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Infectious bursal disease virus infection induces macrophage activation via p38 MAPK and NF-κB pathways

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Abstract

In the present study, we show that infection with infectious bursal disease virus (IBDV) causes activation of macrophages, the key cells involved in inflammatory and immune-regulatory functions. Exposure of cultured spleen macrophages (SM) from SPF chickens to IBDV resulted in the production of nitric oxide (NO). In addition, there was upregulation of mRNA expression of inducible nitric oxide synthase (iNOS), IL-8 and cyclooxygenase-2 (COX-2). The signal transduction pathways involved in macrophage activation were examined. The role of mitogen-activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB) was tested by using specific pharmacological inhibitors. Addition of p38 MAPK inhibitor, SB-203580 and NF-κB inhibitor Bay 11-7082, suppressed IBDV-induced NO production and mRNA expression of iNOS, IL-8 and COX-2. The results suggest that IBDV uses cellular signal transduction machinery, in particular the p38 MAPK and NF-κB pathways, to elicit macrophage activation. The increased production of NO, IL-8 and COX-2 by macrophages may contribute to bursa inflammatory responses commonly seen during the acute IBDV infection.

Keywords: IBDV; p38MAPK; NF-κB; Proinflammatory cytokines; COX-2

1. Introduction

Infectious bursal disease is one of the most important naturally occurring viral diseases of commercial chickens worldwide (Lukert and Saif, 2003). The causative agent, IBDV, belongs to the family Birnaviridae. This virus causes an acute, highly contagious and immuno-suppressive disease in chickens (Lukert and Saif, 2003). The virus infects and destroys actively dividing IgM-bearing B cells in the bursa of fabricius (Hirai et al., 1981; Rodenberg et al., 1994).

Although B cells are the principal targets for IBDV, recent data show that the virus also infects and possibly replicates in macrophages. Infection with IBDV causes production of proinflammatory mediators and cytokines in the macrophages which peak during active virus replication. The production of proinflammatory cytokines by macrophages correlates with the extensive inflammatory response in the bursa (Khatri et al., 2005; Kim et al., 1998; Palmquist et al., unpublished results).

Macrophages constitute a major component of innate immunity against infection, particularly against virus infections. Macrophages internalize the pathogens into intracellular compartments via endocytosis and destroy them. The enzymolysis of exogenous particles in the acidic environment of endosomal–lysosomal vesicles finally leads to the induction of various cellular responses (Bidani and Heming, 1995; Geisow et al., 1981; Mellman et al., 1986). At the local sites of infection, macrophages induce proinflammatory responses aiming at the elimination of invading pathogens and infected cells. During virus infection, the activated macrophages secrete various mediators, such as proinflammatory cytokines, including interleukin-1β (IL-1β) and IL-6; chemokines; nitric oxide (NO) (Glass et al., 2003; Heitmeier et al., 1998; Maggi et al., 2000). Signaling pathways mediated by the mitogen-activated protein kinase (MAPK) superfamily have been shown to play pivotal roles in regulating cellular activation and associated functions (Garrington and Johnson, 1999). For mammalian cells, several different subgroups of the MAPK subfamily have been identified, such as

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the extracellular signal-regulated kinases 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK) and p38 MAPK (Garrington and Johnson, 1999; Kyriakis and Avruch, 2001).

A number of stimuli including lipopolysaccharide (LPS), osmotic shock and growth factors, viral infections and double stranded RNA can activate the p38 MAPK pathway (Chen et al., 2000; Derijard et al., 1995; Han et al., 1994; Hashimoto et al., 2000). Activation of this kinase leads to the induction of various inflammatory cytokines, initiation of innate immune responses and activation of the adaptive immune lymphocytes (Dong et al., 2002; Fearns et al., 2000; Hedges et al., 2000; Lee et al., 1994).

Another well-studied signaling molecule that has been reported to activate many genes, including those for inflammatory cytokines is NF-κB. Previous reports in mammals have shown that viral infections and dsRNA are potent activators of NF-κB (Chen et al., 2000; Imani et al., 1999; Kumar et al., 1994; Rager et al., 1998; Uetani et al., 2000). We, therefore, considered it important to determine if similar activation of NF-κB also occurs in chicken macrophages in response to IBDV infection.

