Veterinary Immunology and Immunopathology 156 (2013) 107–113

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Inhibition of virus replication and induction of human tetherin gene expression by equine IFN-α1

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ARTICLE INFO

Article history:
Received 12 June 2013
Received in revised form 21 August 2013
Accepted 16 September 2013

Keywords:
Equine IFN-α
Antiviral activities
Equine infectious anemia virus
Equine arteritis virus
Tetherin

ABSTRACT

Type I interferons (IFNs) play important roles in the defense of host cells against viral infection by inducing the expression of a diverse range of antiviral factors. IFNs from different animals likely share similar features with human IFNs, and some of them have cross-species activities. Equine IFN-α was proved effective in both equine and human cells. However, the previous studies mostly focused on the inhibition of virus induced cytopathic effects. In this study, we used virus-specific assays to demonstrate the antiviral activities of equine IFN-α1 in both equine and human cells. Equine IFN-α1 inhibited the expression of viral structural proteins and the production of virions of equine infectious anemia virus (EIAV) and equine arteritis virus (EAV) in equine cells. In addition, equine IFN-α1 inhibited the production of EIAV virus-like particles (VLP) from human 293T cells. An IFN-inducible human gene, tetherin, was induced in 293T cells by equine IFN-α1. Its induction correlated with the inhibition of VLP release from the cell membrane. This result indicates that equine IFN-α1 shares a similar mechanism of action with human IFN-α in regulating antiviral genes expression in human cells.

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1. Introduction

Type I interferons (IFNs), which mainly include IFN-α, IFN-β, and IFN-ω, are a growing protein family. IFNs are well recognized as the cytokines are induced at the early stage of pathogen infection and mediate innate immune response. Type I IFNs have a broad spectrum of antiviral activity against a variety of DNA and RNA viruses. However, this effect is not direct; it is achieved by inducing the production of host factors in the innate immune response. For example, the expressions of the APOBEC3G (apolipoprotein B mRNA-editing catalytic polypeptide 3) (Chen et al., 2006; Peng et al., 2006; Tanaka et al., 2006; Wang et al., 2008), TRIM5α (a member of the tripartite interaction motif family of proteins) (Sakuma et al., 2007), and tetherin (Blasius et al., 2006; Neil et al., 2008) genes can be induced by IFNs and have been shown to inhibit the replication, uncoating, and release of retroviruses.

The alpha interferons (IFN-α), comprising various subtypes, are abundantly produced by a number of different cell types, such as monocyte/macrophages and plasmacytoid dendritic cells (Fitzgerald-Bocarsly, 2002; Fitzgerald-Bocarsly et al., 2008), after viral infection or stimulation by exogenous nucleic acids. Fibroblasts and epithelial cells rarely produce IFN-α, but mainly produce IFN-β (Detournay et al., 2013). IFN-β is animal species-specific; however, IFN-α has been shown to exert antiviral activity in heterologous cells (1983; Kubes et al., 1994; Liu et al., 1996; Nagano and Maehara, 1975; Roberts et al., 1998). Crude feline IFN-α was proved to have antiviral activity against vesicular stomatitis virus (VSV) in heterologous animal cells (RK-13, MDBK, MDCK and L-929 cells) (Taira et al., 2005). Canine IFN-α1 exhibited antiviral activity in chicken embryo fibroblast cells, but not in Mardin–Darby bovine kidney cells (MDBK)

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http://dx.doi.org/10.1016/j.vetimm.2013.09.009

(Ruttanapumma et al., 2006). Porcine IFN-α had inhibitory effects on pocine pseudorabies virus (PRV) and porcine reproductive and respiratory syndrome virus (PRRSV) in MDBK and MARC-145 cells (Huang et al., 2012). Chicken IFN-α reduced avian influenza virus (AIV) infection in cells of both turkey and duck species (Jiang et al., 2011). Human IFN-α inhibited VSV and Murine leukemia virus (MLV) infection in MDBK, rhesus monkey, and African green monkey cells (Carthagena et al., 2008; Sakuma et al., 2007). So far, most knowledge on the characterization, immunomodulation and antiviral mechanism of IFN-α is obtained from the studies on humans. In terms of antiviral function, the induction of IFN-α is stimulated by virus infection. IFN-α binds to its receptor and activates the expression of a wide range of interferon-stimulated genes (ISGs), these ISGs are capable of inhibiting virus infection by different mechanism (Sadler and Williams, 2008). Tetherin gene is an ISG and its expression is strongly induced by human IFN-α throughout the JAK/STAT (the janus kinase-signal transducer and activator of transcription) pathway (Kessler et al., 1988; Levy et al., 1988). Tetherin is a transmembrane protein at cell surface and discovered to inhibit the release of nascent enveloped virons (Neil et al., 2008). Tetherin also binds to immunoglobulin-like transcript 7 (ILT7) and inhibits the production of interferon in a negative feedback loop (Cao et al., 2009).

