Virus Research 157 (2011) 54–60

Contents lists available at ScienceDirect

Virus Research

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

Infection of cultured bovine cells with bovine herpesvirus 1 (BHV-1) or Sendai virus induces different beta interferon subtypes

Leticia Frizzo da Silva, Clinton Jones*

School of Veterinary Medicine and Biomedical Sciences, Nebraska Center for Virology, University of Nebraska, Lincoln, Fair Street at East Campus Loop, Lincoln, NE 68583-0905, United States

ARTICLE INFO

Article history:
Received 8 January 2011
Received in revised form 3 February 2011
Accepted 3 February 2011
Available online 23 February 2011

Keywords:
BHV-1
Sendai virus
Beta-interferon induction
IRF3
IRF7

ABSTRACT

In contrast to mice or humans, cattle contain three beta interferon (IFN-β) genes with distinct transcriptional promoters suggesting IFN-β gene expression is not stimulated the same by different viruses. To test this hypothesis, we compared expression of the three IFN-β subtypes after infection with a RNA virus, Sendai, versus a large DNA virus, bovine herpesvirus 1 (BHV-1). Infection of low passage bovine kidney (BK) or established bovine kidney cells (CRIB) with Sendai virus has consistently led to high levels of IFN-β1 RNA. Conversely, infection of CRIB cells, but not BK cells, with BHV-1 increased IFN-β3 RNA levels and to a lesser extent the other two IFN-β subtypes. Inhibition of de novo protein synthesis with cycloheximide resulted in higher levels of IFN-β1 and IFN-β2 RNA levels after BHV-1 infection. Further studies demonstrated that BHV-1 immediate early and/or early genes were primarily responsible for inhibiting the IFN response in BK cells. The three bovine IFN-β promoters were cloned upstream of a reporter gene construct, and their properties analyzed in transient transfection assays. Only the IFN-β3 promoter was trans-activated by IRF3 (interferon responsive factor 3). IRF7 and double stranded RNA (polyI:C) stimulated IFN-β1 and IFN-β3 promoter activity, but not IFN-β2. Relative to the human IFN-β promoter, the IFN-β3 promoter contained fewer nucleotide differences in the positive regulatory domain III (PRD III), PRD IV, and PRD I compared to the IFN-β1 and IFN-β2 promoter. Collectively, these studies provide evidence that virus infection differentially stimulates expression of the three bovine IFN-β genes.

1. Introduction

Stimulation of beta-interferon (IFN-β) transcription is an early response to virus infection (Au et al., 1995; Goodbourn et al., 1985; King and Goodburn, 1994; Munshi et al., 1998; Sharma et al., 2003; Yoneyama et al., 1998). In contrast to humans or mice, cattle contain three IFN-β genes with distinct promoters (Valarcher et al., 2003; Wilson et al., 1983). All three bovine IFN-β isotypes have anti-viral activity, but it is not clear whether virus infection differentially induces the three subtypes. Viral regulation of IFN responses is crucial for survival in nature and pathogenesis. For example, mice lacking type I and type II IFN receptors in combination with RAG-2 gene deletions die within a few days following infection with bovine herpesvirus 1 (BHV-1) (Abril et al., 2004), an alpha-herpesvirinae subfamily member. Conversely, infection of wt mice with BHV-1 does not lead to clinical symptoms. BHV-1 induces an IFN response, in part due to its high CpG content (Lundberg et al., 2003). It is assumed that DNA "sensors" and/or toll like receptors that recognize unmethylated CpG DNA motifs, TLR9 for example (Bowie and Unterholzner, 2008), recognize incoming BHV-1 DNA and induces an IFN response. During the course of productive infection, viral encoded products, including infected cell protein 0 (bICP0), interfere with innate immune responses (reviewed by Jones (2009)).

