγ binds to the common type 1 IFN receptors, a hetero dimeric form of IFNaR1 (19) and IFNaR2 (2). However, IFNγ is produced mainly from trophoblasts of ruminant conceptuses during the blastocyst stage when the elongated trophoblast attaches to the uterine wall. The secretion of IFNγ prevents the destruction of the corpus luteum and helps in maternal recognition of pregnancy. In ruminant reproduction, IFNγ constitutes an actual pregnancy signal (20-24). In the pregnant period, IFNs produced by trophoblast are detected in other mammals but antiviral activity is almost non-detectable (25).

Type 1 IFNs induced antiviral activity via inhibiting viral gene transcription. The common ISGs associated with antiviral activities are 1) double stranded RNA-dependent protein kinase (PKR) suppressing translation initiation through the phosphorylation of protein synthesis initiation factor eIF-2α, 2) 2’,5’-oligoadenylate synthetase (OAS)-1 family and RNase L nuclease, which mediate RNA degradation, and 3) Mx dynamin-like GTPase (Mx) family targeting viral nucleocapsids resulted in inhibition of RNA synthesis (26-29). In the present study, we cloned porcine IFN8 from domestic pig (Sus scrofa domestica), a main strain in Korea, and compared its antiviral activities to human IFNa2 using different species of cell lines.
MATERIALS AND METHODS

Cells and reagents
Human amnion (fibroblast) Wistar Institute Susan Hayflick (WISH) cells, Madin-Darby bovine kidney (MDBK) epithelial cells, Madin-Darby canine kidney (MDCK) epithelial-like cell, and vesicular stomatitis virus (VSV; Indiana strain) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). WISH, MDBK, and MDCK cells were cultured in a medium containing 1% penicillin-streptomycin (Life Technologies, Grand Island, NY, USA), and 10% fetal bovine serum (Hyclone, Logan, UT, USA) according to the ATCC’s instructions. Human recombinant IFNα2 protein was obtained from LG biotech (Seoul, Korea). The IgG-horseradish peroxidase (HRP) conjugated secondary antibodies and anti-actin primary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

RNA extraction and RT-PCR for TA cloning
Total RNA was isolated from whole blood of domestic pig (Sus scrofa domestica) with the QIAamp RNA Blood Mini Kit (Qiagen, Valencia, CA, USA). Total RNA (2 µg) was reverse-transcribed with 0.5 µl of Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) in 20 µl reaction volume as described previously (30). The 2 µl of cDNA was propagated to perform PCR of porcine IFNα8 (accession No. ACV42397) with sense primer: 5′-AGCATCTGCAAGGTTCCCAA-3′; reverse primer: 5′-CAGGTGTCTGTCACTCCTTC-3′. PCR mixture was denatured at 94°C for 20 s, annealed at 59°C for 40 s, elongated at 72°C for 1 min, and reacted 30 cycles. The PCR products were resolved by 1%-agarose gel electrophoresis. The PCR product was visualized under ultra-violet (UV) trans-illuminator.

Construction of IFNα8 expression vector
The RT-PCR product of porcine IFNα8 was ligated into T&A cloning vector (RBC Bioscience, Xindian City, Taiwan) and the insert of cDNA was verified with DNA sequencing analysis (COSMO Genetech, Seoul, Korea). The region of mature IFNα8 without signal sequence was amplified by PCR method with primers containing EcoRI recognition site at 5′ end and XbaI recognition site at 3′ end (sense primer: 5′-ATATGAATTCACGCCATGCAGAACGAAA-3′; reverse primer: 5′-TATACTCTAGATCACCTTCTTCCTTGGG-3′). The PCR product was trimmed with EcoRI and XbaI, and ligated into pProEx/HTa expression vector (Invitrogen). The sequence of mature porcine pProEx/HTa-IFNα8 was verified by DNA sequencing prior to using for protein expression.

Expression and purification of recombinant proteins
The mature porcine pProEx/HTa-IFNα8 Escherichia coli (E. coli) expression vector was transferred into BL21/Codon and BL21/Rosetta (Promega, Fitchburg, WI, USA) because the yield of recombinant porcine IFNα8 in DH5α was not good enough (data not shown). The clones of porcine IFNα8 BL21/Codon plus and BL21/Rosetta were cultured in 1 ml volume of Luria-Bertani (LB) broth. The pilot experiment examined the level of recombinant protein in insoluble pellet or soluble supernatant by western blotting as described (31).

