

Original Article



Species Specific Antiviral Activity of Porcine Interferon- α 8 (IFN α 8)

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Conflict of Interest

The authors declare no potential conflicts of interest.

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 IFN α , IFN β , IFN τ , and IFN ω whereas the type 2 IFN consists of only IFN γ , which is a key cytokine driving T helper cell type 1 immunity. IFN λ 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 IFN τ of bovine was first discovered in an ungulate ruminant recognition hormone. IFN τ 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 IFN α 8 from peripheral blood mononuclear cells of Korean domestic pig (*Sus scrofa domestica*). The newly cloned IFN α 8 amino acid sequence from Korean domestic pig shares 98.4% identity with the known porcine IFN α 8 in databank. The recombinant porcine IFN α 8 showed potent antiviral activity and protected bovine Madin-Darby bovine kidney epithelial (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 IFN α 8.

Keywords: Porcine IFN α 8; 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 IFN α , IFN β , IFN τ , and IFN ω . It is known that these 4 members of type 1 IFNs activate common cell surface receptors, IFN α R1 signal transducing chain and IFN α R2 ligand binding chain, respectively (1-3). Unlike vitamin and some hormone, cytokines need cell surface receptors to activate downstream signaling in

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).

IFN γ is only type 2 IFN that binds to distinct receptors and locates a separate chromosomal locus. Initially, IFN γ 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). IFN γ 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 IFN γ in the acquired immunity although IFN γ is produced by NK cells. Possibly professional APCs are likely to be a part of function in early host defense against infections (11,13).

IFN λ s 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 IFN λ 1 and IFN λ 2/3 in similar patterns. The IFN λ s were characterized as interferon-stimulated genes (ISGs) unlike direct induction of IFN α / β by viral infection, thus identifying type 3 IFNs. *In vitro* assays revealed that IFN λ s 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 IFN α (14).

The cytosolic part of receptor is associated with Janus kinase (JAK) activating after type 1 IFN binding to IFN α 1/2 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 IRF-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).

IFN τ also belongs to type 1 IFN that present only in ruminants. Among type 1 IFNs, IFN τ amino acid sequence exhibits the highest homology with IFN ω , but also shares a limited identity with IFN α / β . The amino acid sequence of IFN τ shares 50% and 25% identity with IFN α / β , respectively (18). IFN τ binds to the common type 1 IFN receptors, a hetero dimeric form of IFN α 1 (19) and IFN α 2 (2). However, IFN τ is produced mainly from trophoblasts of ruminant conceptuses during the blastocyst stage when the elongated trophoblast attaches to the uterine wall. The secretion of IFN τ prevents the destruction of the corpus luteum and helps in maternal recognition of pregnancy. In ruminant reproduction, IFN τ 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-2 α , 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 IFN α 8 from domestic pig (*Sus scrofa domestica*), a main strain in Korea, and compared its antiviral activities to human IFN α 2 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 IFN α 2 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 μ g) was reverse-transcribed with 0.5 μ l of Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) in 20 μ l reaction volume as described previously (30). The 2 μ l of cDNA was propagated to perform PCR of porcine IFN α 8 (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 IFN α 8 expression vector

The RT-PCR product of porcine IFN α 8 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 IFN α 8 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'-ATATGAATTCTGCGACCTGCCTCAGA-3'; reverse primer: 5'-TATATCTAGATCACTCCTTCCTCCTG-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-IFN α 8 was verified by DNA sequencing prior to using for protein expression.

