Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Beta interferons from the extant camélids: Unique among eutherian mammals

Avinash Premraj, Abi George Aleyas, Binita Nautiyal, Thaha Jamal Rasool *

Camel Biotechnology Center, Presidential Camels & Camel Racing Affairs Centre, Department of the President’s Affairs, PO Box 17292, Al Ain, United Arab Emirates

**Article Info**

**Keywords:** Antiviral, Zoonosis, Interferon, Dromedary camel, Poxvirus, Coronavirus

**Abstract**

The COVID-19 pandemic is a wake-up call on the zoonotic viral spillover events and the need to be prepared for future outbreaks. Zoonotic RNA viruses like the Middle East respiratory syndrome coronavirus (MERS-CoV) are potential pathogens that could trigger the next pandemic. Dromedary camels are the only known animal source of MERS-CoV zoonotic infections, but little is known about the molecular antiviral response in this species. IFN-β and other type-I interferons provide the first line of defense against invading pathogens in the host immune response. We identified the IFNB gene of the dromedary camel and all extant members of the family Camelidae. Camelid IFN-β is unique with an even number of cysteines in the mature protein compared to other eutherian mammals with an odd number of cysteines. The viral mimetic poly(I:C) strongly induced IFN-β expression in camel kidney cells. Induction of IFN-β expression upon infection with camelpox virus was late and subdued when compared to poly(I:C) treatment. Prokaryotically expressed recombinant dromedary IFN-β induced expression of IFN-responsive genes in camel kidney cells. Further, recombinant IFN-β conferred antiviral resistance to camel kidney cells against the cytopathic effects of the camelpox virus, an endemic zoonotic pathogen. IFN-β from this unique group of mammals will offer insights into antiviral immune mechanisms and aid in the development of specific antivirals against pathogens that have the potential to be the next zoonotic pandemic.

1. Introduction

The emergence and global impact of three highly pathogenic coronaviruses of zoonotic origin within the last two decades have necessitated immediate attention on anthropogenic drivers of such pathogen evolution and preventing potential future zoonotic outbreaks. Many zoonotic viruses are among the leading pathogen candidates that could emerge as the next pandemic after SARS-CoV-2. Among these, the Middle East respiratory syndrome coronavirus (MERS-CoV), a coronavirus similar to SARS-CoV is one of great concern. The unique geographic position of the Middle East makes the region a vital travel hub for millions of people and migratory birds. Besides, the sociopolitical conditions like huge congregation of people, large-scale human displacement due to conflict and political unrest create a human-livestock-wildlife interaction that can lead to potential pathogen spill-over and rapid global dissemination (Kheirallah et al., 2021). The One Health approach emphasizes cross-species holistic health interventions targeted control measures and research for controlling emerging infectious diseases. Reduction of virus transmission from animals to humans through vaccination of camels is proposed as one of the control measures for MERS-CoV. For this, an in-depth understanding of local immune mechanisms and antiviral immunity in camels is essential. Although there has been a considerable amount of research on camel immunoglobulins, research on the cellular component of the camélid immune system is still in infancy (Hussen and Schuberth, 2021).

In the vertebrate innate immune system, the interferons constitute the first line of defense against viral pathogens. These proteins are secreted early in response to the detection of viral infection by various cellular sensors (pattern recognition receptors) and limit the spread of viral infection by inhibiting viral replication and by modulating the virus-specific host immune response. Interferons are classified into types I, II, and III based on their structural and functional properties. Among these, type I is the largest group, which includes major antiviral interferons like IFN-α, IFN-β, and others like IFN-δ, IFN-ɛ IFN-κ. (Li et al., 2018). In camélids, type I IFN genes are intronless and arranged in tandem on a ~400 kb region in the genome. In the type I IFN locus, many IFN genes extensively duplicated during evolution to give rise to different subtypes (IFN-α, IFN-δ etc), but a few genes like IFN-β...
remained unique with only a single copy (Krause and Pestka, 2015). Like other type I IFNs, IFN-β also uses the same heterodimeric receptor comprising of IFNAR1 and IFNAR2 subunits. The binding of IFN-β to the receptor triggers the activation of Jak1, which in turn phosphorylates STAT1 and STAT2. Phosphorylated STAT1 and STAT2 join with IRF9 to form a trimer termed interferon-stimulated growth factor-3 (ISGF3). The ISGF3 trimer, a transcription factor, then translocates to the nucleus where it binds to the interferon-stimulated response elements (ISRE) to drive the transcription of many interferon-stimulated genes (ISGs) (Honda et al., 2005). In the arms race with the host antiviral immune response, zoonotic viruses have evolved multiple countermeasures to evade the host type I IFN response. MERS-CoV suppresses the early drive the transcription of many interferon-stimulated genes (ISGs) where it binds to the interferon-stimulated response elements (ISRE) to form a trimer termed interferon-stimulated growth factor-3 (ISGF3). The receptor triggers the activation of JAK1, which in turn phosphorylates ISGF3 trimer, a transcription factor, then translocates to the nucleus genome encodes a protein CMS-252, which is similar to the Vaccinia virus B19R protein with potent IFN-αβ antagonistic activity. Other type I IFNs, IFN-αβ remained unique with only a single copy (Krause and Pestka, 2015). Like IFN-αβ, MERS-CoV has a single copy of the IFNB gene. The genomic DNA of the camelids were isolated as described previously (Premraj et al., 2015). Gene-specific primers for dromedary IFN-β RACE were designed based on the IFN-β ORF sequence identified earlier (Table 1). For R 5’ RACE, we conducted two rounds of PCR with gene-specific (CdIFNb-5RCFR/CdIFNb-5RCNR) and SMARTer RACE adaptor specific (UPM/UPM short) primers. The 3’ RACE PCR was carried out with a single round of PCR with CdIFNb-3RCFF and UPM primers.

2. Materials & methods

2.1. Identification of the dromedary camel IFN-β CDS and complete cDNA

We identified potential IFNB gene coding region of the dromedary camel genome using homology BLAST. We designed PCR primers CdIFNb- NF & CdIFNb-NR (Table 1) at the flanking sequences of the putative protein-coding region of the IFNB. PCR amplification was performed using Phusion Green Hot Start II High-Fidelity DNA polymerase (Thermo Fisher Baltics, Vilnius, Lithuania) using genomic DNA as template from two camels. Independent amplicons were cloned into pGEM-T Easy vector (Promega, Madison, WI, USA) and sequenced as described previously (Premraj et al., 2020).