Porcine IFNα8 was expressed in BL21/Codon plus since this host strain produced the great amount of recombinant protein. The cells were cultured at 37°C. When the OD of cultured broth at 600 nm reached 0.6 OD, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce the target protein expression for 3 h at 37°C. After 3 h, the cells were collected by centrifugation at 8,000 rpm for 15 min at 4°C, resuspended in basic buffer (8 M Urea,
20 mM Tris-Hcl, pH 9.0), and subjected to ultrasonication. After 3 h incubation at 37°C, the supernatant was collected by centrifugation at 10,000 rpm for 20 min at 4°C and followed by sonication (pulse 30 s and interval 30 s for 2 min). It was then centrifuged at 10,000 rpm for 10 min at 20°C, when the supernatant was collected for purification. The recombinant porcine IFNα8 protein was purified with a Talon affinity column (Invitrogen) using his6-tag at N-terminus of the recombinant protein. Before loading the collected supernatant, the basic buffer was loaded onto Talon column for washing and equilibrium. The supernatant was loaded onto the column twice, and 0.1% Triton X 114 in basic buffer was loaded to remove lipopolysaccharide (LPS) (32). After washing with basic buffer, the protein was eluted with elution buffer (8 M Urea, 20 mM Tris-Hcl, pH 9.0, 150 mM imidazole). Four fractions (1 ml per fraction) were collected. After sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the protein bands were visualized by staining with Coomassie brilliant blue. After Talon affinity-purified porcine IFNα8 dialyzed in 20 mM Tris-Hcl, pH 9.0 then applied to anion exchange chromatography (GE Healthcare, Marlborough, MA, USA). The recombinant protein was analyzed by western blot using mouse monoclonal antibody against his6-tag (R&D system, Minneapolis, MN, USA) as described (4).

Cytopathic effect of antiviral assay
To determine the antiviral activity of recombinant porcine IFNα8, vesicular stomatitis virus (VSV) inhibition assay was performed with canine MDCK, bovine MDBK and human WISH cells. These cells were cultured in 96-well plates until the cells reached monolayer status. Then the cells were washed and bovine porcine IFNα8 proteins were added to the plate. Human IFNα2 was used as a positive control. Porcine IFNα8 proteins and human IFNα2 were used to treat the cell by a 2-fold serial dilution. The treated cells were incubated for 6 h and then VSV was added for infection. After 22 h of VSV infection, the media was removed and the cells were stained with crystal violet solution.

RT-PCR
MDBK, WISH, and MDCK cells (1×10^6 cells/well in 6-well plates; TPP Techno Plastic Products AG, Trasadingen, Switzerland) were treated with human porcine IFNα8 (20 ng/ml) at different time points (3 or 6 h) and then harvested for RNA extraction. Total RNA was isolated with Tri reagent (Sigma-Aldrich, St. Louis, MO, USA) according to manufacturers manual. Total RNA (2 µg) samples were used for RT-PCR. 2 µl of cDNA was used to perform PCR of canine MX dynamin-like GTPase 1 (Mx-1, accession No. NM_001003134) with sense primer: 5′-TGGAGGCTCTGTCAGGAGTT-3′; reverse primer: 5′-TTGCCTTCAGTCCCTCTGTC-3′, canine OAS-1 (accession No. AY863104) with sense primer: 5′-ATCTCCTGCCAGACACAG-3′; reverse primer: 5′-GTGAAGCAGGTGGAGAACTC-3′, canine β-actin (accession No. NM_001195845) with sense primer: 5′-ACCAACTGGGACGATGG-3′; reverse primer: 5′-GCCAATAGTGATGACCTGCC-3′. The rest of primers for Mx-1, OAS-1, and β-actin have been described in previous study (33).