BHV-1 infections can lead to conjunctivitis, pneumonia, genital disorders, abortions, and an upper respiratory infection known as bovine respiratory disease (BRD) (Chowdhury and Jones, 2010; Jones and Chowdhury, 2008; Tikoo et al., 1995). BHV-1 initiates BRD by immunosuppressing cattle (Carter et al., 1989; Griebel et al., 1987a,b, 1990; Yates, 1982), which leads to secondary bacterial infections and life-threatening pneumonia (Yates, 1982). BRD costs the cattle industry more than $1 billion/year in the United States ((NASS), 1996; Bowland and Shewen, 2000; Carter et al., 1989; Griebel et al., 1987a,b, 1990; Ishmael, 2001; Powell, 2005; Tikoo et al., 1995). Modified live vaccines are available, and in general, they prevent clinical disease in adults. However, vaccine strains are immunosuppressive, can cause serious disease in young calves or abortions in pregnant cows, and can reactivate from latency.

* Corresponding author at: University of Nebraska, VBMS, EAST Campus Loop, Rm 104, Lincoln, NE 68583-0905, United States. Tel.: +1 402 472 1890; fax: +1 402 472 9690.
E-mail address: cjones@unlnotes.unl.edu (C. Jones).

0168-1702/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2011.02.004

Thus, there is a need to better understand the mechanism by which BHV-1 suppresses immune responses, in particular innate immune responses.

In contrast to large double stranded DNA viruses, RNA viruses generally initiate an IFN response by novel mechanisms, in large part due to high levels of double stranded RNA produced during their replication cycle (Bowie and Unterholzner, 2008). For example, Sendai virus, a small negative single stranded RNA virus that belongs to the Paramyxovirinae subfamily, activates the IFN-β promoter primarily through a RIG-I (retinoic acid inducible gene I) dependent manner (Strahle et al., 2007). The Sendai virus encoded C protein, in part, inhibits IFN responses indicating that inducing and inhibiting IFN responses following infection is a normal process following infection of mammalian cells with a RNA virus.

In this study, we examined expression of the three IFN-β subtypes following infection with BHV-1 or Sendai virus. In established or low passage bovine cells, Sendai virus consistently increased IFN-β1 RNA levels. In contrast, BHV-1 productive infection led to an increase in IFN-β3 RNA levels in established BK cells. When low passage bovine cells were infected, IFN-β RNA levels were not detected unless protein synthesis was blocked. The promoters of each IFN-β subtype were cloned and transient transfection assays performed to begin to understand how these promoters were activated. Only the IFN-β3 promoter construct was trans-activated by IRF3 (interferon responsive factor 3). IRF7 and double stranded RNA (polyI:C) stimulated IFN-β1 and IFN-β3 promoter activity. The IFN-β3 promoter was more similar to the human IFN-β promoter relative to the IFN-β1 and IFN-β2 promoter. Collectively, these studies suggested that IFN-β subtype RNA levels are differentially regulated by BHV-1 versus Sendai virus. Furthermore, cell type specific induction of IFN-β RNA levels were observed following infection with BHV-1.

2. Materials and methods

2.1. Cells

Human epithelial 293 (293) cells, murine neuroblastoma cells (neuro-2A), low passage bovine kidney (BK) cells, low passage bovine turbinate cells, low passage bovine testicle cells, and established bovine kidney cells (CRIB) were cultured in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin (10 U/ml), and streptomycin (100 μg/ml) in a humidified 5% CO_2 atmosphere at 37 °C. Low passage BK cells were prepared from a healthy calf.

2.2. Viruses

The Cooper strain of BHV-1 (wt virus) was obtained from the National Veterinary Services Laboratory, Animal and Plant Health Inspection Services, Ames, IA. BHV-1 stocks were prepared in bovine cells (CRIB).

Sendai virus (Cantell strain) was obtained from Dr. Fernando Osorio (University of Nebraska, Lincoln, NE). The designated cells were infected with 40-hemagglutinin units/ml.