2.1.1. Identification of the 5’ and 3’ untranslated regions (UTRs) of the IFN-β cDNA using RACE

CAMEL kidney cell lines of fibroblastic lineage (Aleyas et al., 2015a) were cultured in vitro and transfected with 5 μg/mL poly(I:C) using FuGENE HD transfection reagent (Promega). After 24h, total RNA was isolated and 5’ and 3’ RACE cDNA were synthesized with SMARTer RACE Kit, (Takara Bio, Shiga, Japan) as described previously (Premraj et al., 2015). Gene-specific primers for dromedary IFN-β RACE were designed based on the IFN-β ORF sequence identified earlier (Table 1). For 5’ RACE, we conducted two rounds of PCRs with gene-specific (CdIFNb-5RCFR/CdIFNb-5RCNR) and SMARTer RACE adaptor specific (UPM/UPM short) primers. The 3’ RACE PCR was carried out with a single round of PCR with CdIFNb-3RCFF and UPM primers.

2.2. Identification of the promoter of dromedary IFNB gene by gene walking

We made a dromedary camel genomic DNA - genome walker library (Premraj et al., 2020) and used this library as the template to amplify the 5’ end of the IFNB gene, containing the promoter region, using CdIFNb-5RCNR and library-specific primer. Amplicons were cloned into the pGEM-T Easy vector and sequenced.

2.3. Cloning of the IFNB gene from the Bactrian camel and all four South American camels (Alpaca, Vicuna, Llama and Guanaco)

The genomic DNA of the camels were isolated as described previously (Premraj et al., 2013). As the IFNB gene is intronless, we used the genomic DNA from these five camels and primers CdIFNb–NF & CdIFNb-NR to amplify the coding sequence with Phusion High-Fidelity DNA Polymerase. Independent amplicons were cloned and sequenced. All clones were sequenced and contigs assembled with Sequencher 4.9 program (Gene Codes, Ann Arbor, MI, USA). We used the BioEdit 7.0 program to identify the ORF and predicted protein sequences of the cloned IFN-β sequences. CLUSTAL-W and MEGA-X programs were used for multiple sequence alignments and phylogenetic analysis, respectively.

The I-TASSER homology modeling server (Yang and Zhang, 2015) was used to predict the 3D-structure of mature dromedary camel IFN-β protein. We chose the most appropriate homology model based on the Template Modeling Score (TM-score) and the C-score. PyMol 2.5.2 was used to visualize the homology models.

| Primer Name | Sequence - 5’ to 3’ | Annealing Temperature | Product size | Remarks |
|-------------|---------------------|----------------------|--------------|---------|
| CdIFNb-NF   | GTGTTGAAACAGATCATTGTC | 58 °C | 646 bp | Cloning of IFN-beta gene/cDNA |
| CdIFNb-NR   | AGAACCTCACTCATTTGAGCA | 58 °C | 307 bp | 5’ RACE (5’RACE) (Takara Bio) |
| CdIFNb-5RCF | GTCCATCCTGTCCTTGAGGCAATACTG | 65 °C | 307 bp | 5’ RACE (5’RACE) (Takara Bio) |
| CdIFNb-5RCFR| CAAAGTGCGCTGCTCTTGGTACCC | 65 °C | 307 bp | 5’ RACE (5’RACE) (Takara Bio) |
| CdIFNb-5RCNR| TGGATGTTGCGATGAGACATGGTGA | 65 °C | 307 bp | 5’ RACE (5’RACE) (Takara Bio) |
| CdIFNb-3RCFF| CCTGGGCGAATTATGAAAGAG | 60 °C | 505 bp | 3’ RACE with SMARTer RACE kit (Takara Bio) |
| CdIFNb-3RCF | CAGCCCGCTGTATTTGACC | 60 °C | 117 bp | Real-time qPCR |

Table 1: List of oligonucleotide primers used for IFN-β cloning or qPCR.
2.4. Analysis of IFN-β mRNA expression in camel kidney cells upon poly (I:C) treatment and camelpox virus infection

Camel kidney cell lines were cultured in vitro in 6-well plates in advanced DMEM (Thermo) with 10% fetal calf serum as described earlier (Premraj et al., 2020). The cells were transfected with 5 μg/mL of poly(I:C) (Merck-Sigma) using FuGENE HD transfection reagent and incubated for 2, 4, 6, 12, or 24 h. Appropriate controls (Untransfected cells, FuGENE HD transfection reagent alone and direct addition of poly (I:C) into the medium without transfection reagent) were also included. Total RNA was isolated from the poly(I:C) transfected/control cells using easy-spin Total RNA extraction kit (iNtRON Biotechnology Gyeonggi-do, South Korea). To remove contaminating genomic DNA from purified total RNA samples before cDNA synthesis, we used the engineered double-strand-specific DNase (dsDNase) provided with the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Baltics, Vilnius, Lithuania). As per the manufacturer’s instructions, 2 μg of total RNA was incubated with 1X dsDNase buffer and 1 μL of dsDNase enzyme in a 10 μL reaction volume for 2 min at 37°C. The entire RNA after DNase treatment (10 μL) was reverse transcribed to cDNA using Maxima first-strand cDNA synthesis kit in a 20 μL volume (Thermo Fisher Baltics, Lithuania). Quantitative real-time PCR was used to determine the mRNA expression levels of IFN-β using GoTaq qPCR Master Mix (Promega) in a QuantStudio 5 real-time PCR system (Applied Biosystems-Thermo Fisher). Relative quantification of IFN-β mRNA was performed using GAPDH mRNA as the housekeeping gene by the 2^(-ΔΔCt) method.

2.4.1. Camelpox virus propagation, infection

The viral assays were performed using the same Camelpox virus (CMLV) isolate used in our previous reports on Camel IFN-α (Premraj et al., 2020) and Camell II-26 (Premraj et al., 2015). The virus was initially isolated from camels infected with camelpox following an endemic outbreak in Al Ain, Abu Dhabi, UAE in 2012. PCR and sequencing of five different CMLV genes (ATIP, L1R, B5R, A27, and A33R) were done to verify the isolate as camelpox virus (Aleyas et al., 2015b). Routine viral passages were performed using cultured camel kidney cells (Aleyas et al., 2015a) grown in DMEM with 10% fetal calf serum (Sigma) until 70% confluence and infected with CMLV at 0.1 MOI. The cells were incubated at 37°C at 5% CO2 until extensive cytopathic effects were evident (typically around 24–48 h). The flask containing the cells and medium overlaid was then freeze-thawed three times to release the virus. To separate the virus from the cell debris, the thawed lysate was centrifuged at 1000g. The supernatant containing viruses was stored at ~80°C as aliquots. According to Reed and Muench’s method, viral titer was determined by TCID50 (50% tissue culture infective dose) assay. We infected camel kidney cells grown in T-25 flasks with CMLV (MOI 0.1) and incubated the cells for 2–24 h to study the expression of IFN-β upon CMLV infection. RNA was isolated from the cells 2, 4, 6, 12, or 24 h post-infection using easy-spin Total RNA extraction kit. We quantified the relative expression of IFN-β mRNA by qRT-PCR as described previously.