RESULTS
Constitutive expression of IFNa in porcine peripheral whole blood cells
First, we examined the regulation of porcine IFNa8 with fresh peripheral whole blood cells from Korean domestic pig (Sus scrofa domestica). The whole blood cells were treated with different stimuli as indicated on the top. Interestingly, constitutive expression of porcine IFNa8 was detected in unstimulated control and there was no significant induction of
the transcript (Fig. 1). The control of β-actin exhibited at bottom panel to show that the difference is not due to varying amount of transcript. We used the RT-PCR product of the untreated control transcript for T&α cloning and its DNA sequence was verified as described in Materials and Methods section. As shown in Fig. 2, porcine IFN open reading frame (ORF) contains 570 base pair including first codon for “Met” residue and the last stop codon “TGA”. The 23 amino acid sequence of hydrophobic signal peptide was highlighted with green color.

**Figure 1.** The regulation of porcine IFNα8 transcript. RT-PCR was performed with the whole blood RNA from Korean domestic pig (Sus scrofa domestica) under stimulation of LPS, polyinosinic-polycytidylic acid (Poly I:C), and VSV at time point of 3 h. The control β-actin showed no difference in transcript. The data represents one of 4 independent experiments. 

Ctl, control; LPS, lipopolysaccharide.

**Figure 2.** Translated amino acid sequence of porcine IFNs. The nucleotide sequence was used to convert into amino acid sequence. The hydrophobic signal sequence of 69 base pairs nucleotide sequences were highlighted by green color with underlined 23 amino acid residues. This sequence was deposited in databank and obtained accession number as “KX275310”.

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ATGCGCTCAACTCAGCTCTGCCTTCGTCATCGATGAAAGGCTGGATGCTGCTGAACTCAGATATCTG
1 MAPTSAFFTALVLLSCNAIC
21 CTCGACTGCTGCTGCACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
SLGCDCLPQTHSALHTRALRL
121 CTGCGAGAATGGAATTCTGCTGCCCTCTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
LAQMRISPFSCLDHRRDFG
181 TTCGCCAGGCTCGTGGGGCAGAGCGAGCTCGAGCTCGAGCTCGAGCTCGAGCTCGAGCT
FPQEqALGOGNVQKAAQAMALV
241 GTGAGATTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
HEWLMQQTFOFLSFEQGSAAAW
301 GTGAGATGGAATTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
DESLLHQFCTGLDQQLROLE
361 CGCTGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
ACVMQEGVLEGTPPLEEDSI
421 CGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
LAVKYFHRLTLYLQEKYS
481 CGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
PACAWEIIRAEVMRAFSSSN
541 CGTGAAGAATGGAATTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG
LQDRLARKER*
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Amino acid sequence of IFNa8 from Korean domestic pig

The obtained amino acid sequence of porcine IFNα was aligned with porcine IFNα8 in Fig. 3A. The amino acid sequence of T&A cloned the porcine IFNα8 from Korean domestic pig shared 98.4% identity with the known porcine IFNα8 (accession No. ACV42397) as shown in Fig. 3. Three amino acid residues are different from the known porcine IFNα8 and the different residues are highlighted by yellow. The newly obtained Korean porcine IFNα8 was registered in databank as accession number (KX275310). The region of mature protein without signal sequence (Met/I-Gly/23) was subcloned into pProEx/HTa vector for E. coli expression. Actually, 2 amino acid residues of Korean porcine mature IFNα8 are different from that of the known IFNα8 in databank.

Expression of recombinant porcine IFNa8 protein

In order to compare the expression efficiency of recombinant IFNa8 protein in E. coli, the expression vectors were transformed into E. coli BL21/Codon and Rosetta strain. The production of recombinant proteins was induced by adding IPTG and the result was confirmed by western blot analysis by using anti-his6-tag antibody (Fig. 4A). The anti-his6-tag antibody recognized the IPTG-induced 25 kDa band as indicated by arrow. A large-scale expression was processed with BL21/Codon plus strain and the production of recombinant porcine IFNα8 proteins was present in an insoluble fraction (not shown).

