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Original article

Bovine parainfluenza virus type 3 accessory proteins that suppress beta interferon production

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Abstract

The paramyxovirus P gene encodes accessory proteins antagonistic to interferon (IFN). Viral proteins responsible for the IFN antagonism, however, are distinct among paramyxoviruses. Here we determine bovine parainfluenza virus type 3 (bPIV3) IFN antagonists that suppress IFN-β production, and investigate the underlying molecular mechanism. Of bPIV3 P gene products, C and V proteins were found to suppress double-stranded RNA-stimulated IFN-β production. The V protein of bPIV3 and Sendai virus in the same genus Respirovirus significantly inhibits double-stranded RNA-stimulated IFN-β production and the IFN-β promoter activation enhanced by overexpression of MDA5 but not RIG-I, and yet does not suppress IFN-β production induced by TRIF, TBK1, and IKKi. The V protein of both viruses specifically binds to MDA5 but not RIG-I. These results suggest that the V protein targets MDA5 for blockage of the IFN-β gene activation signal. On the other hand, both bPIV3 and Sendai virus C proteins modestly inhibited IFN-β production irrespective of a species of the signaling molecules used as an inducer. Interestingly, reporter gene expression driven by various promoters was also suppressed by the C proteins irrespective of the promoter species. These results demonstrate that the target of the respirovirus C protein is undoubtedly different from that of the V protein.

Keywords: Bovine parainfluenza virus type 3; Sendai virus; C protein; V protein; Interferon; MDA5; RIG-I

1. Introduction

Viruses in the subfamily *Paramyxovirinae* have acquired the ability antagonistic to interferon (IFN) during evolution to evade the host innate immunity [1,2]. The IFN antagonistic ability discovered so far in the *Paramyxovirinae* is classified into two categories. One is to block Janus tyrosine kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, thereby inhibiting establishment of an antiviral state by suppressing induction of IFN-stimulated gene products including double-stranded (ds) RNA-dependent protein kinase and 2′,5′-oligoadenylate synthetase [1,2]. Another is to interfere with signaling pathways that lead to IFN-β production through IFN regulatory factor 3 (IRF3) activation in infected cells [3–5].

Most paramyxoviruses largely employ the V protein for both IFN-antagonistic activities [3,4,6,7], whereas Sendai virus (SeV) (mouse parainfluenza virus type 1) in the genus Respirovirus uses the C protein for the blockage of the JAK-STAT pathway [8–10], and both V and C proteins for the negative regulation of the IFN-β production [5]. However, it is not

Abbreviations: IFN, interferon; JAK, Janus tyrosine kinase; STAT, signal transducer and activator of transcription; ds, double-stranded; IRF3, interferon regulatory factor 3; SeV, Sendai virus; bPIV3, bovine parainfluenza virus type 3; ORF, open reading frame; RIG-I, retinoic acid inducible gene I; MDA5, melanoma differentiation-associated gene 5; TBK1, TANK binding kinase 1; IKKi, inducible IkB kinase; IPS-1, IFN-β promoter stimulator; TLR3, Toll-like receptor 3; TRIF, Toll-interleukin-1 receptor domain-containing adaptor-inducing beta interferon; TK, thymidine kinase; SV, simian virus; CMV, cytomegalovirus; poly(I):poly(C), polyinosinic–polycytidylic acid.

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apparent whether this unique feature seen in SeV is common to members of the Respirovirus genus. To address this question, we decided to investigate the IFN antagonism of another respirovirus, bovine parainfluenza virus type 3 (bPIV3).

The P gene of bPIV3 as well as SeV expresses the V protein by a process known as RNA editing [11]. The V protein is translated from the edited mRNA, in which additional one G residue is inserted at the editing site during *de novo* viral mRNA synthesis. Since the P protein is translated from a faithful copy of the P gene, N-terminal portion of V and P proteins before the editing site is identical to each other. Another edited mRNA in which two G residues are inserted at the editing site produces the D protein for bPIV3 and the W protein for SeV. Although there is a high degree of sequence homology (~59%) in cysteine-rich extreme C terminus (53 residues) of the V protein of both viruses, there is a great difference in the C-terminal region between the D and W proteins [12]. The D protein consists of the P-V common region (141 aa) plus a long C-terminal region (226 aa), while the W protein is essentially truncated form of the P protein since the W open reading frame (ORF) is terminated by the stop codon shortly after the editing site. Both bPIV3 and SeV express the C protein encoded by means of overlapping ORFs, but there is a lesser degree of sequence homology (~35%) of the C protein, compared with a high degree of sequence homology (~70%) between SeV and human parainfluenza virus type 1 [12]. From these characteristic differences, it is not so clear that the C and V proteins of bPIV3 really function like those of SeV as IFN antagonists. It is even possible that the D protein might participate in the IFN antagonistic functions of bPIV3.