2.5. Expression of recombinant cameldasydromedary IFN-β in E.coli and purification

The dromedary IFN-β mature protein-coding region was cloned into pET28a vector to create a N-terminal His6-tagged fusion protein expression construct. Rosetta 2(DE3) E.coli strains (Novagen, Madison, WI, USA) harboring the CdIFNβ-pET28 construct were grown in LB Kanamycin broth at 37°C to an optical density of ~0.5 (600 nm). Isopropyl-β-D-thiogalactoside (IPTG, Promega) was added to the log-phase culture at 0.2 mM final concentration and recombinant protein expression induction performed at 12°C for 18 h. The cells were harvested by centrifugation and resuspended in a lysis buffer (20 mM Tris HCl, 0.5M NaCl, 20 mM imidazole, pH 7.5) containing 1X BugBuster protein extraction reagent (Novagen), 100 μg/mL lysozyme and 1X Halt Protease inhibitor cocktail (Thermo-Pierce). The soluble and insoluble fractions were separated and recombinant protein purification from insoluble inclusion bodies was carried out as described earlier (Premraj et al., 2020). For affinity purification of the soluble fraction, the sample was applied on a HisTrap FF Crude 5 ml Ni-Sepharose column connected to AKTA Avant 25 FPLC system (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and protein bound to the column was initially washed with a wash buffer (40 mM imidazole, 20 mM Tris HCl, 0.5M NaCl, pH 7.5). After washing, His-tagged protein bound to the column were eluted with a linear gradient of 100–500 mM imidazole containing elution buffer (20 mM Tris HCl, 0.5M NaCl, pH 7.5). Elution fractions were analyzed by SDS-PAGE and fractions containing the recombinant protein of interest were pooled. We used Amicon Ultra-15 centrifugal filter (Millipore) for desalting and concentration of the pooled fraction. Endotoxin removal was carried out using Endotoxin Removal beads (Milenyi Biotech, Gladbach, Germany). Recombinant proteins were further sterilized using 0.22 μm Millex GF filter (Merck Millipore, Cork, Ireland) and stored with 50% glycerol.

2.6. Treatment of camel kidney cells with recombinant dromedary IFN-β and analysis of expression of ISGs

To determine the biological activity of the recombinant dromedary IFN-β, camel kidney cells were cultured as described previously and treated with 100 ng/mL of recombinant camel IFN-β, IFN-α1 (Premraj et al., 2020), a His6-tagged control protein, or phosphate-buffered saline (PBS) for 4 h. We chose the concentration and duration of treatment based on previous reports using recombinant IFN-β in kidney cells (Liang et al., 2015) and our previous report of camel IFN-α (Premraj et al., 2020). As a control, we used a ~21 kDa His-tagged protein with a thioredoxin (Trx) fusion partner, which is commonly used as an inert fusion tag in protein production. It was expressed in E. coli and purified in the same manner as the recombinant camel IFN-β. The same His-tagged protein was used as the control protein in our previous report of camel IFN-α (Premraj et al., 2020), since it did not show significant induction of interferon-stimulated genes (ISGs) or antiviral activity against CMLV. After treatment, the total RNA was isolated and mRNA expression of ISGs (ISG15, Viperin, Mxi1, OAS1, RIG-1, and IRF1) was determined by RT-qPCR as described in section 2.4. The fold induction of these ISGs was quantified with that of the corresponding untreated control using GAPDH mRNA as the housekeeping gene.

2.7. Antiviral activity assay of recombinant dromedary IFN-β against CMLV in camel kidney cells

Camel kidney cells cultured in 6-well plates were pretreated with 100 ng/mL recombinant camel IFN-β, IFN-α1, a His6-tagged control protein or culture medium (Untreated control) for 1 h and then infected with CMLV (MOI 0.1). After 1 h of infection, the overlaying medium with the viral inoculum was removed, fresh growth medium was added and the cells were grown for further 24 h. Cells were observed using a Nikon Eclipse Ti-S inverted microscope and images captured with the NIS-Elements D 3.10 Software (Nikon Instruments, Tokyo, Japan). CMLV DNA was quantified by the TaqMan qPCR assay, which recognized the CMLF48L gene of CMLV (Ourafour et al., 2011a). The CMLV standard was made using plasmid DNA (pGEM-T Easy, Promega) containing a portion of the CMLV CMLF48L gene. In quantitative RT-PCR with TaqMan probe, tenfold serial dilutions of the CMLV standard were used at 10000, 1000, 100, 10 and 1 fg/μL concentrations to estimate the viral DNA quantity in the samples as described previously (Premraj et al., 2020).

2.8. Statistical analysis

GraphPad Prism software was used to analyze the experimental values from three replicates. To determine the significant differences
between the control and treated samples, we used one-way ANOVA with Tukey’s or Games-Howell multiple comparisons test using GraphPad Prism.

3. Results

3.1. Molecular cloning of the Camelus dromedarius IFNB gene

We designed PCR primers flanking the potential IFNB gene coding region based on the Camelus dromedarius whole-genome sequencing contigs available at the NCBI Genbank. We amplified a 646 bp fragment using CdIFNbNF and CdIFNbNR primers from dromedary camel genomic DNA, cloned and sequenced. The cloned fragment contained a 561 bp Open Reading Frame (ORF) encoding a 186 amino acid protein. When compared with the IFNB from well-studied model mammalian counterparts, at the nucleotide level, the dromedary camel IFN-β shared 83.2%, 77.4%, and 62% identity with porcine, human & murine orthologs. At the protein level, the corresponding amino acid identity values were 89.2%, 81.3% & 60.7%.

SignalP-5.0 Server analysis revealed that the first 21 amino acids formed the eukaryotic signal peptide. The mature protein is hence predicted to consist of 165 amino acids with an estimated molecular weight of 19.6 kDa and a predicted pl of 5.75. Interestingly in the mature protein, four cysteine residues are present, whereas most mammalian IFN-β has only three.