The first step of purification was performed by using a mini-Talon affinity column. The eluted fractions from the Talon affinity column were analyzed by Coomassie blue staining (Fig. 4B). The molecular weight of porcine IFNα8 appeared to be approximately 25 kDa band (see the arrow in Fig. 4B). The purified recombinant IFNα8 protein by the Talon affinity column was further purified by anion exchange chromatography after overnight dialysis against Tris-Hcl (20 mM, pH 9.0). The elution peak of recombinant porcine IFNα8 protein was observed mainly in bound fractions (Fig. 5A). The anion exchange chromatography fractions were

98.4% identity (100.0% similar) in 189 aa overlap (1-189:1-189)

|   | IFNa8 | ref |
|---|-------|-----|
| 10 | MAPTSAFFTALVLLSCNAICSLGCDLPQTHSLAHTRALRLLAQMRRISPFSCLDHRRDFG | MAPTSAFLTALVLLSCNAICSLGCDLPQTHSLAHTRALRLLAQMRRISPFSCLDHRRDFG |
| 20 | | |
| 30 | | |
| 40 | | |
| 50 | | |
| 60 | | |
| 70 | FPQEALGGNQVQKAQAMALVHEMLQQTFQLFSTEGSAAAWDESLLHQFCTGLDQQLRDLE | FPQEALGGNQVQKAQAMALVHEMLQQTFQLFSTEGSAAAWDESLLHQFCTGLDQQLRDLE |
| 80 | | |
| 90 | | |
| 100 | | |
| 110 | | |
| 120 | | |
| 130 | ACVMQEVGLEGTPLLEEDSILAVRKYFHRLTLYLQEKSYSPCAWEIIRAEVMRAFSSSTN | ACVMQEAGLEGTPLLEEDSILAVRKYFHRLTLYLQEKSYSPCAWEIVRAEVMRAFSSSTN |
| 140 | | |
| 150 | | |
| 160 | | |
| 170 | | |
| 180 | | |

Figure 3. Comparison of porcine IFNa8 to the known porcine IFNα. The ORF of porcine IFNa8 mRNA from Korean domestic pig was analyzed and newly identified as porcine IFNα8 (accession No. KX275310). The amino acid sequence was deduced by newly obtained the DNA sequence of porcine IFNa8 and the different amino acid residue of Korean porcine IFNa8 compared to porcine IFNα8 (accession No. ACV42397). Three distinct amino acid residues are highlighted with yellow color. ORF, open reading frame.
visualized by silver staining to confirm the purity of the recombinant porcine IFNα8 protein (Fig. 5B). The anion exchange chromatography purified recombinant porcine IFNα8 protein appeared to be approximately 25 kDa and faint 38 kDa band in silver staining. We confirmed 38 kDa band is porcine IFNα8 by western blot (data not shown). The fractions were pooled as unbound fractions for quantification comparing to bovine serum albumin (BSA) in Fig. 5C.

**Antiviral activity of porcine IFNα8 against VSV**
The antiviral assay was first performed with human WISH cells by using both porcine IFNα8 and the control human IFNα2. As shown in Fig. 6A, human IFNα2 protected the cells from VSV infection while porcine IFNα8 failed to protect the cells. Next, we used bovine MDBK cells for porcine IFNα8 antiviral assay and both porcine IFNα8 and human IFNα2 protected MDBK cells (Fig. 6B). We further examined canine MDCK cells and human IFNα2 and porcine IFNα8 failed to protect MCBK cells from VSV infection (Fig. 6C).

**The antiviral activity of porcine IFNα8 via Mx-1 and OAS-1**
To investigate the antiviral mechanism of porcine IFNα8, the induction of ISGs was tested with RT-PCR. The levels of Mx-1 and OAS-1 were increased in bovine MDBK cells treated with porcine IFNα8 at 3 and 6 h (Fig. 7A) however the induction of antiviral genes in human WISH cells increased with only human IFNα2 (Fig. 7B). Interestingly, the high constitutive expression of Mx-1 and OAS-1 in canine MCBK was observed in the absence of IFNs stimulation (Fig. 7C). The control β-actin transcript showed that the induction of ISGs is not due to experimental variation (Fig. 7).

**DISCUSSION**
In the present study, we cloned porcine IFNα8 from domestic pig (*Sus scrofa domestica*), main strain of pig in Korea. The recombinant porcine IFNα8 protein was expressed in *E. coli* and examined for its antiviral activity with different species of cell line. Interestingly, the amino
The acid sequence of porcine IFNα8 is different from that of the known porcine IFNα8 in databank (accession No. ACV42397). The amino acid sequence of porcine IFNα8 shares 98.4% identity with the known porcine IFNα8. We deposited newly cloned porcine IFNα8 from Korean domestic pig (Sus scrofa domestica) in gene bank and obtained its accession number (KX275310).