Expression and purification of recombinant proteins

The mature porcine pProEx/HTa-IFN α 8 *Escherichia coli* (*E. coli*) expression vector was transferred into BL21/Codon and BL21/Rosetta (Promega, Fitchburg, WI, USA) because the yield of recombinant porcine IFN α 8 in DH5 α was not good enough (data not shown). The clones of porcine IFN α 8 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 IFN α 8 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 IFN α 8 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 IFN α 8 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 IFN α 8, 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 IFN α 8 proteins were added to the plate. Human IFN α 2 was used as a positive control. Porcine IFN α 8 proteins and human IFN α 2 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 (1×10⁶ cells/well in 6-well plates; TPP Techno Plastic Products AG, Trasadingen, Switzerland) were treated with human porcine IFN α 8 (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 manufactures manual. Total RNA (2 μ g) samples were used for RT-PCR. 2 μ l 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'-TTGCCTTC AGTCCCTCTGTC-3', canine OAS-1 (accession No. AY863104) with sense primer: 5'-ATCTCCTGCCAGACACA CAG-3'; reverse primer: 5'-GTGAAGCAGGTGGAGAACTC-3', canine β -actin (accession No. NM_001195845) with sense primer: 5'-ACCAACTGGGACGACATGGA-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 IFN α in porcine peripheral whole blood cells

First, we examined the regulation of porcine IFN α 8 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 IFN α 8 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&A 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.

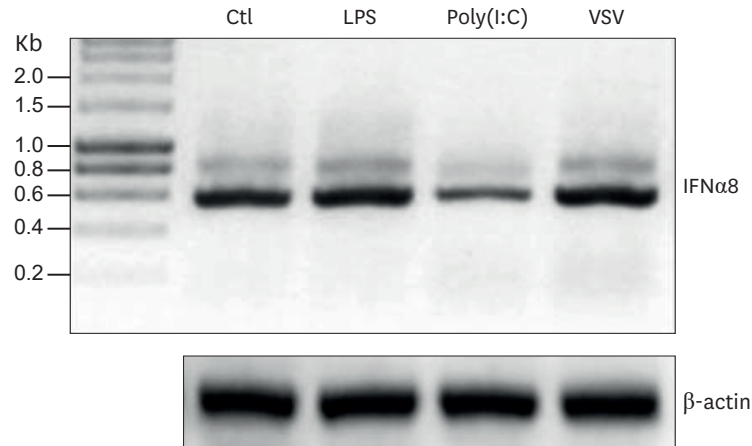


Figure 1. The regulation of porcine IFN α 8 transcript. RT-PCR was performed with the whole blood RNA from Korean domestic pig (*Sus scrofa domestica*) under stimulation of LPS, polyinosinic-polycytidylic acid (Poly I:C), and VSV at time point of 3 h. The control β -actin showed no difference in transcript. The data represents one of 4 independent experiments.
Ctl, control; LPS, lipopolysaccharide.

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1  ATGGCCCCAACCTCAGCCTTCTTCACAGCCCTGGTGCTACTCAGCTGCAATGCCATCTGC
1  M A P T S A F F T A L V L L S C N A I C

