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Short communication

Antiviral and antiproliferative effects of canine interferon-λ1

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ABSTRACT

Interferon (IFN)-λs, members of the type III IFN group, were recently identified in several vertebrates. Although IFN-λs have the potential to be utilized as antiviral and antitumor agents in veterinary medicine, the biological properties of IFN-λs have not yet been studied in companion animals. In this study, we analyzed the expression of canine IFN-λs and their receptors, produced a recombinant canine IFN-λ1 protein, and investigated its antiviral and antiproliferative activities using a canine kidney epithelial cell line, MDCK cells. MDCK cells were found to express type III IFN molecules, *IFN-λ1* and *IFN-λ3*, and the receptors, *IFNλR1* and *IL10R2*. *IFN-λ1* was induced faster than *IFN-λ3* by stimulation with poly (I:C). His-tagged IFN-λ1 protein expressed in *Escherichia coli* inhibited cytolytic plaque formation by influenza A virus infection, and induced the expression of interferon-stimulated genes, *Mx1* and *OAS1*, in MDCK cells. In addition, recombinant IFN-λ1 inhibited the proliferation of MDCK cells slightly. These effects were observed in a dose-dependent manner. These results indicate that canine IFN-λ1 has antiviral effect, and suggest the potential applicability of canine IFN-λ1 as a therapeutic agent.

1. Introduction

Animals possess innate and adaptive immune mechanisms that contribute to the clearance of viral infection. Interferons (IFNs) are one of the key mediators that activate both the innate and adaptive immune responses against viral pathogens. IFNs belong to a large family of structurally related class II cytokines that includes interleukin (IL)-10, IL-19, IL-20, IL-22, IL-24 and IL-26 (George et al., 2012). IFNs are classified into three types based on their structural features and receptor usage; that is, type I (IFN-α/β), type II (IFN-γ) and type III (IFN-λ). IFN-λs were recently identified in several mammals including humans and mice (Kotenko et al., 2003; Sheppard et al., 2003). The type III IFN family consists of some isotypes in each species. In humans, three functional IFN-λ genes, that encoding IFN-λ1, IFN-λ2 and IFN-λ3 proteins, also known as IL-29, IL-28A and IL-28B respectively, were identified (Donnelly and Kotenko, 2010). In mice, the *Ifnl2* and *Ifnl3* genes, encoding IFN-λ2 and IFN-λ3 proteins, respectively, were identified (Donnelly and Kotenko, 2010). IFN-λs are known to be expressed in a variety of cells both in vitro and in vivo in response to viral infection. (Kotenko, 2011). IFN-λs have also been shown to induce a number of biological activities. For example, IFN-λs inhibit the replication of human hepatitis virus B and human hepatitis virus C in vitro (Marcello et al., 2006; Robek et al., 2005). IFN-λs also exhibit antiviral

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activity against other viruses, such as encephalomyocarditis virus and vesicular stomatitis virus, in several cell lines (Ank et al., 2006; Kotenko et al., 2003; Sheppard et al., 2003). Further, IFN-λs have been shown to induce antiproliferative activity in several cell lines including intestinal epithelial cells (Brand et al., 2005) and glioblastoma cells (Meager et al., 2005).

The biological activities of IFN-λs are similar to those of type I IFNs. As IFN-λs bind to unique heterodimeric receptors consisting of IFN-λR1 and IL-10R2, they activate the phosphorylation of STATs and induce the transcription of IFN-stimulated genes (ISGs) similar to that seen in type I IFNs (Donnelly and Kotenko, 2010; Kotenko, 2011). Although IFN-λR1 and IL-10R2 are expressed in a broad range of tissues and cells, the response to IFN-λs was mainly observed in the epithelial cells of the lung, intestine and kidney (Ank et al., 2008; Mordstein et al., 2010; Sommereyns et al., 2008; Zhou et al., 2007). In addition, fibroblasts, endothelial cells, monocytes and T lymphocytes do not respond to IFN-λs in spite of IFN-λ receptor expression (Lasfar et al., 2006; Witte et al., 2009). These reports suggest that the clinical application of IFN-λs to the treatment of infectious diseases and tumors would result in lower less toxicity than type I IFN. Indeed, preliminary human clinical studies have revealed that IFN-λ1 may possess antiviral activity against hepatitis C virus infection comparable to that of type I IFN but with a potential reduction in side effects (Muir et al., 2010). Therefore, IFN-λs have the potential to be used as antiviral and antitumor agents in veterinary medicine. To date, the biological properties of IFN-λs in companion animals have not been reported. In the present study, we performed molecular cloning of canine IFN-λ1 cDNA and produced a recombinant IFN-λ1 protein, then investigated its antiviral and antiproliferative activities using a canine kidney epithelial cell line.