Porcine recombinant IFNα8 was examined for its biological activity using a common antiviral assay. The antiviral activity of porcine IFNα8 was weaker in its cytopathic effect compared to human IFNα2 in bovine MDBK cells. The antiviral activity of porcine IFNα8 was obtained only with MDBK cells however human IFNα2 exhibited antiviral activity in both human WISH and bovine MDBK cells (Fig. 6). In addition, both porcine IFNα8 and human IFNα2 failed to show antiviral activity in canine MDCK cells (Fig. 6). In addition, the results of RT-PCR in Fig. 7 corresponded to the antiviral activity of IFNs in Fig. 6. These data suggested the species specificity with different IFNs.

It has been reported that human type 1 IFNs is superior in their activities in different species whereas mouse type 1 IFN inferior to other species such as human cells. This species-specific
activity provided Uzé et al. (19), to isolate IFNaR1 more than 20 years ago. However, there was no difference of activity of porcine IFNα8 and human IFNα2 in canine MDCK cell and both IFNs failed to show the antiviral activities in this cell line (**Fig. 6C**). This result may be
explained by RT-PCR data that the high constitutive expression of antiviral genes without IFN stimulation (Fig. 7C).

Although IFNα, IFNβ, IFNo, and IFNτ bind to common type 1 receptors, IFNaR1 and IFNaR2, their bindings and biological activities clearly exhibit species-, tissue-, and cell- specific differences probably due to 3-dimensional conformation differences among type 1 IFN ligands (18). Thus, the result indicated that the low activity of porcine IFNα8 in MDBK cells could be due to the difference of amino acid sequence between porcine IFNα8 and human IFNα2. However, we cannot exclude that there are additional unknown receptor components contributing for the species-specific antiviral activity of porcine IFNα8. The biological activity of porcine IFNα8 would be optimized in cells of their own strain bearing high-affinity receptors. It is necessary to investigate whether there is an additional receptor like IL-1 family cytokine ligands and receptors possessing 11 members of ligands and receptors (5) while 4 distinct ligands of type 1 IFN has only 2 receptors components.

Type 1 IFNs induce the synthesis of antiviral factors involving OAS-1 and Mx-1 genes via activation of the cellular JAK-STAT signaling pathway (2,34). The IFN-inducible OAS leading to the degradation of RNA requires 2 enzymes, OAS, and RNase L (35). Three isoforms of OAS, designated as OAS-1, OAS-2, and OAS-3, have been identified in human cells by immunoblotting and by characterization of cDNA and genomic clone analysis (36,37), however it is known that the oligomerization of OAS-1 and OAS-2 appears necessary for enzymatic activity (38-41). RNase L, a latent endonuclease, becomes active by binding 2–5 A oligonucleotides and digests the unusual RNAs. We found that porcine IFNα8 and human IFNα2 increased the synthesis of the cellular antiviral factors, such as OAS and Mx-1 proteins (Fig. 7). These results suggest that porcine IFNα8 induces the antiviral activity through similar pathways like other type 1 IFNs.

This study describes the cloning and characterization of porcine IFNα8 from domestic pig in Korea. Unexpectedly, the amino acid sequence of the porcine IFNα8 differs by about 2% from that of the known porcine IFNα8 in databank. We expect that the recombinant porcine IFNα8 would prevent different virus infection with lower cytotoxicity than other existing therapies. A large quantity of biologically active recombinant IFNα8 protein can be also used to develop protein drug for preventing early viral infection in industrial baby pig.

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REFERENCES

1. Kim SH, Cohen B, Novick D, Rubinstein M. Mammalian type I interferon receptors consists of two subunits: IFNaR1 and IFNaR2. Gene 1997;196:279-286.
2. Novick D, Cohen B, Rubinstein M. The human interferon alpha/beta receptor: characterization and molecular cloning. Cell 1994;77:391-400.

3. Novick D, Nabioullin RR, Ragsdale W, McKenna S, Weiser W, Garone L, Burkins C, Kim SH, Rubinstein M, Tepper MA, et al. The neutralization of type I IFN biologic actions by anti-IFNAR-2 monoclonal antibodies is not entirely due to inhibition of Jak-Stat tyrosine phosphorylation. J Interferon Cytokine Res 2000;20:971-982.