The 5′ and 3′ UTRs of camel IFN-β cDNA were amplified by RACE-PCR using RNA from poly(I:C) treated camel kidney cells. 5′RACE for IFN-β cDNA produced a 307 bp fragment that contained 88 bp 5′UTR and the first 174 bp of the ORF. The 505 bp 3′ RACE fragment contained the last 227 bp of the ORF and the 231 bp 3′UTR with poly(A) tail (NCBI GenBank Accession No ON256405). In the 3′ UTR of the cDNA, three (ATTTA) instability motifs and a polyadenylation signal sequence (AATAAA) upstream of the poly(A) tail were identified. Also, the key nonamer AU-Rich sequence motif UUAUUUUUUU that mediates mRNA degradation is present in the 3′ UTR (Fig. 1).

3.2. Identification of the promoter of the dromedary IFNB gene

Using gene walking, we amplified and cloned a 1274 bp fragment from a Dral-digested genomic DNA library. The fragment contained 108 bp of the IFNB CDS and 1166 bp 5′ regulatory region upstream of the start codon (Fig. 2). In this upstream regulatory region, a TATA box was identified ~110 bp upstream of the start codon. Upstream of the TATA box (~120 to ~190), the Virus-Responsive Elements (VRE) containing all four positive regulatory domains (PRDs) I, II, III, and IV were identified in the camel IFNB promoter region also. The dromedary camel IFNB gene sequence with its promoter is deposited in the NCBI GenBank (Accession number ON256404).

3.3. IFNB gene sequences from all other living members of Camelidae

As the IFNB gene is intronless, we were able to amplify the gene from genomic DNA from the Bactrian camel, and all four new world camelids (alpaca, vicuña, guanaco and llama). Like the dromedary camel IFNB, all

Fig. 1. Nucleotide sequence of the cloned dromedary camel IFN-β cDNA sequence: We amplified a 646 bp fragment with CdIFNbNF and NR primers. This fragment contained a 561 bp ORF (1–561) encoding a 186 amino acid protein. The start and the stop codons of the ORF are highlighted and the translation of the amino acid is provided below the nucleotide sequence. Later the 5′ and 3′ UTR regions were identified by Rapid amplification of cDNA ends (RACE). The complete IFN-β cDNA sequence (NCBI GenBank Accession No ON256405) consists of 88 bp 5′UTR, 561 bp ORF, and 231 bp 3′UTR with poly-A tail. Analysis of the predicted protein indicated that the first 21 amino acids (underlined) formed the eukaryotic signal peptide. The four conserved cysteine residues in the mature protein are also marked in yellow highlight. The predicted polyadenylation signal sequence (AATAAA) sequence located 187 bp downstream of the stop codon is underlined. The nonamer AU-rich sequence motif TTATTTATT located 156 bp after stop codon that mediates ARE-dependent mRNA decay is also marked in underline.
other camelids also featured a 561 bp ORF encoding 186 amino acid protein (NCBI GenBank Accession numbers ON256399 to ON256403). The dromedary and Bactrian camel IFNB protein-coding sequences were identical. At the protein level, the IFN-β sequence of new world camels had five amino acid changes when compared with the old-world camels – T18A, K54E, E108Q, R115Q, and R143K. Four of these changes were in the mature protein and one in the signal peptide (Fig. 3). The predicted IFN-β amino acid sequences of vicuña, llama, and guanaco were identical, whereas the alpaca sequence had one amino acid change compared with the rest of the new world camels. For alpaca, the amino acid residue at 115 is R as in old world camels, but Q is observed in the other three new world camelids.

It is interesting to note that the IFN-β sequence from all six living representatives of Camelidae has four cysteines in the mature protein due to an additional unique cysteine amino acid position 184, which is not present in any eutherian mammals (Fig. 3). The predicted 3-D homology model of the IFN-β with the I-TASSER server was predominantly alpha-helical with five major helices (Supplementary Fig. S1).

Phylogenetic analysis of the protein sequences reveals that the camelid IFN-β cluster as a unique group from other members of the Superorder Cetartiodactyla. The tylopoda (Camels), ruminants, suiformes (pig), cetaceans form distinct clades within this superorder (Fig. 4).

3.4. Poly(I:C) treatment upregulates IFN-β mRNA expression in camel kidney cells

We analyzed the induction of IFN-β mRNA expression upon poly(I:C) treatment by RT-qPCR. The IFN-β mRNA expression was around ~15000-fold in cells transfected with poly(I:C). However, direct addition of poly(I:C) into the medium at the same concentration without any transfection reagent for 24h did not induce any significant IFN-β mRNA expression (Fig. 5A). Within 2h of transfection of poly(I:C) IFN-β mRNA expression was increased ~1500 fold, by 6h it increased to ~27000 fold and at 24h it was 94,000 fold (Fig. 5B).

The induction of IFN-β mRNA in camel kidney cells upon CMLV infection was subdued and delayed when compared to transfection with the viral mimetic poly (I:C). Only a two-fold increase in the expression of IFN-β mRNA was observed 6h post-infection. The IFN-β expression increased 10-fold and ~300-fold by 12h and 24h post-infection, respectively (Fig. 5C).

3.5. Expression of recombinant dromedary IFN-β protein in E.coli and purification by affinity chromatography

The mature protein-coding sequence of the dromedary IFN-β was cloned into pET28a vector to express the protein as an N-terminal His6-tagged protein. The IFN-β mature protein coding sequence contained sixteen E.coli rare codons that could limit the recombinant expression in E.coli. Hence, we tried recombinant expression in both BL21 (DE3) as
well as Rosetta 2(DE3) E.coli that contained a plasmid to supplement the limiting rare codons. Better expression of the recombinant protein was noted in the Rosetta 2(DE3) strain of E.coli with rare codon supplementation (Supplementary Fig. 2A). The recombinant proteins were characterized by western blotting with an anti-His-tagged antibody. The anti-His-tag antibody detected a protein of ~24 kDa (Supplementary Fig. 2B).

Although the maximum amount of recombinant protein was produced in inclusion bodies, the recombinant protein after purification and in vitro refolding was found to be functionally inactive. Hence, we resorted to inducing the recombinant protein in Rosetta 2(DE3) E.coli at a lower temperature (12°C for 18h). Although the amount of protein expressed in the soluble fraction was lower even in low-temperature induction, we could obtain some purified protein by affinity chromatography (Supplementary Fig. 2C), which was concentrated using Amicon centrifugal filter and confirmed by western blot before use in in vitro assays (Supplementary Fig. 2D).