In horse, there are 8 classes in the equine IFN family mainly including 6 IFN-α, 4 IFN-β, and 8 IFN-ω genes (Detournay et al., 2013). Both natural and recombinant equine IFN-α exhibited broad antiviral activity in both domestic animals and human cells (Himmler et al., 1986; Steinbach et al., 2002; Yilma et al., 1982). The antiviral activity of equine IFN-α was various depending on the cell types and/or virus system used (Steinbach et al., 2002). Most of these observations are based on the inhibition of cytopathic effects induced by the viruses. Moreover, the specific equine IFN-α induced virus-blocking mechanism is still not clear. As a result, we have a limited understanding of equine IFN-α-mediated antiviral activity against different viruses, and its cross species function. In this study, we determined specific virus production and the expression levels of a human IFN-inducible gene, tetherin, to further evaluate the antiviral and biological activities of equine IFN-α1.

2. Materials and methods

2.1. Cells and viruses

Human embryonic kidney (HEK) 293T cells, Madin–Darby canine kidney (MDCK) cells, rabbit kidney (RK-13) cells, primary fetal equine dermal(FED) cells and fetal donkey dermal (FDD) cells (Shen et al., 2006) were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (p/s) (D10). All cells were maintained in 37 °C in a 5% CO_2 incubator.

An attenuated vaccine strain of EIAV, was propagated on FDD cells (Shen et al., 2006). EAV was grown, and its infectious titers were tested, in RK-13 cells. Vesicular stomatitis virus tagged with green fluorescent protein (VSV-GFP) was kindly provided by Dr Bu Zhigao (Bai et al., 2010).

2.2. Interferon

The full-length of equine interferon-alpha 1 gene (eIFN-α1), including the secretion signal peptide coding region, was cloned into a modified pcDNA3.1 (+) vector tagged with HA at the N terminal, pcDNA-vHA, to generate the recombinant eIFN-α1-encoding plasmid peIFN-α1. To obtain soluble interferon, 293T cells were transiently transfected with 1 μg peIFN-α1 using 3 μl Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's recommendations. The supernatants were collected 48 h post-transfection and separated on 12% SDS-PAGE gels. Separated proteins were transferred onto nitrocellulose (NC) membranes. The membranes were blocked with 5% dry milk in TBS for 2 h at room temperature, and subsequently incubated with either mouse anti-HA or β-actin monoclonal antibodies (Sigma, USA) at a 1:10,000 dilution for 2 h. DLight800-conjugated anti-mouse IgG (KPL, USA) was used as the secondary antibody at 1:10,000 dilution. The NC membrane was scanned using an Odyssey infrared imaging system (Odyssey, UK) at a wavelength of 800 nm. The resulting IFN-containing supernatant (eIFN-α1) was used for inducing cells. As a control, we employed the culture medium from cells transfected with pcDNA-vHA. In order to ensure consistency across all eIFN-α1 tests, the collected eIFN-α1 was aliquoted and stored at −80 °C, freezing and thawing only once before use.

2.3. Definition of activity unit of equine IFN-α1

The antiviral activity of equine IFN-α was usually assessed on Madin–Darby bovine kidney (MDBK) cells (Wagner et al., 2008). However, considering equine IFN-α was more active on FED as compared with MDBK cells (Steinbach et al., 2002), FED cells were seeded in 6-well plates to achieve 90% confluence overnight. 1.5 ml of $10^{0.5}$-fold serially diluted eIFN-α1 or control media was added into each well. Each dilution was performed in triplicates. After 24 h treatment, cells were infected with 15,000 PFU VSV-GFP for 1 h in 2 ml DMEM containing 2% FBS. Cells received no interferon treatment but VSV-GFP infection was used as virus control while cells without interferon or virus treatments were set for blank control (BC). After 18–24 h of infection, the expression of GFP in wells was observed with a Leica DMIRE2 fluorescence microscope (Leica, Germany). Subsequently, the cells were washed three times with PBS and split into 1.5 ml eppendorf tube for flow cytometry (BD) analysis. The highest dilution, where the expression of GFP was half the virus control wells, was determined as one laboratory unit (LU).

2.4. Antiviral activity of equine IFN-α1 against equine infectious anemia virus and equine arteritis virus

FED cells were seeded in a 6-well plate at a density of 8×10^5 cells/well and incubated overnight. Cells were then treated with 2×10^4 LU of eIFN-α1 or control media for 24 h, and subsequently infected with EIAV

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