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Short communication

Induction and sequencing of Rousette bat interferon α and β genes

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Abstract

Bats are considered to be natural reservoirs for several viruses of clinical importance, including rabies virus, Nipah virus, and Hendra virus. Type I interferons (IFNs) is an important part of the immune system in the defense against viral infection. To investigate the function of type I IFNs upon viral infection in bats, the nucleic acid, and amino acid sequences of Egyptian Rousette (*Rousettus aegyptiacus*) IFN-α and -β were characterized. Sequence data indicated that bat IFN-α consists of 562-bp encoded 187-aa, and IFN-β consisted of 558-bp encoded 186-aa. Phylogenetic analysis of the overall identity of IFN-β shared the highest sequence homology with pig IFN-β in both nucleotide and amino acid level. Stimulation of bat primary kidney cells (BPKCs) and bat lung cell lines, Tb-1 Lu, with polyinosinic–polycytidylic acid (poly(I:C)) or exogenous bat type I IFNs resulted in increased type I IFNs mRNA expression in BPKCs, but not in Tb-1 Lu. Characterization of the bat IFN-α and -β genes allows understanding of the immune responses upon stimulation in different tissues, thus providing practical strategies for control and treatment of clinically important diseases. These results are important especially for the virus infection, and suggest that future molecular studies on virus infection experiment of bats *in vitro* will require careful consideration of the differences of type I IFN expression patterns in different cell types.

Keywords: Bat; Type I interferons; Bat kidney primary cells; Tb-1 Lu

1. Introduction

Bats, Chiropteras, are well-known vectors of rabies and some studies indicate that they may also naturally harbor some emerging viruses such as Nipah virus, Hendra virus, bat-SARS-CoV and Ebola virus (Chua et al., 2002; Halpin et al., 2000; Lau et al., 2005; Leroy et al., 2005; Mayen, 2003; McColl et al., 2000; Normile, 2005). Bat has two suborders, Megachiroptera (flying fox) and Microchiroptera (insectivorous bat). Many emerging or re-emerging viruses, such as rabies, Nipah virus, and Hendra virus, were isolated form Megachiroptera. In particular, European bat lyssavirus type I was also isolated from *Rousettus* sp. (Van der Poel et al., 2000; Wellenberg et al., 2002; Wong et al., 2007). Bats were thought to have an important role for the infection cycle of these emerging and re-emerging viruses. *In vivo* experiment,

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Ebola virus inoculation studies showed that both flying foxes and insectivorous bats support viral replication and circulation with high viral titers without becoming ill (Swanepoel et al., 1996). Studies *in vitro* have shown that Ebola virus VP35 protein blocks the activation of interferon regulatory factor 3 (IRF-3) and Ebola virus VP24 protein inhibits interferon (IFN) signaling (Basler et al., 2003; Reid et al., 2006). These data suggested that Ebola virus might evade the anti-viral activity of IFNs in bat cells. Therefore, it is crucial to investigate IFN regulation and function in bats because few immunological studies have been reported for this animal species.

Cells have many responses to viral infection. One of the responses is the secretion of type I IFNs which are composed of multiple α subtypes and a single β subtype (Sen, 2001). Type I IFNs expression utilize two signal transduction pathways; the Toll-like receptor (TLR)-dependent pathway and TLR-independent pathway. In TLR-dependent pathway, cells recognize viral double-strand RNA, single-strand RNA and CpG DNA via TLR, and subsequently IFN-β is induced. In TLR-independent pathway, intracellular sensors such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene-5 (mda-5) detect viral components in the cytoplasm, and transactivate IFN-β mRNA (Hiscott et al., 2006). Expressed IFN-β binds to the type I IFN receptors and activates numerous IFN-stimulated genes, such as the protein kinase R (PKR) gene, the 2′–5′ oligoa-denylate synthetases (OAS) gene, and the myxovirus resistance (Mx) gene. The product of these genes controls viral infection (Samuel, 2001). Viral double-stranded RNA (dsRNA), a viral intermediate in the proliferation of many RNA viruses, is known as an IFN-inducer through these sensors (Gitlin et al., 2006). Polyinosinic–polycytidylic acid (poly(I:C)) is a synthetic mimetic of viral dsRNA and a strong inducer of type I IFNs *in vivo* and *in vitro* via these sensors (Hertzog et al., 2003).

Type I IFNs stimulate anti-viral activity as mentioned above; however, such studies in bat have not been possible because the bat IFN related genes had not been previously identified. In this study, we determined the sequence of a subtype of IFN-α and IFN-β from *Rousettus aegyptiacus*, including the full open reading frames (ORFs), and analyzed phylogenetically based on IFNs from other mammals. In addition, the up-regulation of these mRNAs in both bat primary kidney cells (BPKCs) and a bat lung cell line, Tb-1 Lu was examined using poly(I:C) or bat type I IFNs derived from BPKCs.

2. Materials and methods

2.1. Preparation of cDNA from bat genomic DNA

Fresh liver sample and whole blood of *R. aegyptiacus* under anesthesia with ketamine (5 mg/ml/kg) and medetomidine (0.2 mg/ml/kg) were collected by heart puncture. Bat liver was fixed with 10% neutral buffer formalin. Bat genomic DNA was isolated from fixed liver with the Wizard Genomic DNA Purification kit (Promega, Madison, WI) and stored at −20 °C until usage.

2.2. Sequencing of bat IFN genes

Bat genomic DNA sample was used as a template of polymerase chain reaction (PCR) using TaKaRa Ex Taq (Takara Bio, Ohtsu, Shiga, Japan). Forward and reverse primers of IFN-α and IFN-β for PCR were designed from the sequence data of human, mouse, cat, pig, and horse IFN-α and IFN-β (Table 1). The accession numbers of these data in GenBank are as follows: IFN-α

Table 1
Sequence of each PCR primers

Primer name		Sequence (5′–3′)
IFN-α	Forward	CTC TCT AGG ATG TGA CCT GCC TCA GA
	Reverse	ACA GGG GCT GTG TTT CTT CTC
IFN-β	Forward	GCT TGG ATT CCA ACT AAG AAG CAG C
	Reverse	ACA GAC GCT GTA CTC CTT GGC CTT CA
GAPDH	F	GAT GGA GCA TCA TAC TGA TCC
	R	GAC CTT CTA CCA CTA CCC AAA
IFN-α	F3	ACA GAG GCA GGT CTT CAC AAC CTA GA
	R2	GAG AAG CAT TTC CAT GTT GAA CCA G
IFN-β	cdsF	TAG GTG ATA GTA GGC ACC ACT GTT CC
	cdsR	CTT TCT CAG AAG TAC AGG CGG AGA GA