4. Lee S, Choi DK, Kwak A, Kim S, Nguyen TT, Gil G, Kim E, Yoo KH, Kim IA, Lee Y, et al. IL-32-induced inflammatory cytokines are selectively suppressed by α1-antitrypsin in mouse bone marrow cells. Immune Netw 2017;17:116-120.

5. Kwak A, Lee Y, Kim H, Kim S. Intracellular interleukin (IL)-1 family cytokine processing enzyme. Arch Pharm Res 2016;39:1556-1564.

6. Kim S. Interleukin-32 in inflammatory autoimmune diseases. Immune Netw 2014;14:123-127.

7. Kim B, Lee Y, Kim E, Kwak A, Ryoo S, Bae SH, Azam T, Kim S, Dinarello CA. The interleukin-1α precursor is biologically active and is likely a key alarmin in the IL-1 family of cytokines. Front Immunol 2013;4:391.

8. Bach EA, Aguet M, Schreiber RD. The IFN gamma receptor: a paradigm for cytokine receptor signaling. Annu Rev Immunol 1997;15:563-591.

9. Carnaud C, Lee D, Donnars O, Park SH, Beavis A, Koezuka Y, Bendelac A. Cutting edge: cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. J Immunol 1999;163:4647-4650.

10. Young HA. Regulation of interferon-gamma gene expression. J Interferon Cytokine Res 1996;16:563-568.

11. Frucht DM, Fukao T, Bogdan C, Schindler H, O'Shea JJ, Koyasu S. IFN-gamma production by antigen-presenting cells: mechanisms emerge. Trends Immunol 2001;22:556-560.

12. Gessani S, Belardelli F. IFN-gamma expression in macrophages and its possible biological significance. Cytokine Growth Factor Rev 1998;9:117-123.

13. Sen GC. Viruses and interferons. Annu Rev Microbiol 2001;55:255-281.

14. Ank N, West H, Bartholdy C, Eriksson K, Thomsen AR, Paludan SR. Lambda interferon (IFN-lambda), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections in vivo. J Virol 2006;80:4501-4509.

15. Gray CA, Abbey CA, Beremand PD, Choi Y, Farmer JL, Adelson DL, Thomas TL, Bazer FW, Spencer TE. Identification of endometrial genes regulated by early pregnancy, progesterone, and interferon tau in the ovine uterus. Biol Reprod 2006;74:383-394.

16. Binelli M, Subramaniam P, Diaz T, Johnson GA, Hansen TR, Badinga L, Thatcher WW. Bovine interferon-tau stimulates the Janus kinase-signal transducer and activator of transcription pathway in bovine endometrial epithelial cells. Biol Reprod 2001;64:654-665.

17. Roberts RM. Interferon-tau, a type 1 interferon involved in maternal recognition of pregnancy. Cytokine Growth Factor Rev 2007;18:403-408.

18. Roberts RM, Liu L, Alexenko A. New and atypical families of type I interferons in mammals: comparative functions, structures, and evolutionary relationships. Prog Nucleic Acid Res Mol Biol 1997;56:287-325.

19. Uzé G, Lutfalla G, Gresser I. Genetic transfer of a functional human interferon alpha receptor into mouse cells: cloning and expression of its cDNA. Cell 1990;60:225-234.

20. Bazer FW, Spencer TE, Ott TL. Interferon tau: a novel pregnancy recognition signal. Am J Reprod Immunol 1997;37:412-420.
21. Chen Y, Green JA, Antoniou E, Ealy AD, Mathialagan N, Walker AM, Availle MP, Rosenfeld CS, Hearne LB, Roberts RM. Effect of interferon-tau administration on endometrium of nonpregnant ewes: a comparison with pregnant ewes. *Endocrinology* 2006;147:2127-2137.

22. Roberts RM, Cross JC, Leaman DW. Interferons as hormones of pregnancy. *Endocr Rev* 1992;13:432-452.

23. Spencer TE, Becker WC, George P, Mirando MA, Ogle TF, Bazer FW. Ovine interferon-tau regulates expression of endometrial receptors for estrogen and oxytocin but not progesterone. *Biol Reprod* 1995;53:732-745.

