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Borna disease virus P protein inhibits nitric oxide synthase gene expression in astrocytes

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Abstract

Borna disease virus (BDV) is one of the potential infectious agents involved in the development of central nervous system (CNS) diseases. Neurons and astrocytes are the main targets of BDV infection, but little is known about the roles of BDV infection in the biological effects of astrocytes. Here we reported that BDV inhibits the activation of inducible nitric oxide synthase (iNOS) in murine astrocytes induced by bacterial LPS and PMA. To determine which protein of BDV is responsible for the regulation of iNOS expression, we co-transfected murine astrocytes with reporter plasmid iNOS-luciferase and plasmid expressing individual BDV proteins. Results from analyses of reporter activities revealed that only the phosphoprotein (P) of BDV had an inhibitory effect on the activation of iNOS. In addition, P protein inhibits nitric oxide production through regulating iNOS expression. We also reported that the nuclear factor kappa B (NF-κB) binding element, AP-1 recognition site, and interferon-stimulated response element (ISRE) on the iNOS promoter were involved in the repression of iNOS gene expression regulated by the P protein. Functional analysis indicated that sequences from amino acids 134 to 174 of the P protein are necessary for the regulation of iNOS. These data suggested that BDV may suppress signal transduction pathways, which resulted in the inhibition of iNOS activation in astrocytes.

Keywords: Borna disease virus; Nitric oxide synthase; Astrocytes; Central nervous system; Phosphoprotein; Gene regulation; NF-kappa B

Introduction

Borna disease virus (BDV), a nonsegment negative-strand RNA virus, belongs to the family *Bornavirida*, characterized by low productivity, neurotropism, and nuclear localization of transcription and replication products (Jordan and Lipkin, 2001; Tomonaga et al., 2002). It has been shown that BDV can cause diseases of central nervous system (CNS) in sheep and horses originally. Recent studies suggested that BDV infection even occurs in a wide range of vertebrate species (Rott and Becht, 1995). Epidemiological researchers have demonstrated a higher prevalence of BDV infection in human psychiatric patients than in controls (Bode and Ludwig, 2003; Miranda et al., 2006). However, more recent reports indicated that BDV may not play significant roles in human health (Wolff et al., 2006; Durrwald et al., 2007).

The phosphoprotein (P) of BDV can bind to the N, X, and L proteins of the virus and regulate the active polymerase complex of BDV (Poenisch et al., 2004; Schneider et al., 2004; Schneider, 2005). It has been reported that P protein can be phosphorated by protein kinase Cϵ and casein kinase II (Schwemmle et al., 1997). The P protein is abundantly expressed in infected animal brains and directly binds to a multifunctional protein (HMGB-1) and inhibits the functions of HMGB-1 in cultured neural cells (Kamitani et al., 2001; Zhang et al., 2003). In addition, expression of BDV P protein resulted in the induction of behavioral and neurological abnormalities in transgenic mice (Kamitani et al., 2003). This viral protein can also counteract TBK-1-dependent IFN-β expression in cells and, hence, the establishment of an antiviral state (Unterstab et al., 2005).

The inducible isoform of nitric oxide synthase (iNOS) is not typically expressed in CNS under physiological conditions, but it

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is expressed in abnormal conditions such as infection, trauma, or in many neurological disorders (Kleinert et al., 2003). It is recently reported that neuronal nitric oxide synthase plays a key role in CNS demyelination (Linares et al., 2006). Glial cells are the major places of iNOS expression and consequently, high-output production of nitric oxide (NO), a product of iNOS, in the CNS. Overproduction of NO may lead to the generation of highly reactive species, such as peroxynitrite and stable nitrosothiols that may cause irreversible cell damage in pathological conditions (Kleinert et al., 2003). Several studies have suggested that NO may be involved in the pathogenesis of various neuroinflammatory/degenerative diseases. Increased concentrations of NO in the CNS are usually attributed to an increase in the iNOS usually produced by inflammatory cells. Transcriptional regulation of iNOS gene is complex and not fully understood, but appears to be regulated in part by NF-κB (Davis et al., 2005).

In animal model, the mechanism of impaired immune responses by the virus has not been fully determined. In this report, we studied the effects of BDV and its P protein on the regulation of iNOS gene. The roles of NF-κB, ISRE, and AP-1 binding elements in the expression of iNOS gene regulated by the P protein were also investigated. The potential molecular mechanisms involved in the effects of the P protein of BDV on the regulation of iNOS gene expression and therefore the production of NO were also discussed.