2.3. Construction of bovine IFN-β1, 2 and 3 reporter constructs

IFN-β-chloramphenicol acetyltransferase (CAT) reporter constructs containing the bovine IFN-β promoters were generated by PCR using DNA extracted from CRIB cells as a template. Specific primers for regions flanking the enhanceosome of these IFN-β promoters were designed using the following oligonucleotide primers. For the bovine IFNβ-1 promoter, the forward primer was 5′-AAGGTACCCCTCTCCCCAAATCTC-3′ and the reverse primer was 5′-CTCGAGGTCCTTCGTCCTTAAGTG-3′. To amplify the bovine IFN-β3 promoter, the forward primer was 5′-GGTACCTGTTCTCGGAAAGTTGAGC-3′, and the reverse primer was 5′-CTCGAGGATGAAAACAGGCACAGGG-3′. The respective amplification products were cloned into the CAT vector, pCAT-basic reporter plasmid (Promega, catalog no. E1871) at the unique XhoI and KpnI restriction enzyme sites. The bovine IFN-β2 promoter construct was synthesized (IDT), because it was difficult to amplify a specific fragment containing its promoter region. The flanking region of the bovine IFN-β2 promoter is almost identical to the flanking region of bovine IFN-β1.

The human IRF-3 and IRF-7 expression constructs were obtained from Luwen Zhang (University of Nebraska, Lincoln, NE). The dsRNA (PolyI:C) was purchased from Invivogen, (catalog # tlrl-pic).

2.4. CAT assays

The respective cells were transfected with the designated plasmids. Human epithelial 293, BK, or CRIB cells were transfected with TransIT (Mirus, catalog no. MIR2000) according to the manufacturer's instructions. Neuro-2A cells were transfected with NeuroTransit (Mirus, catalog no. 2145), according to the manufacturer's instructions. Forty hours after transfection, cells were lysed by three freeze–thaw cycles in 250 mM Tris–HCL (pH7.4). CAT assays were performed with 0.2 μCi (7.4 KBq) {^{14}C}-chloramphenicol (Amersham Biosciences, catalog no. CFA754) and 0.5 mM acetyl coenzyme A (Sigma, catalog no. A2181). Chloramphenicol and its acetylated forms were separated by thin-layer chromatography and CAT activity measured with a PhosphorImager (Molecular Dynamics, CA). CAT activity was expressed as the fold of induction relative to the vector control. Transfection experiments for CAT assays were repeated at least three times to confirm the results.

For virus infection studies, the designated cells were cultured in 60 mm^2 dishes and then transfected with Lipofectamine™ 2000 transfection reagent (Invitrogen, catalog no. 11668-019) according to the manufacturer's instructions. Cells were incubated with the transfection mix for 5 h and then replaced with fresh media supplemented with 10% FBS. At 24 h after transfection, cells were infected with wt BHV-1 using a multiplicity of infection (moi) of 5 or with 40 hemagglutinin units of Sendai virus/ml. Cell lysate was harvested at the designated time points, and CAT activity measured as described previously (Meyer and Jones, 2008; Workman et al., 2009; Zhang and Jones, 2001, 2005; Zhang et al., 2006).

2.5. RNA extraction and RT-PCR

RNA extraction and reverse transcription (RT)-PCR was performed as described previously using primers that specifically amplify the three bovine IFN-β subtypes (Perez et al., 2008). The IFN-β1 primers are: forward, AGGAGCTACAGCTTGCTTCG and reverse, TGACCAATATGGCATCTTCC. The IFN-β2 primers are: forward, AATGGACAGTTAAATACTGTAAGC and reverse, TAAATGTCTCCAGGGTGCTC. The IFN-β3 primers are: forward, TCCTCTTCTACTTTCTGCCAAA and reverse, AAGGGCTTGCAGAGTGAATG. PCR products were electrophoresed on 2% agarose gels and stained with ethidium bromide.

3. Results

3.1. Analysis of bovine IFN-β RNA levels after infection

To understand whether specific viral pathogens differentially stimulate bovine IFN-β RNA expression, established CRIB or low passage BK cells were infected with Sendai virus or BHV-1 and expression of the three IFN-β subtypes examined by RT-PCR. RT-PCR was used for these studies because there are no subtype specific