61  TCTCTGGGCTGTGACCTGCCTCAGACCCATAGCCTGGCGCACACAGGGCCCTGAGGCTC
21  S L G C D L P Q T H S L A H T R A L R L

121 CTGGCACAATGAGGAGAATCTCCCTTCTCTGCCTGGACCAGAAAGGACTTTGGA
41  L A Q M R R I S P F S C L D H R R D F G

181 TTCCCCAAGAGGCCTTGGGGGCAACAGGTCCAGAAGGCTCAAGCCATGGCTCTGGT
61  F P Q E A L G G N Q V Q K A Q A M A L V

241 CATGAGATGCTCCAGCAGACCTTCCAGCTTTGACGACAGAGGCTCGGCTGCTGCCTGG
81  H E M L Q Q T F Q L F S T E G S A A A W

301 GATGAGAGCCTCCTGCACGAGTTCTGCACTGGACTGGATCAGCAGCTCAGGACCTGGAA
101 D E S L L H Q F C T G L D Q Q L R D L E

361 GCCTGTGTCATGCAGGAGGTGGGGCTGGAAGGACGCCCTGCTGGAGGAGGACTCCATC
121 A C V M Q E V G L E G T P L L E E D S I

421 CTGGCTGTGAGGAAATACTTCCACAGACTCACCTCTATCTGCAAGAGAAGAGCTACAGC
141 L A V R K Y F H R L T L Y L Q E K S Y S

481 CCCTGTGCCTGGGAGATCATCAGGCGAGAAGTCATGAGAGCCTTCTTCTCCACAAAC
161 P C A W E I I R A E V M R A F S S S T N

541 CTGCAAGACAGACTCAGGAGGAAGGAGTGA
181 L Q D R L R R K E *

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Figure 2. Translated amino acid sequence of porcine IFN α . 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”.

Amino acid sequence of IFN α 8 from Korean domestic pig

The obtained amino acid sequence of porcine IFN α was aligned with porcine IFN α 8 in **Fig. 3A**. The amino acid sequence of T&A cloned the porcine IFN α 8 from Korean domestic pig shared 98.4% identity with the known porcine IFN α 8 (accession No. ACV42397) as shown in **Fig. 3**. Three amino acid residues are different from the known porcine IFN α 8 and the different residues are highlighted by yellow. The newly obtained Korean porcine IFN α 8 was registered in databank as accession number (KX275310). The region of mature protein without signal sequence (Met/1-Gly/23) was subcloned into pProEx/HTa vector for *E. coli* expression. Actually, 2 amino acid residues of Korean porcine mature IFN α 8 are different from that of the known IFN α 8 in databank.

Expression of recombinant porcine IFN α 8 protein

In order to compare the expression efficiency of recombinant IFN α 8 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 IFN α 8 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 IFN α 8 appeared to be approximately 25 kDa band (see the arrow in **Fig. 4B**). The purified recombinant IFN α 8 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 IFN α 8 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)

	10	20	30	40	50	60
IFN α 8	MAPTSAF	TALVLLSCNAI	CSLGCCLPQTHSLA	HTRALRLLAQMRR	SPFSCDLHRRDFG	
ref	MAPTSAF	TALVLLSCNAI	CSLGCCLPQTHSLA	HTRALRLLAQMRR	SPFSCDLHRRDFG	
	70	80	90	100	110	120
IFN α 8	FPQEALGGNQVQKAQAMALVHEMLQQTQFLFSTEGSAAAWDESLLHQFCTGLDQQLRDLE					
ref	FPQEALGGNQVQKAQAMALVHEMLQQTQFLFSTEGSAAAWDESLLHQFCTGLDQQLRDLE					
	130	140	150	160	170	180
IFN α 8	ACVMQE	GLEGTPLLEEDS	ILAVRKYFHLRLTYLQEKSYSPCAME	IAEVMRAFSSSTN		
Ref	ACVMQE	GLEGTPLLEEDS	ILAVRKYFHLRLTYLQEKSYSPCAME	IAEVMRAFSSSTN		
	130	140	150	160	170	180
IFN α 8	LQDRLRAKE					
Ref	LQDRLRAKE					

Figure 3. Comparison of porcine IFN α 8 to the known porcine IFN α . The ORF of porcine IFN α 8 mRNA from Korean domestic pig was analyzed and newly identified as porcine IFN α 8 (accession No. KX275310). The amino acid sequence was deduced by newly obtained the DNA sequence of porcine IFN α 8 and the different amino acid residue of Korean porcine IFN α 8 compared to porcine IFN α 8 (accession No. ACV42397). Three distinct amino acid residues are highlighted with yellow color.
ORF, open reading frame.