24. Wolfsdorf KE, Diaz T, Schmitt EJ, Thatcher MJ, Drost M, Thatcher WW. The dominant follicle exerts an interovarian inhibition on FSH-induced follicular development. *Theriogenology* 1997;48:435-447.

25. Robinson RS, Mann GE, Lamming GE, Wathes DC. The effect of pregnancy on the expression of uterine oxytocin, oestrogen and progesterone receptors during early pregnancy in the cow. *J Endocrinol* 1999;160:21-33.

26. Pontzer CH, Bazer FW, Johnson HM. Antiproliferative activity of a pregnancy recognition hormone, ovine trophoblast protein-1. *Cancer Res* 1991;51:5304-5307.

27. Pontzer CH, Torres BA, Vallet JL, Bazer FW, Johnson HM. Antiviral activity of the pregnancy recognition hormone ovine trophoblast protein-1. *Biochem Biophys Res Commun* 1988;152:801-807.

28. Skopets B, Li J, Thatcher WW, Roberts RM, Hansen PJ. Inhibition of lymphocyte proliferation by bovine trophoblast protein-1 (type I trophoblast interferon) and bovine interferon-alpha II. *Vet Immunol Immunopathol* 1992;34:81-96.

29. Soos JM, Subramaniam PS, Hobeika AC, Schiffernauer J, Johnson HM. The IFN pregnancy recognition hormone IFN-tau blocks both development and superantigen reactivation of experimental allergic encephalomyelitis without associated toxicity. *J Immunol* 1995;155:2747-2753.

30. Kim H, Gil G, Lee S, Kwak A, Jo S, Kim E, Nguyen TT, Kim S, Jhun H, Kim S, et al. Cytokine-like activity of liver type fatty acid binding protein (L-FABP) inducing inflammatory cytokine interleukin-6. *Immune Netw* 2016;16:296-304.

31. Lee S, Kim E, Jhun H, Hong J, Kwak A, Jo S, Bae S, Lee J, Kim B, Lee J, et al. Proinsulin shares a motif with interleukin-1α (IL-1α) and induces inflammatory cytokine via interleukin-1 receptor 1. *J Biol Chem* 2016;291:14620-14627.

32. Aida Y, Pabst MJ. Removal of endotoxin from protein solutions by phase separation using Triton X-114. *J Immunol Methods* 1990;132:191-195.

33. Kang D, Ryoo S, Chung B, Lee J, Park S, Han J, Jeong S, Rho G, Hong J, Bae S, et al. Amino acid differences in interferon-tau (IFN-τ) of Bos taurus Coreanae and Holstein. *Cytokine* 2012;59:273-279.

34. Uzé G, Schreiber G, Pfehler J, Pellegrini S. The receptor of the type I interferon family. *Curr Top Microbiol Immunol* 2007;316:71-95.

35. Kerr IM, Brown RE. pppA2’p5’A2’p5’A: an inhibitor of protein synthesis synthesized with an enzyme fraction from interferon-treated cells. *Proc Natl Acad Sci U S A* 1978;75:256-260.

36. Rebouillat D, Hovanessian AG. The human 2′,5′-oligoadenylate synthetase family: interferon-induced proteins with unique enzymatic properties. *J Interferon Cytokine Res* 1999;19:295-308.

37. Rebouillat D, Hovananian A, Marié I, Hovanessian AG. The 100-kDa 2′,5′-oligoadenylate synthetase catalyzing preferentially the synthesis of dimeric pppA2’p5’A molecules is composed of three homologous domains. *J Biol Chem* 1999;274:1557-1565.
39. Ghosh A, Sarkar SN, Guo W, Bandyopadhyay S, Sen GC. Enzymatic activity of 2'-5'-oligoadenylate synthetase is impaired by specific mutations that affect oligomerization of the protein. *J Biol Chem* 1997;272:33220-33226.

40. Sarkar SN, Bandyopadhyay S, Ghosh A, Sen GC. Enzymatic characteristics of recombinant medium isozyme of 2'-5' oligoadenylate synthetase. *J Biol Chem* 1999;274:1848-1855.

41. Sarkar SN, Ghosh A, Wang HW, Sung SS, Sen GC. The nature of the catalytic domain of 2'-5'-oligoadenylate synthetases. *J Biol Chem* 1999;274:25535-25542.