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Original article

Flavivirus induces interferon-beta gene expression through a pathway involving RIG-I-dependent IRF-3 and PI3K-dependent NF-κB activation

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Abstract

In this study, we found that infection with flaviviruses, such as Japanese encephalitis virus (JEV) and dengue virus serotype 2 (DEN-2), leads to interferon-β (IFN-β) gene expression in a virus-replication- and de novo protein-synthesis-dependent manner. NF-κB activation is essential for IFN-β induction in JEV- and DEN-2-infected cells. However, these two viruses seem to preferentially target different members of the interferon regulatory factor (IRF) family. The activation of constitutively expressed IRF-3, characterized by slower gel mobility, dimer formation, and nuclear translocation, is more evident in JEV-infected cells. Other members of the IRF family, such as IRF-1 and IRF-7 are also induced by DEN-2, but not by JEV infection. The upstream molecules responsible for IRF-3 and NF-κB activation were further studied. Evidently, a cellular RNA helicase, retinoic acid-inducible gene I (RIG-I), and a cellular kinase, phosphatidylinositol-3 kinase (PI3K), are required for flavivirus-induced IRF-3 and NF-κB activation, respectively. Therefore, we suggest that JEV and DEN-2 initiate the host innate immune response through a molecular mechanism involving RIG-I/IRF-3 and PI3K/NF-κB signaling pathways.

Keywords: Flavivirus; Japanese encephalitis virus; Dengue virus, Interferon-β; NF-κB; IRF-3; RIG-I; PI3K

1. Introduction

Interferon-α/β (IFN-α/β), the so-called type I IFN, are produced by eukaryotic cells in response to viral infection, and play a pivotal role in innate immunity directed against viral infection [1,2]. Synthesized IFNs are secreted and act in an autocrine or paracrine manner by binding to specific cell-surface receptors and triggering the Janus kinase-signal transducer and activation of transcription (Jak-Stat) signaling pathway. This results in the induction of cellular proteins that mediate the diverse functions of the IFNs [3,4]. There are about a dozen IFN-α proteins and only one IFN-β protein in humans and mice. IFN-α/β can be further subdivided into two groups: immediate–early-type IFN genes, including IFN-β, human IFN-α1, and murine IFN-α4, the expression of which does not require de novo protein synthesis, and delayed-type IFN genes, which include other IFN-α subtypes and are induced by the upregulation of transcription factors produced after the immediate–early IFN-response [4].

The induction of the immediate–early IFN-β gene occurs primarily at the level of transcription initiation, through the coordinate activation of several preexisting transcription factors [4,5]. Three families of transcription factors participate in the IFN-β induction process. Members of the IFN regulatory factor (IRF) family, namely IRF-3 and IRF-7, bind to the cognate IFN-response element; NF-κB binds to the κB site; and the c-Jun/ATF-2 heterodimer binds to the AP-1 site. Following viral infection, IRF-3, NF-κB, and c-Jun/ATF-2 are posttranslationally activated by inducer-mediated phosphorylation. The cooperative binding of these transcription

Abbreviations: DEN-2, dengue virus serotype 2; dsRNA, double-stranded RNA; IRF, interferon regulatory factor; Jak-Stat, Janus kinase-signal transducer and activation of transcription; JEV, Japanese encephalitis virus; PI3K, phosphatidylinositol-3 kinase; RIG-I, retinoic acid-inducible gene I; TLR-3, Toll-like receptor 3.

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factors, together with the architectural protein of the mammalian high-mobility group, HMG-I(Y) [6,7], results in the assembly of a multicomponent enhanceosome complex on the IFN-β promoter. The enhanceosome then recruits the transcriptional coactivator, CREB-binding protein (CBP)/p300 [8–11], and the RNA polymerase II holoenzyme to enhance the rate of IFN-β transcription [12].

IRF-3 is constitutively expressed in the cytoplasm in the latent form and is regulated by posttranslational modification. Phosphorylated IRF-3 undergoes dimerization, nuclear translocation, and association with the coactivator CBP/p300, then primarily activates the IFN-β promoter [8–11]. Once IFN-β is produced, it binds to its receptor, activates the Jak-Stat signaling pathway, and induces the formation of IFN-stimulated gene factor 3 (ISGF3), which comprises Stat1, Stat2, and IRF-9 [13,14]. Unlike IRF-3, IRF-7 is short-lived and its expression is dependent on activated ISGF3 [15]. Newly synthesized IRF-7 then undergoes virus-induced phosphorylation and activates the IFN-α and IFN-β promoters. Thus, a biphasic IFN-α/β gene induction mechanism is thought to ensure a rapid and effective response in the host upon viral infection [16]. In the early phase, the expression of immediate–early type of IFN is induced through the action of constitutively expressed IRF-3 protein. In the late phase, IRF-3 and IFN-induced IRF-7 cooperate to amplify the induction of IFN-β and other IFN-α family genes [17,18].