Results

BDV represses the expression of iNOS mRNA and protein induced by LPS and PMA

In order to investigate the roles of BDV in the regulation of iNOS expression activated by LPS and PMA, we examined the effects of BDV on the activation of iNOS mRNA and its protein in murine astrocytes C6 by RT-PCR and Western blot analyses. Total mRNA and protein extracts were prepared from C6 cells infected with BDV or mock-infected and treated with bacterial LPS and PMA. RT-PCR results showed that the levels of iNOS-specific mRNA were significantly reduced in cells infected with BDV comparing to that of mock-infected cells (Fig. 1A). Similar results were obtained from Western blot analyses of iNOS protein, which showed that BDV inhibited the levels of iNOS protein expression activated by LPS and PMA (Fig. 1B). To ensure BDV protein is expressed in infected cells, the expression status the BDV P gene was used as an indicator. Results indicated that both P gene mRNA (Fig. 1A) and its protein (Fig. 1B) were expressed in C6 cells infected with BDV, but not in mock-infected cells. Results also showed that the levels of β-actin mRNA (Fig. 1A) and its protein (Fig. 1B) remained relatively unchanged in the presence or absence of BDV (Figs. 1A and B, lanes 1 and 2).

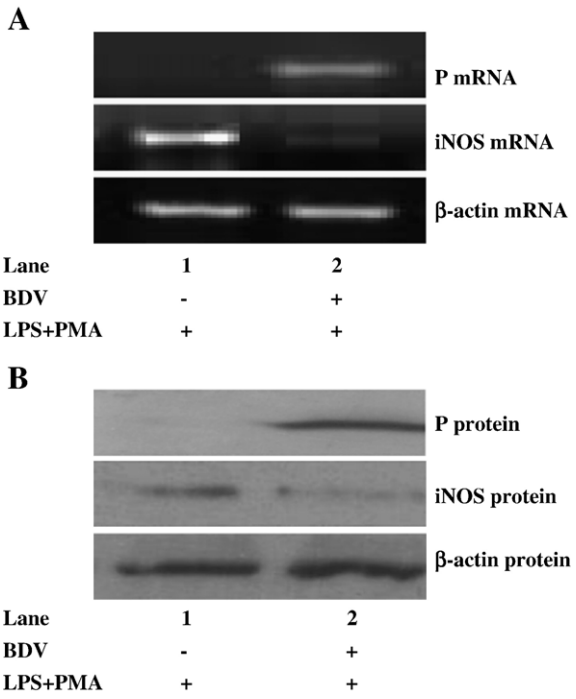

Fig. 1. Determination of the roles of BDV in the regulation of iNOS mRNA and its protein expression. C6 cells infected with BDV strain H1766 or mock infected were treated with LPS and PMA. (A) Total mRNA was prepared from treated C6 cells and used for semi-quantitative RT-PCR using primers specific for P, iNOS, and β-actin genes, respectively. (B) Protein extracts were prepared from treated C6 cells and used for Western blot analysis using antibodies specific to P, iNOS, and β-actin proteins, respectively.

P protein inhibits the activation of iNOS promoter induced by LPS and PMA

To study the effects of BDV proteins on iNOS gene expression, we investigated effects of three major gene products (P, N, and P10) of the virus on the regulation of the iNOS gene promoter activated by LPS and PMA. C6 rat glioma cells were co-transfected with reporter plasmid (pGL-iNOS-Luc) containing the luciferase reporter gene driven by the iNOS gene promoter and control plasmid (pCDNA-3) or three individual plasmids (pCDNA-P, pCDNA-N, pCDNA-P10) expressing the P, N, and P10 protein, respectively. After 24 h post-transfection, cells were treated with bacterial LPS and PMA. For additional 24 h, the effect of each viral protein on the expression of the iNOS gene activated by LPS and PMA was determined by the measurement of luciferase activity in transfected cells. Results revealed that among the three candidate proteins tested, only the P protein of BDV showed significant inhibitory effect on the activation of iNOS induced by LPS and PMA (Fig. 2A).

To determine if the effect of P protein on iNOS promoter expression was specific, C6 cells were co-transfected with the reporter plasmid and plasmid pCDNA-P at different concentrations, respectively. After the treatment of bacterial LPS and PMA for additional 24 h, cells were analyzed for luciferase activity. Results clearly showed that the levels of iNOS promoter activity were gradually decreased as the concentration of P protein increased (Fig. 2B), which indicated that the inhibitory effect of P protein on the activation of iNOS promoter regulated by LPS and PMA was in a P protein concentration-dependent fashion.