3.6. Recombinant dromedary IFN-β induces transcriptional activation of interferon-stimulated genes in camel kidney cells

To investigate the IFN-β-dependent induction of interferon-stimulated genes (ISGs), we treated camel kidney cells with recombinant dromedary IFN-β, dromedary IFN-α1 (Premraj et al., 2020), a His6-tagged control protein, or phosphate-buffered saline (PBS) for 4h. The mRNA expression of six candidate ISGs – Mx1, ISG15, Viperin (VIP), RIG-1, OAS1, and IRF1 was quantified by RT-qPCR. Among these, the most highly upregulated mRNA upon IFN-β treatment were Viperin (~130 fold), RIG-1 (~105 fold), and Mx1 (~100 fold). ISG15, OAS1, & IRF1 mRNA expression also increased ~40 fold, ~20 fold, and ~8 fold respectively (Fig. 6). The recombinant dromedary IFN-α1 protein also induced the expression of these ISGs, whereas the His6-tagged control protein and PBS-treated cells did not have significant changes in the mRNA expression of these ISGs.
3.7. Recombinant dromedary IFN-β shows antiviral activity against camelpox virus (CMLV) in vitro

We assessed the antiviral activity of dromedary IFN-β in vitro using CMLV and camel kidney cell lines. Cells pretreated with IFN-β showed resistance to CMLV infection. The IFN-β and IFN-α1 treated cells showed little cytopathic effects compared with the control protein treated or untreated control cells infected with CMLV (Fig. 7A).

To quantify the antiviral activity, we used real-time qPCR to estimate the reduction of CMLV DNA upon IFN-β treatment. Cells pretreated with IFN-β had less amount of CMLV DNA compared to untreated cells or treated with His₆-tagged control protein (Fig. 7B).

4. Discussion

The devastating COVID-19 pandemic is a wake-up call on the looming threat of potential zoonotic disease spillover and the need for global preparedness for the prevention and mitigation of such events. Among these, the Middle East respiratory syndrome coronavirus (MERS-CoV) is of great concern, and dromedary camels are so far identified as the sole source of this zoonotic infection. In 2018, even before the SARS-CoV-2 outbreak, the World Health Organization included MERS in a blueprint of severe emerging diseases that lacked sufficient medical countermeasures or effective drugs and needed urgent accelerated research attention (Mehand et al., 2018). A recent report indicating that the strains of MERS-CoV established in the Arabian peninsula have
acquired increased replication competence and pathogenic potential in the human lung is worrying (Zhou et al., 2021). As the first line of defense against invading pathogens, type I IFNs play a key role in host antiviral response. IFN-β treatment has been reported to be effective in the inhibition of replication of MERS-CoV (Hart et al., 2014). In the current study, we report the cloning of IFN-β from all living camels. To determine the functional activities of camelid IFN-β, recombinant dromedary camel IFN-β was produced in a prokaryotic expression system. The upregulation of IFN-stimulated genes upon IFN-β treatment in camel kidney cells indicates that the recombinant protein activated IFN antiviral response. IFN-β pretreatment conferred antiviral resistance to an endemic camelid zoonotic virus CMLV as evidenced by the reduction of CPE, as well as the pathogen load.

Sequence analysis of the IFN-β orthologs from all extant members of Tylopoda reveals unique beta interferon not seen among any other eutherian mammals. In placental mammals, the mature IFN-β protein sequence contains three conserved cysteine residues corresponding to Cys-38, Cys-52, and Cys-161 in the alignment. Mouse IFN-β is an exception as it has only one Cysteine - Cys-38. All six camelid IFN-β proteins have an additional cysteine at Cys-183 very close to the C-terminus, taking the total number of cysteines to four in the mature protein (Fig. 3 and Supplementary Fig. 1). Camelids seem to be the only eutherian mammals with an even number of cysteines in the IFN-β protein.

The Cys-38 is unpaired in human IFN-β, whereas Cys-52 and Cys 162 are connected by a disulfide linkage (Hosoi et al., 1988). Two disulfide bonds are predicted in Camelid IFN-β, Cys 52-162 like the human and a novel Cys-38-183, which is not present in human or any other known IFN-β. Using an in-silico method for predicting stability changes (∆∆G) of a protein with the STRUM server (Quan et al., 2016), we found that the stability of IFN-β is predicted to be increased due to the presence of the cysteine at 183 amino acid position, in place of tyrosine, which is present in most mammalian orthologs. Functional studies with engineered mouse IFN-β mutants with a total of three and two cysteines have revealed a higher activity than the wild type with one cysteine. The mutant with a pair of cysteines had the most antiviral activity, almost 15-fold of the wild type (Day et al., 1992). Also, in the production of human recombinant IFN-β, it has been shown that the free unpaired cysteine can lead to protein aggregation through intermolecular disulfide scrambling. Aggregation was reduced by mutation of the unpaired cysteine to serine without affecting the activity (Runkel et al., 1998). Crystallographic, structural and mutational studies on the role of the additional cysteines in camelid IFN-β would reveal their functional significance.

A cysteine similar to the camelid IFN-β Cys-184 is found in the marsupial Tammar wallaby (Macropus eugenii). The mature Tammar wallaby IFN-β however, has an odd number of cysteines (five) (Harrison et al., 2004). On the basis of predicted IFN-β sequences from GenBank, three other marsupials - brushtail possum, koala, and common wombat also possess the Cys-184 present in Camelidae. But, they have additional cysteine residues at positions 72 and 159 (Tammar wallaby), 72 (brushtail possum and koala) or 98 (common wombat). IFN-β Cys-52 and Cys-161 are conserved among marsupials and all eutherian species (Supplementary Figure -S3). Although the camels and some marsupial feature four cysteines in the mature IFN-β sequence, phylogenetic analysis reveals that the marsupial IFN sequences are very divergent from the rest of the mammals. In the phylogenetic analysis, it is noteworthy that only two divergent groups of mammals - all extant camels and some marsupials contain four cysteines in mature IFN-β proteins (Fig. 4). The phylogenetic relationship of dromedary IFN-β and camelid IFN-β are connected by a disulfide linkage (Hosoi et al., 1988). Two disulfide...
other mammalian orthologues is in line with our earlier report of dromedary IFN-α (Premraj et al., 2020) and other immune genes (Premraj et al., 2013, 2015). Within the living tylopoda, the new world and old world camelids form distinct subgroups in the phylogenetic analysis. (Fig. 4). Other reported type I camel IFNs also have an odd number of cysteines in the mature protein. There are five conserved cysteine residues in all dromedary camel IFN-α, whereas IFN-ε has three (Supplementary Fig. S4).