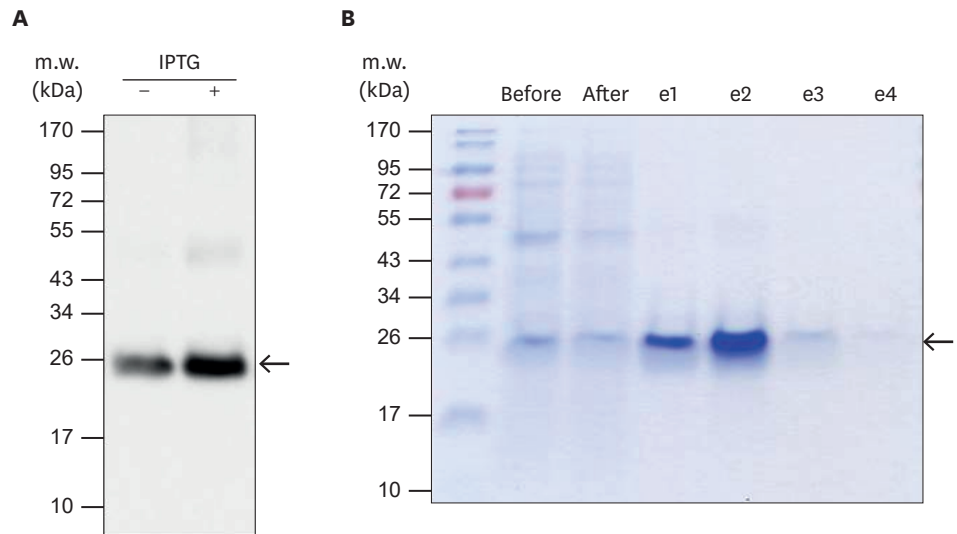


Figure 4. The expression and purification of recombinant porcine IFN α 8 with Talon affinity chromatography. The recombinant porcine IFN α 8 mRNA from Korean domestic pig was expressed in *E. coli* and purified by a mini-Talon metal affinity column. (A) The IPTG-induced recombinant porcine IFN α 8 detected with mouse anti-his⁶-tag monoclonal antibody. The arrow indicated porcine IFN α 8 induced in the presence of IPTG for 3 h. (B) The a mini-Talon metal affinity chromatography purified recombinant porcine IFN α 8 was visualized by Coomassie blue staining. The data represents one of 5 independent experiments.

visualized by silver staining to confirm the purity of the recombinant porcine IFN α 8 protein (**Fig. 5B**). The anion exchange chromatography purified recombinant porcine IFN α 8 protein appeared to be approximately 25 kDa and faint 38 kDa band in silver staining. We confirmed 38 kDa band is porcine IFN α 8 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 IFN α 8 against VSV

The antiviral assay was first performed with human WISH cells by using both porcine IFN α 8 and the control human IFN α 2. As shown in **Fig. 6A**, human IFN α 2 protected the cells from VSV infection while porcine IFN α 8 failed to protect the cells. Next, we used bovine MDBK cells for porcine IFN α 8 antiviral assay and both porcine IFN α 8 and human IFN α 2 protected MDBK cells (**Fig. 6B**). We further examined canine MDCK cells and human IFN α 2 and porcine IFN α 8 failed to protect MCBK cells from VSV infection (**Fig. 6C**).

The antiviral activity of porcine IFN α 8 via Mx-1 and OAS-1

To investigate the antiviral mechanism of porcine IFN α 8, 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 IFN α 8 at 3 and 6 h (**Fig. 7A**) however the induction of antiviral genes in human WISH cells increased with only human IFN α 2 (**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 IFN α 8 from domestic pig (*Sus scrofa domestica*), main strain of pig in Korea. The recombinant porcine IFN α 8 protein was expressed in *E. coli* and examined for its antiviral activity with different species of cell line. Interestingly, the amino