2. Materials and methods

2.1. Cell culture

The canine kidney epithelial cell line, MDCK cells, were cultured in Eagle's minimum essential medium (Sigma–Aldrich, St. Louis, MO, USA) containing 5% fetal bovine serum (Life Technologies, Carlsbad, CA, USA) and 1% penicillin/streptomycin solution (Nacalai Tesque, Tokyo, Japan) at 37 °C in 5% CO_2.

2.2. Reverse transcription (RT)-PCR and real-time PCR

Total RNA was isolated from MDCK cells treated with or without 50 μg/ml poly (I:C) (GE Healthcare, Uppsala, Sweden) using Trizol reagent (Life Technologies). Reverse transcription was performed with 1 μg total RNA, SuperScript III reverse transcriptase (Life Technologies), and an oligo dT primer, according to the manufacturer's recommended protocol. PCR was performed with 1 μl of first-strand cDNA, a primer pair (listed in Table 1), and HybriPol DNA Polymerase (Bioline, London, UK). Real-time PCR was performed with first strand cDNA, a primer pair (listed in Table 1), and Fast SYBR Green Master Mix (Life Technologies) in a StepOne system (Life Technologies) according to the manufacturer's recommended protocol. All PCR products were cloned into a pGEM-T Easy vector (Promega, Madison, WI, USA) and confirmed by sequencing using an ABI 3130 Genetic Analyzer (Life Technologies).

2.3. Expression and purification of recombinant canine IFN-λ1

Recombinant canine IFN-λ1 was expressed in *Escherichia coli* using a previously described experimental procedure (Masuda et al., 2012). The cloned cDNA of canine IFN-λ1, amplified by RT-PCR with the primers listed in Table 1, was ligated into a prokaryotic 6×His-tagged protein expression vector, pET-28a (Merck, Darmstadt, Germany), and used for transformation.

2.4. Plaque formation assay

Confluent cultures of MDCK cells in 6-well plates were treated with or without recombinant canine IFN-λ1 for 12 h, inoculated with 54 pfu/well of influenza virus A/whistling swan/Shimane/499/83 (H5N3) for 1 h, and incubated in Eagle's minimal essential medium (Sigma Aldrich) containing 0.3% BSA, 1% agarose and 1 μg/ml trypsin for 3 days at 37 °C in 5% CO_2. The cells were fixed with 10% formalin for 1 h and stained with 2% crystal violet, and the formation of plaques was then observed.

2.5. Cultured cell proliferation assay

MDCK cells were seeded at 1×10^3 cells/well in 100 μl of culture medium with or without recombinant canine IFN-λ1 in a 96-well microplate, and incubated for 72 h at 37 °C in 5% CO_2. Cells were then treated with WST-1 reagent (Roche Applied Science, Mannheim, Germany) according to the manufacturer's recommended protocol, and the OD at 450 nm was measured using a Model 680 microplate reader (Bio-Rad Laboratories Hercules, CA, USA).

2.6. Statistics

Statistical analyses were performed by one-way analysis of variance with Tukey's post hoc test and Kruskal–Wallis test with Dunn's multiple comparison test using GraphPad Prism 6 software (GraphPad software, La Jolla, CA, USA).

3. Results and discussion

3.1. Expression of IFN-λs and their receptors in MDCK cells

Previous reports revealed that epithelial cells, including those in the lung, intestine and kidney of the mouse or human, responded to IFN-λs (Ank et al., 2008; Mordstein et al., 2010; Sommereyns et al., 2008; Zhou et al., 2007). Therefore, we used a canine kidney epithelial cell line, MDCK cells, to examine the biological properties of canine IFN-λ1. We firstly examined the expression of IFN-λR1 and IL-10R2 in MDCK cells. RT-PCR analyses revealed