The mature IFN-β protein is predicted to be alpha-helical (65% of the total secondary structure). Like the IFN-α, the protein is composed of five helices A, B, C, D, and E, which are interconnected by loops (Fig. 3 and Supplementary Fig. 1). The putative IFNAR1-binding residues are located in helices A and C. The AB loop and helix D feature the IFNAR2-binding motifs. In human IFN-β, the IFNAR2-binding residues are located on helix A, the loop between helices AB and helix E. Helices B, C and D harbor residues that interact with the IFNAR1 binding sites (Piehler et al., 2012). Among camelids, IFNAR2 binding residues are all conserved. Amino acid changes between the old world and new world camelids are noted in three IFNAR1 binding residues (Fig. 3). Compared to human IFN-β, many amino acid changes are noted in IFNAR1 and IFNAR2 binding residues of the camelid IFN-β. Due to the lack of species-specific reagents or biologicals, human recombinant interferons and other cytokines are used in domestic animals like camels. However, amino acid substitutions at the receptor binding sites may have an effect

![Fig. 6. Expression of interferon-stimulated genes (ISG) in dromedary camel kidney cells in vitro after interferon treatment: Camel kidney cells were treated with 100 ng/mL of recombinant camel IFN-β, IFN-α1, a His-tagged control protein, or phosphate-buffered saline (PBS) for 4 h. After treatment, total RNA was isolated and mRNA expression of ISGs (ISG15, Viperin (VIP), Mx1, OAS1, RIG1, and IRF1) was determined by RT-qPCR. The fold induction of the ISGs was estimated with that of the corresponding untreated control using GAPDH mRNA as the housekeeping gene. The data is represented as the mean ± standard deviation (n = 3). Statistical significance (one-way ANOVA with Games-Howell’s multiple comparisons test) is indicated for pairwise comparisons compared to the untreated control: *P-value < 0.05, **P-value < 0.01, ***P-value < 0.0001 and ns for no significance. Capped lines connecting two data bars indicate significance between the IFN-β, and IFN-α1 treatments.](image-url)
Fig. 7. - Antiviral activity of recombinant camel IFN-β: A) Camel kidney cells were pre-treated with medium (Untreated control), His-tagged control protein, recombinant dromedary IFN-α1, or recombinant dromedary IFN-β for 1h and then infected with CMLV (MOI 0.1). The cells were grown for a further 24h and observed, and images were captured with the NIS-Elements D 3.10 software connected to Nikon Eclipse Ti-S inverted microscope. B) Reduction in CMLV DNA after infection of IFN treated cells with CMLV. The culture lysate was collected 24h post infection and viral DNA isolated. CMLV DNA was estimated by TaqMan qPCR assay using primers CLMV-qTF/CLMV-qTR and 5’-FAM-labeled CMLV QT Probe which recognized the CMP48L gene of CMLV. A standard curve made from 10-fold serial dilutions of viral DNA standard run under the same conditions in qPCR was used to estimate the viral DNA quantity. The results are shown as mean ± SD (n = 3). Statistical significance (one-way ANOVA with Games-Howell’s multiple comparisons test) annotations above the data bars indicate significance values relative to the Untreated Control. Statistical significances between other groups are indicated by lines. ***P < 0.001.
on the biological activity and specificity. This emphasizes the need for the development of specific IFN-β proteins and reagents for such divergent mammalian groups as camels.

During infection, pathogen-associated molecular patterns (PAMPs) are recognized by pattern-recognition receptors (PRRs) such as toll-like receptors (TLRs) and RIG-I-like receptors (RLRs), which induce a signaling cascade that activates the assembly of an IFN-β enhancosome comprising transcription factors ATF-2/c-Jun, NFκB, and interferon response factors. The Virus-Responsive element of the IFNB contains four key cis-regulatory elements PRD I, PRD II, PRD III, and PRD IV. In the enhancosome, interferon regulatory factors (IRF-7/IRF-3) activate the PRD III and PRD I, PRD II by nuclear factor κB (NF-κB) and PRD I by ATF-2/c-Jun to induce the transcription of IFN-β (Honda et al., 2005). ATF-2/c-Jun binding site 5′-TGACTAG-3′ is strictly conserved in the PRD-IV of the IFN-β enhancer of camel IFNB (Fig. 2). Both PRD III and PRD I feature the consensus IRF-3 binding site 5′-AANNGAAA-3′ (Panne et al., 2004).

The last element PRD II has the NF-κB binding consensus sequence 5′-GGGRNNYYYCC-3′ almost conserved in the camel promotor (Fig. 2). In comparison, the dromedary camel IFNA promoter does not contain PRD II and PRD IV, but only PRD I and PRD III-like regulatory elements (Premraj et al., 2020).

In camel kidney cells cultured in vitro, very low basal expression of IFN-β was observed, but upon poly(I:C) transfection, induction of IFN-β mRNA expression was high. In human cells cultured in vitro, poly(I:C) transfection has been reported to increase IFN-β mRNA expression by 10^3–10^6 fold, whereas direct addition of poly(I:C) did not cause a significant upregulation (Yang et al., 2013).

TLRs or RLRs of host cells detect viral RNA or dsRNA like poly(I:C) based on the route of entry. Through endocytosis, extracellular RNA enters the endosome and activatesTLR3 on the endosomal membrane, whereas cytosolic dsRNA is recognized by RLRs. Transfected poly(I:C) causes the activation of RIG-I-like receptors (RLRs) which leads to robust production of IFN-β via the IFR3 and NF-κB signaling pathways (Yang et al., 2013). In bat cell lines also, basal IFN-β expression was very low but was highly induced by transfection with poly(I:C). Upon poly(I:C) transfection, bat cells produced IFN-β early, and their expression of RLR was significantly increased, indicating dsRNA recognition in the cytosol (Sarkis et al., 2018). While cellular receptors for dsRNA are well established, extracellular sensing of dsRNA to initiate an antiviral response has long been a mystery. Now there is significant evidence that a group of cell surface receptors known as class A scavenger receptors (SR-As) serves as pathogen sensors of extracellular dsRNA. SR-As bind to viral dsRNA extracellularly and facilitate entry into cells via clathrin-mediated endocytosis, delivering to known intracellular dsRNA sensors (TLR3, RIG-I, and MDA5) that initiate type-I IFN responses (DeWitte-Orr et al., 2010). Induction of IFN production in response to extracellular poly(I:C) in different cell types or cell lines depend on the expression of these SR-As on the cell surface.