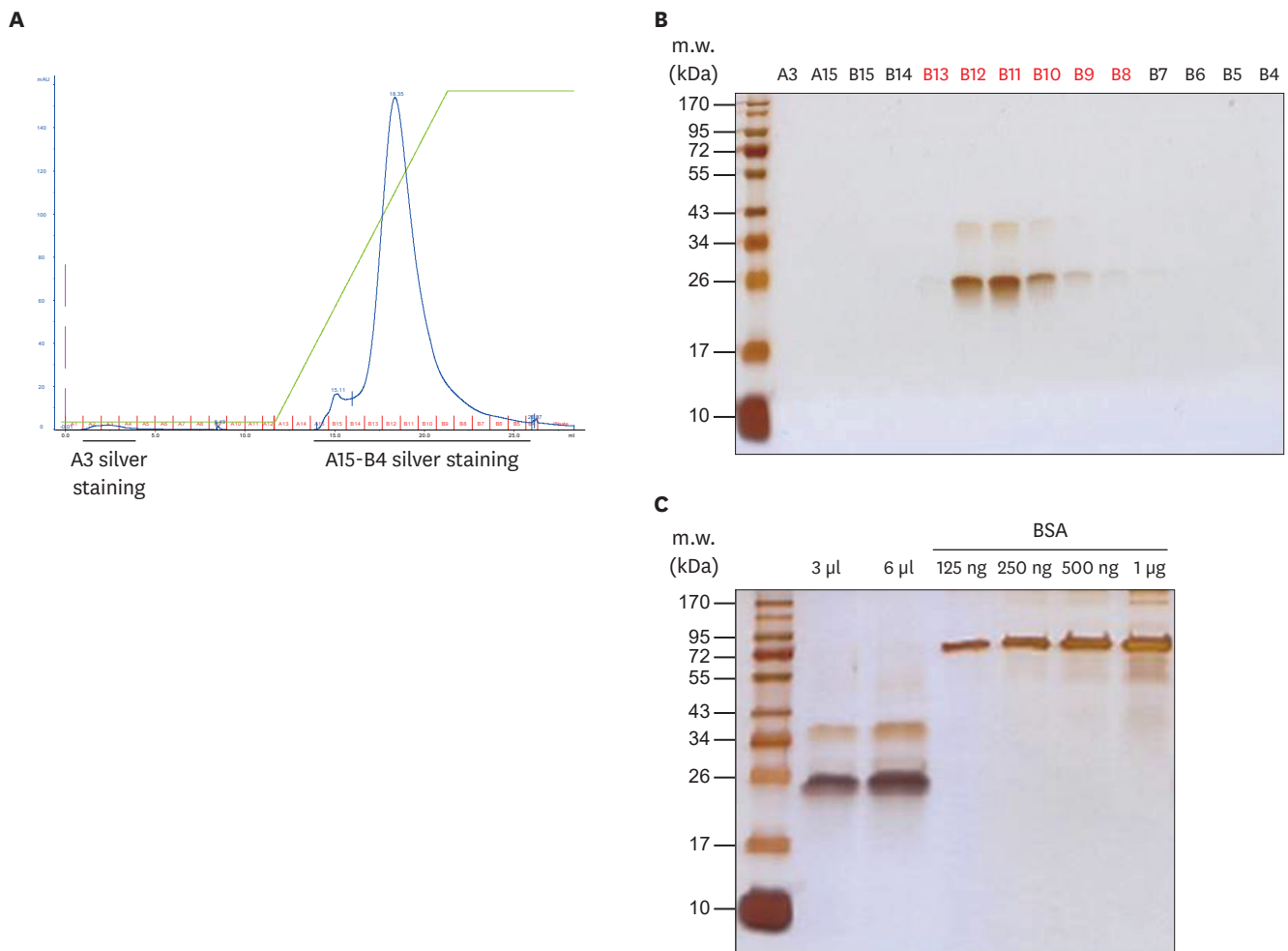


Figure 5. The purification of porcine IFN α 8 by anion exchange chromatography. (A) UV absorbance at 280 nm was recorded during the porcine IFN α 8 the second step purification process with anion chromatography. (B) The purified recombinant porcine IFN α 8 fractions were visualized by silver staining. (C) The bound fractions of recombinant porcine IFN α 8 were pooled for quantification to compare with BSA in silver staining. The data represents one of 5 independent experiments. BSA, bovine serum albumin; UV, ultra-violet.

acid sequence of porcine IFN α 8 is different from that of the known porcine IFN α 8 in databank (accession No. ACV42397). The amino acid sequence of porcine IFN α 8 shares 98.4% identity with the known porcine IFN α 8. We deposited newly cloned porcine IFN α 8 from Korean domestic pig (*Sus scrofa domestica*) in gene bank and obtained its accession number (KX275310).