The activation of IRF-3 involves the phosphorylation of a stretch of serine (Ser) and threonine (Thr) residues in the C-terminal region of the protein. Recent studies have shown that IKKε and TBK-1 are required for the activation of IRF-3 and the induction of IFN-β expression [19,20]. The binding of double-stranded RNA (dsRNA) to Toll-like receptor 3 (TLR-3) leads to the recruitment of the TIR-domain-containing adaptor, TRIF and the activation of TBK-1 and IKKε [19,20]. The full phosphorylation and activation of IRF-3 by dsRNA also requires the activity of phosphatidylinositol-3 kinase (PI3K) [21]. Intracellular viruses might use a TLR-3-independent mechanism to activate IRF-3 [22]. Retinoic acid-inducible gene I (RIG-I), which encodes an N-terminal region possessing tandem motifs with limited homology to the caspase activation and recruitment domain (CARD) and a downstream DExD/H box RNA helicase domain, is an essential component of the detection system upstream from IRF-3 phosphorylation and is required for IFN-β production in response to Newcastle disease virus (NDV) infection [23]. The helicase domain of RIG-I has also been shown to bind the hepatitis C virus (HCV) 5′ and 3′ untranslated region RNAs to trigger the IRF-3 activation signal [24].

The family *Flaviviridae*, the genome of which is a single-stranded positive-sense RNA, contains three genera, *Hepacivirus*, *Flavivirus*, and *Pestivirus*. Infections with flaviviruses such as yellow fever virus (YFV), dengue virus (DEN), Japanese encephalitis virus (JEV), and West Nile virus (WNV) are emerging worldwide. The noncytopathic biotype of bovine viral diarrhea virus (BVDV), a member of the Pestivirus, does not induce type I IFN in vitro [25] and blocks the induction of IFN by other activators [26], whereas cytopathic BVDV induces type I IFN. In human hepatoma cells, the replication of a subgenomic replicon of HCV stimulates the activation of the IFN-β promoter through an increase in NF-κB binding and IRF-3 nuclear translocation [27]. WNV infection has been shown to induce IFN-β and several IFN-stimulated genes late in the infection of cultured cells, due to the delayed activation of IRF-3 [28]. High levels of IFN-α have been detected in patients with dengue fever or dengue hemorrhagic fever [29] and in cell cultures infected with DEN or cocultured with DEN-infected cells [30,31]. IFN-α activity was also detected in the plasma and cerebrospinal fluid specimens from patients with Japanese encephalitis [32]. However, the molecular mechanisms that trigger the induction of the type I IFN gene by JEV and DEN are largely unknown.

In the present study, we investigated the IFN-β induction mechanisms involved in infections of JEV and DEN serotype 2 (DEN-2). We demonstrate that JEV infection leads to IFN-β gene expression through an IRF-3- and NF-κB-mediated pathway. The replicative virus, but not the UV-inactivated virus, activates IRF-3 phosphorylation, dimerization, and translocation into the nucleus to drive IFN-β gene transcription. In DEN-2 infection, other members of the IRF family, such as IRF-1 and IRF-7, are induced in addition to IRF-3. The importance of different IRF-3 regions involved in JEV activation was also studied, and the N-terminal DNA-binding domain and C-terminal Ser/Thr stretch of IRF-3 are required for viral activation. RIG-I appears to be essential for flavivirus-induced IRF-3 activation and both its helicase and its CARD domains are indispensable. Flavivirus-induced NF-κB activation is dependent on cellular PI3K activity, since its specific inhibitor LY294002 greatly reduced the nuclear translocation of NF-κB p65, as well as the induction of IFN-β. Overall, flaviviruses such as JEV and DEN-2 initiate IFN innate immunity signaling through a RIG-I-dependent IRF-3 and PI3K-dependent NF-κB pathway.

2. Materials and methods

2.1. Cell cultures and chemicals

A549, an IFN-responsive human lung carcinoma cell line, was cultured in F-12 medium (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco), and 2 mM L-glutamine. SK-N-SH (American Type Culture Collection [ATCC] HTB-11), a human neuroblastoma cell line, was cultured in minimal essential medium (MEM, Eagle) supplemented with 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, and 10% FBS. Chang Liver cells were cultured in basal medium (Eagle) with Earle's balanced salt solution (BSS), and 10% FBS. African green monkey kidney Vero cells, were cultured in MEM (Eagle) with Earle's BSS, 2 mM L-glutamine, 1.5 g/l sodium bicarbonate, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, and 10% FBS. The chemicals such as MG132, cycloheximide, and LY294002 were purchased from Sigma.