Research on the effects of extracellular dsRNA on other cell types of camels, including the role of SR-As, would be interesting given that direct poly(I:C) treatment did not prompt significant IFN-β expression in camel kidney cells.

In bats, a dampened STING-dependent interferon activation has been attributed to innate immune tolerance to host a large number of viruses. The Ser-358 residue highly conserved in most mammalian STING, a key phosphorylation site for the virus-triggered IFR3 activation is replaced in bats and results in a weakened downstream IFN-β induction (Xie et al., 2019). Camels may not have this mechanism of dampening IFN responses since Ser-358 is conserved like other mammalian STING homologs. 致力于 (Premraj et al., 2020). Poxviruses have evolved multiple proteins (e.g. VACV E3, K3, D9, and D10) to counter recognition and signaling by host intracellular sensors like PKR, OAS, RNAS1, RIG-I, and MDA5. VACV proteins C6, E3 and B3 target cytosolic DNA sensing pathways via cGAS, STING, etc. VACV proteins A46, A52, K7, E3, etc. target the TLR-mediated signaling (Yu et al., 2021). In CMLV, genes 32L and 55L encode proteins similar to the ones from VACV which sequesters dsRNA to prevent PKR and OAS activation. A VACV protein encoded by gene E3L targets IFR3, blocking IFN-β mRNA synthesis (Xiang et al., 2002) and CMLV055 has been suggested to be its camelpox viral counterpart (See et al., 2003).

Such poxvirus antagonists prevent the transcription of IFNs, and this may explain the reduced levels of IFN-β mRNA in the early stages of viral infection (Fig. 5C). Poxviruses also have evolved strategies to neutralize antiviral activity even after the host IFN proteins have been produced. The CMLV201 protein-like the VACV B19R binds IFNs/β and acts as a decoy receptor to block type-I IFNs binding to the host cell receptor. CMLV097 gene encodes a homolog of the VACV H1 phosphatase which dephosphorylates STAT1 to prevent IFN-stimulated innate immune responses (Duraffour et al., 2011b; Seet et al., 2003).

The biological activity of the recombinant protein was first evaluated by assessing the mRNA expression of IFN-responsive genes in camel kidney cells. Like camel IFN-α (Premraj et al., 2020), recombinant IFN-β treatment also induced the expression of these genes. Under similar conditions, the fold induction of expression of mRNAs of these ISGs was higher under IFN-β treatment, when compared to IFNα. Only Mx1 mRNA induction was less upon IFN-β treatment, when compared to IFNα treatment. Between IFN-β and IFN-α treatments, statistically significant differences in gene expression were observed for genes including ISG15 (p = 0.0025), Mx1 (p = 0.0023), RIG-I (p = 0.0008), and IRF1 (p = 0.0018). The correspondence changes in VIP (p = 0.0562) and OAS1 (p = 0.187) were not found statistically significant. (Fig. 6). Although different type I IFNs bind to the same cell surface receptor, they show difference in their activities. Compared to IFN-α, IFN-β has been reported to activate a significantly higher number of genes and even at a much lesser concentration. The differential activity has been attributed to the higher binding affinity of IFN-β with IFNAR1 and IFNAR2 when compared to IFN-α. It must also be noted that the differential activity is pleiotropic and has been reported to vary across different cell types (Piechler et al., 2012).

For analyzing the antiviral activity of dromedary IFN-β, we used the Camelpox virus (CMLV) an enzootic pathogen endemic to all camel rearing areas globally. CMLV belongs to the genus Orthopoxviruses in the Poxviridae family was believed to primarily infect camels only, but the recent report of zoonotic infections in human and the similarity with the variola virus has raised concerns (Narnaware et al., 2021). Pretreatment of camel kidney cells treated with recombinant IFN-β resulted in a
reduction in visible cytopathic effects and the viral load estimated using quantitative real-time PCR (Fig. 7A and B). The recombinant camel IFN-α that we reported earlier (Premraj et al., 2020) was used as a control. The antiviral activity of camel IFN-α was more compared to that of IFN-β as evidenced by the lesser amount of CMLV DNA detected in IFN-α treated cells (Fig. 7B). Among domestic animals, there has been a fair amount of research on porcine IFNs. In a comparative study of thirty porcine IFNs against different viral pathogens, it was found that the IFN-α subtypes displayed the greatest activity against PRRSV and VSV in monkey and porcine cells. IFNs like IFN-β, IFN-α, and IFN-κ differed in antiviral activity depending on their target cells and viruses (Jennings and Sang, 2019).

As a consequence of the growing need for camel meat, milk, or sport; camel husbandry has become more intensive and expanded to previously uninhabited areas. This has led to deeper interactions between camels and wild animals and an increase in the number of zoological pathogens reported from camels - especially viruses like Camelpox virus, MERS-CoV, Rift Valley fever virus (RVFV), Crimean–Congo Hemorrhagic Fever Virus (CCHFV), Alkhurma hemorrhagic fever virus (AHFV) etc. The camel-rearing areas in the Middle East and North Africa have the potential to become a hotspot for zoonotic spillover and great risk to human health (Zhu et al., 2019). It is presumed that MERS-CoV had its origin in bats before it infected camels. Recently, a MERS-related coronavirus Ty-BatCoV with potential for human transmission has been isolated from bats and this virus was also sensitive to IFN-β (Lau et al., 2021). In a recent report, high transmission activity of CCHFV was observed in the livestock market of the UAE. The virus was detected in camels and camel ticks, suggesting that the virus might be transported long distances and could spread via zoonotic transmission (Camp et al., 2021). The development of species-specific recombinant interferons and other antivirals in animals like camels with serious zoonotic risk is critical for managing future spillover events.

In summary, we cloned and identified the IFNB gene from the dromedary camel and all other extant members of Camelidae. The cameld IFN-β with two pairs of cysteines in the mature proteins is unique among eutherian mammals. Treatment of camel kidney cells with prokaryotically expressed recombinant dromedary IFN-β produced the expression of ISGs and conferred antiviral resistance to the cytopathic effects of camelpox virus. IFN-β from this unique group of mammals will help elucidate the antiviral immune mechanism and development of specific antivirals against pathogens that have the potential to become the next zoonotic pandemic.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest
The authors declare that they have no competing interests.

CRediT authorship contribution statement
Avinash Premraj: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. Abi George Aleyas: Resources. Binita Nautiyal: Resources. Thaha Jamal Rasool: Conceptualization, Resources, Project administration, Writing – review & editing.