Recent studies on innate immunity have uncovered detailed signaling pathways leading to IFN-β production in response to virus infection [13]. This also prompted us to investigate how bPIV3 and SeV suppress IFN-β production. Intracellular dsRNA produced during viral replication is detected by the cytoplasmic proteins, retinoic acid inducible gene I (RIG-I) [14] and melanoma differentiation-associated gene 5 (MDA5) [15] (Fig. 1). After RIG-I and MDA5 sense intracellular dsRNA, TANK binding kinase 1 (TBK1) and inducible IκB kinase (IKKi) are activated through an adaptor molecule, the IFN-β promoter stimulator (IPS-1) [16–19]. On the other hand, extracellular dsRNA probably released from infected cells with apoptosis is recognized by Toll-like receptor 3 (TLR3) residing in endosomes. This recognition induces activation of TBK1 and IKKi through the adaptor molecule, Toll-interleukin-1 receptor domain-containing adaptor-inducing beta interferon (TRIF). The activated TBK1 and IKKi via these pathways then phosphorylate IRF-3. The phosphorylated IRF3 is dimerized and translocated into the nucleus to activate transcription of the IFN-β gene.

Using information about nucleotide sequences of the newly identified signaling molecules, we here created plasmids that express signaling molecules, RIG-I, MDA5, TRIF, TBK1 and IKKi and examined effect of the bPIV3 and SeV P gene products on IFN-β production induced by these signaling molecules. As a result, we found that the bPIV3 IFN antagonists

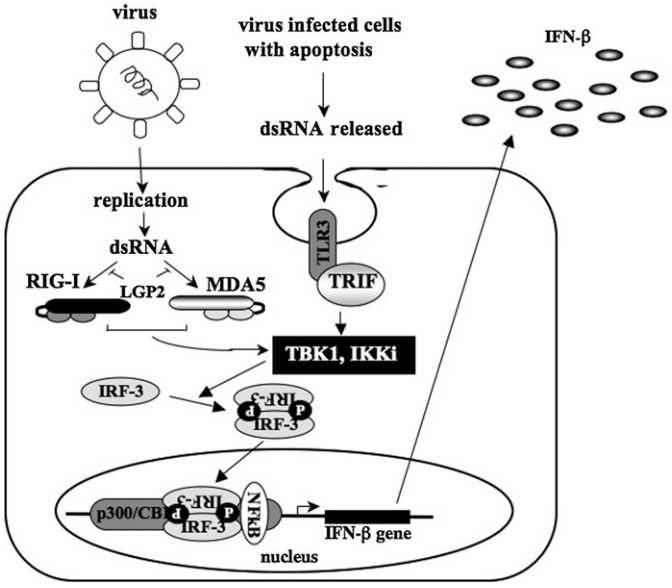

Fig. 1. Signaling pathways that lead to IFN-β production. Both RIG-I and MDA5 detect cytoplasmic dsRNA produced during viral replication, whereas TLR3 recognizes extracellular dsRNA probably released from infected cells with apoptosis. TRIF functions as an adaptor molecule of TLR3. Binding of dsRNA to the dsRNA-sensors, RIG-I and MDA5, results in activation of the kinases, TBK1 and IKKi, which phosphorylate IRF-3. Phosphorylated IRF-3 is dimerized and translocated into the nucleus.

responsible for the suppression of IFN-β production are V and C proteins and demonstrated that the V protein has a specific ability to inhibit MDA5-mediated signaling pathway leading to IFN-β production, whereas the C protein does not target MDA5, but distinct stages for the suppression of IFN-β production.

2. Materials and methods

2.1. Cells, plasmids, and antibodies

HEK293T cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum.

The pIFΔ(-125)lucter [20], a reporter plasmid with a firefly luciferase gene under the control of the human IFN-β promoter, was kindly provided by Dr. S. Goodbourn. The pRL-TK, pRL-SV40, pRL-CMV, and phRL-TK vectors, which express a renilla luciferase under the control of the herpes simplex virus thymidine kinase (TK) promoter, simian virus (SV) 40 promoter, cytomegalovirus (CMV) promoter, and artificial TK promoter, respectively, were purchased from Promega Corp., Madison, WI.

DNA fragments encoding RIG-I, MDA5, TRIF, TBK1, and IKKi were amplified by RT–PCR with total RNA isolated from HeLa cells or IFN-α-treated HeLa cells. The PCR-amplified DNAs were then inserted into the multi-cloning sites downstream of the mammalian EF1α promoter of the pEFneo vector [21]. These expression plasmids were termed pEFneo-RIG-I, pEFneo-MDA5, pEFneo-TRIF, pEFneo-TBK1, and