Porcine recombinant IFN α 8 was examined for its biological activity using a common antiviral assay. The antiviral activity of porcine IFN α 8 was weaker in its cytopathic effect compared to human IFN α 2 in bovine MDBK cells. The antiviral activity of porcine IFN α 8 was obtained only with MDBK cells however human IFN α 2 exhibited antiviral activity in both human WISH and bovine MDBK cells (**Fig. 6**). In addition, both porcine IFN α 8 and human IFN α 2 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

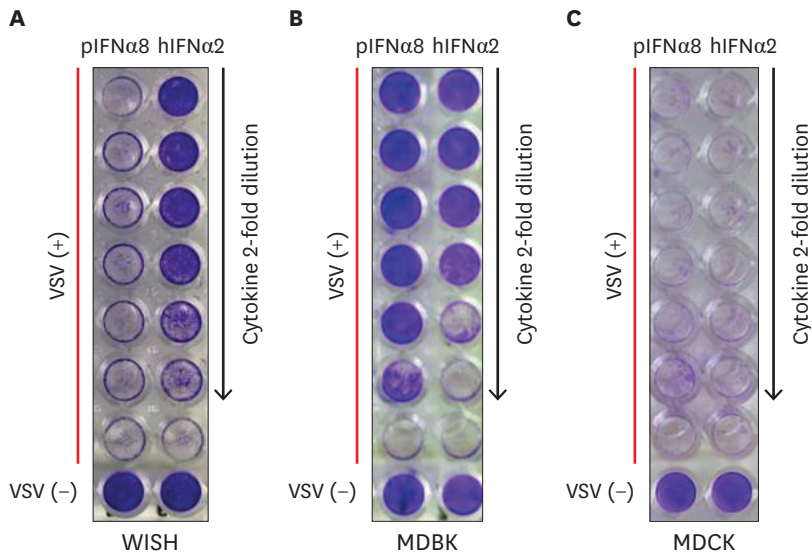


Figure 6. Recombinant porcine IFN α 8 reduces the cytopathic effect caused by VSV. (A) Human epithelial WISH, (B) bovine MDBK, (C) canine MDCK cells were treated with recombinant porcine IFN α 8 including positive control human IFN α 2 then infected with VSV to analyze the reduction of cytopathic effect. The plate was visualized with crystal violet staining. The concentration of porcine IFN α 8 (20 ng/ml) and human IFN α 2 (2 ng/ml) were added by the 2-fold serial dilution. For control, the wells at bottom row were non-infected cells; the rows above non-infected wells were only VSV infected cells which shows the complete cytopathic effect to VSV challenge. The data represents one of 5 independent experiments. hIFN, human interferon; pIFN, porcine interferon.