Acknowledgements
The authors wish to acknowledge the vision and support of His Highness Sheikh Khalifa bin Zayed Al Nayah, the honourable President of the United Arab Emirates (UAE) for establishing the Camel Biotechnology Center under the auspices of the Department of President’s Affairs. His Highness Sheikh Sultan bin Hamdan Al Nayah, Private advisor to the President is gratefully acknowledged for the motivation and providing all resources for this research. Also acknowledged are Ms. Sreelekshmi Puthirikattil Raveendranathan and Ms. Shamma Juma Al Ketbi for their excellent assistance and support in the research process. For help with the statistical analysis, we would also like to thank Dr. Praseetha Kizhakkedath, Department of Genomics, CMHS, UAE University.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.devcim.2022.104443.

References
Premraj, A.G., Nautiyal, B., Premraj, A., Rasool, T.J., 2015a. Development of camel renal cell line using different immortalizing agents. In: Konuspayeva, G. (Ed.), Proceedings of the 4th Conference of ISOCARD – Silk Road Camel: the Camelds, Main Stakes for Sustainable Development. Special Issue of Scientific and Practical Journal Veterinariya #2 (42) 2015, Almaty, Kazakhstan, pp. 95–96.
Premraj, A.G., Premraj, A., Nautiyal, B., Rasool, T.J., 2015b. Endemic camel pox variants of Al Ain region. In: Konuspayeva, G. (Ed.), Proceedings of 4th Conference of ISOCARD “Silk Road Camel: the Camelds, Main Stakes for Sustainable Development.” Special Issue of Scientific and Practical Journal Veterinariya #2 (42) 2015, Almaty, Kazakhstan, pp. 220–230.
Camp, J.V., Weidinger, P., Ramaswamy, S., Kannan, D.O., Osman, B.M., Kolodziejek, J., Kuchroo, V.K., Nowotny, N., 2021. Association of dromedary camels and camel ticks with reassertant Crimean-Congo Hemorrhagic Fever Virus, United Arab Emirates. Emerg. Infect. Dis. 27, 2471–2474. https://doi.org/10.3202/eid2709.210299.
Day, C., Schwartz, B., Li, B.L., Petka, S., 1992. Engineered disulfide bond greatly increases specific activity of recombinant murine interferon-β. J. Res. 12, 139–143. https://doi.org/10.1093/jir/12.1.139.
DeWitte-Orr, S.J., Collins, S.E., Bauer, C.M.T., Bowdish, D.M., Mosman, K.L., 2010. An accessory to the “Triinity”: SRs are essential pathogen sensors of extracellular dsRNA, mediating entry and leading to subsequent type I IFN responses. PLoS Pathog. 6 https://doi.org/10.1371/journal.ppat.1000829.
Durafour, S., Matthys, P., van den Oord, J.J., de Schutter, T., Mittera, T., Sneock, R., Andrei, G., 2011a. Study of camelpox virus pathogenesis in athymic nude mice. PLoS One 6 https://doi.org/10.1371/journal.pone.0021561.
Durafour, S., Meyer, H., Andrei, G., Sneock, R., 2011b. Camelpox virus. Antivir. Res. 92, 167–186. https://doi.org/10.1016/j.antivirres.2011.09.003.
Harrison, G.A., McNicol, R.A., Deane, E.M., 2004. Interferon-α, -β and -κ.
Hussain, J., Chand, A., 2021. Prioritizing zoonotic diseases utilizing the One Health approach: Jordan – a case study. PLoS Negl. Trop. Dis. 15, e0009847. https://doi.org/10.1371/journal.pntd.0009847.
Honda, K., Yanai, H., Takaoscha, T., Taniguchi, T., 2005. Regulation of the type I IFN induction: a current view. Int. Immunol. 17, 1367–1378. https://doi.org/10.1093/ intimm/dxh318.
Hoso, K., Utsunomiya, K., Shirahata, T., Mitaka, T., Koyama, S., 1988. Structural characterization of human fibroblast interferon-β1. J. Interferon Res. 8, 375–384. https://doi.org/10.1093/jir/8.8.375.
Hussen, J., Schubert, H., 2021. Recent advances in camel immunology. Front. Immunol. 11, 1–17. https://doi.org/10.3389/fimmu.2020.01450.
Jennings, J., Sang, Y., 2019. Porcine interferon complex and co-evolution with increasing viral pressure after domestication. Viruses 11 https://doi.org/10.3390/v110606555.
Khabar, K.S.A., Young, H.A., 2007. Post-transcriptional control of the interferon system. Biochim. Biophys. Acta 1769, 761–769. https://doi.org/10.1016/j.biocel.2007.02.008.
Kheirallah, K.A., Al-mistarehi, A., Alswalha, L., Hijazeen, Z., Mahrous, H., Sheikali, S., Al-ramini, S., Mayeeth, M., Dodene, R., 2021. Prioritizing zoonotic diseases utilizing the One Health approach: Jordan – a case study. PLoS Negl. Trop. Dis. 15, e0009847. https://doi.org/10.1371/journal.pntd.0009847.
King, P.H., Chen, C.Y., 2014. Role of KSRP in control of type I interferon and cytokine expression. J. Interferon Cytokine Res. 34, 267–274. https://doi.org/10.1089/ jir.2013.0143.
Krause, C.D., Petka, S., 2015. Cut, copy, move, delete: the study of human interferon genes reveal multiple mechanisms underlying their evolution in amniotes. Cytokine 76, 480–495. https://doi.org/10.1016/j.cytok.2017.05.019.
Lau, S.K.P., Fan, R.Y.L., Zhu, L., Li, K.S.M., Wong, A.C.P., Luk, H.K.H., Wong, E.Y.M., Lam, C.S.F., Lo, G.C.S., Fang, J., He, Z., Fok, F.C.H., Au-Yeung, R.K.H., Zhang, L., Kok, K.H., Yuen, K.Y., Woo, P.C.Y., 2021. Isolation of MERS-related coronavirus from lesser bamboo bats that uses DPP4 and infects human-DPP4-transgenic mice. Nat. Commun. 12, 1–10. https://doi.org/10.1038/s41467-020-20458-x.
Li, S.F., Gong, M.J., Zhao, F.T., Shao, J.J., Xie, Y.L., Zhang, Y.G., Chang, H.Y., 2018. Type I interferons: distinct biological activities and current applications for viral infection. Cell. Physiol. Biochem. 51, 2377–2396. https://doi.org/10.1159/000495897.

Author details
A. Premraj et al. Developmental and Comparative Immunology 133 (2022) 104443