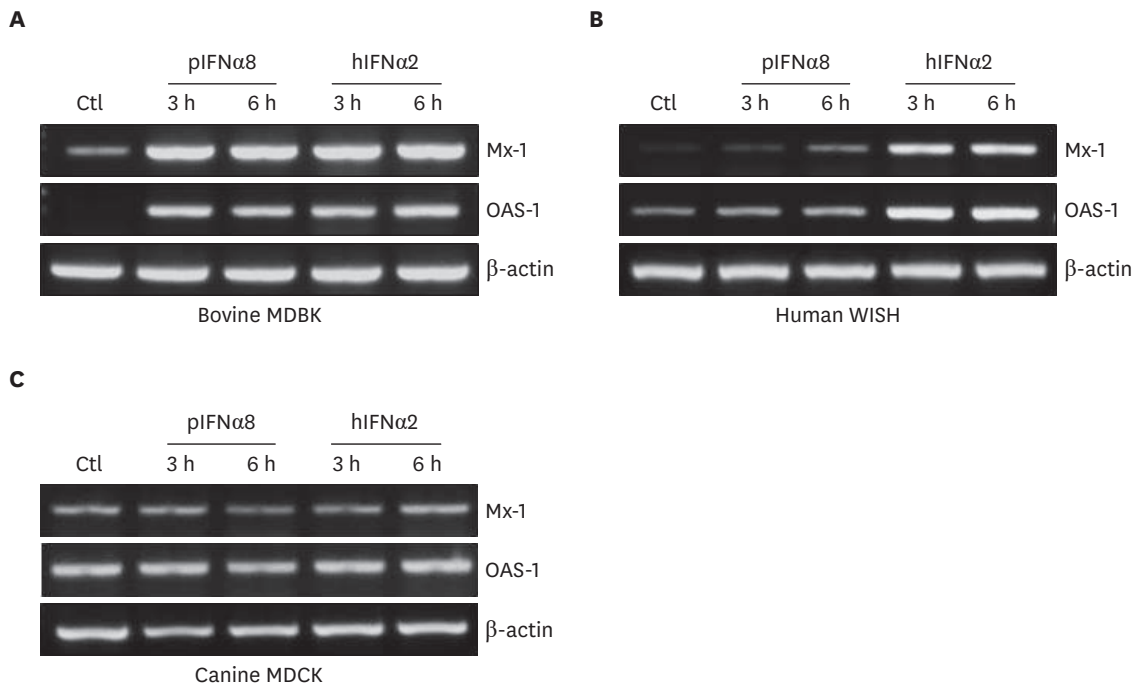


Figure 7. Recombinant porcine IFN α 8 induces antiviral genes. (A) Bovine MDBK, (B) human WISH, and (C) canine MDCK cells were treated with human porcine IFN α 8 (20 ng/ml) and human IFN α 2 (2 ng/ml) at indicated time points (3 and 6 h). Non-treated cells were prepared as negative control. After treatment of IFNs, total RNA was extracted for RT-PCR of Mx-1, OAS-1, and β -actin for normalization. The data represents one of 3 independent experiments. Ctl, control; hIFN, human interferon; pIFN, porcine interferon.

activity provided Uzé et al. (19), to isolate IFN α R1 more than 20 years ago. However, there was no difference of activity of porcine IFN α 8 and human IFN α 2 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 IFN α , IFN β , IFN ω , and IFN τ bind to common type 1 receptors, IFN α R1 and IFN α R2, 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 IFN α 8 in MDBK cells could be due to the difference of amino acid sequence between porcine IFN α 8 and human IFN α 2. However, we cannot exclude that there are additional unknown receptor components contributing for the species-specific antiviral activity of porcine IFN α 8. The biological activity of porcine IFN α 8 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 endoribonuclease, becomes active by binding 2-5 A oligonucleotides and digests the unusual RNAs. We found that porcine IFN α 8 and human IFN α 2 increased the synthesis of the cellular antiviral factors, such as OAS and Mx-1 proteins (**Fig. 7**). These results suggest that porcine IFN α 8 induces the antiviral activity through similar pathways like other type 1 IFNs.

This study describes the cloning and characterization of porcine IFN α 8 from domestic pig in Korea. Unexpectedly, the amino acid sequence of the porcine IFN α 8 differs by about 2% from that of the known porcine IFN α 8 in databank. We expect that the recombinant porcine IFN α 8 would prevent different virus infection with lower cytotoxicity than other existing therapies. A large quantity of biologically active recombinant IFN α 8 protein can be also used to develop protein drug for preventing early viral infection in industrial baby pig.

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