Our main objective in this study was to examine the role of the p38 kinase and NF-κB pathways on NO production and iNOS, COX-2 and IL-8 mRNA expression in chicken macrophages in response to infection with IBDV. Our data show that IBDV induces the production of NO, iNOS, COX-2 and IL-8 that are important mediators of macrophage activation. Further we present evidence that NO, iNOS, COX-2 and IL-8 synthesis is inhibited by pharmacological inhibitors of p38 kinase and NF-κB activity. These results provide evidence that the virus-induced macrophage activation occurs through activation of p38 and NF-κB pathways. Understanding the cellular signaling pathways and factors leading to macrophage gene expression in response to IBDV infection may provide new therapeutic strategies for the treatment of inflammation and extensive bursal destruction associated with IBDV infections.

2. Materials and methods

2.1. Cells and cell lines

Spleens were obtained from 3- to 6-week-old SPF chickens (HyVac., Ames, Iowa). Leukocytes suspension was prepared from spleen by density gradient centrifugation over Ficoll-Hypaque (gradient density 1.090) and washed twice in cold RPMI 1640 (Gibco, Carlsbad, CA). The cells were suspended in complete RPMI medium (RPMI 1640 medium, 5% heat inactivated fetal bovine serum (FBS), antibiotics (100 U penicillin/ml and 100 μg streptomycin/ml) and 2 mM L-glutamine. To obtain the macrophages from leukocyte suspension, cells were cultured in Petri dishes at 41 °C in 5% CO_2. After 2 h, non-adherent cells were removed by gently washing the Petri dishes three times with warm RPMI. The adherent cells were isolated by gentle scrapping with cell scrapper and the viable cells were counted by the trypan blue dye exclusion method. SM thus obtained were cultured for 7 days in RPMI medium containing 10% chicken serum, 10% FBS, antibiotics (100 U penicillin/ml and 100 μg streptomycin/ml) and 2 mM L-glutamine at 41 °C, 5% CO_2.

Cells of a macrophage line, NCSU (Qureshi et al., 1990) (American Type Culture Collection, Manassas, VA), were cultured in flasks according to instructions provided using L-15: McCoy's 5A (1:1) growth medium (invitrogen) containing 10% chicken serum, 10% FBS, antibiotics (100 U penicillin/ml and 100 μg streptomycin/ml) and 2 mM L-glutamine. DF1 cells, a chicken embryo fibroblast cell line, were a gift from Dr. Doug Foster, Department of Animal Sciences, University of Minnesota (Himly et al., 1998). DF1 cells were used for virus isolation and titration. The cells were grown in DMEM supplemented with 10% FBS, antibiotics (100 U penicillin/ml and 100 μg streptomycin/ml) and 2 mM L-glutamine.

2.2. Virus and virus propagation

Virulent IM-IBDV (Winterfield et al., 1972) was passaged 12 times in NCSU cells (Khatri et al., unpublished results). A stock solution of the virus was prepared as follows: NCSU cells were infected with the 11th serial passage of IBDV. After 72 h, the virus infected cultures were freeze thawed three times. The cellular debris was cleared by centrifugation and supernatant was aliquot and stored at −80 °C.

The NCSU cells passaged virus had a titer of 5×10^6 $TCID_{50}$/ml in DF1 cells. The virus was non-pathogenic for chickens but was highly immunogenic and induced high antibody titers and protected chickens against challenge with virulent IBDV (Khatri et al., unpublished results).

2.3. Virus infection of cells

SM (5×10^5 cells/well) were cultured in 24-well plates before infection with IBDV. Each well received 5×10^5 $TCID_{50}$ of IBDV (MOI of 1). After 2 h incubation at 37 °C, the virus inoculum was aspirated and replaced with fresh medium and incubation of cultures at 41 °C was continued. Two to four replicate cultures were set up for each treatment or time point, and replicates were pooled at time of harvest to provide sufficient sample to run multiple analyses.

2.4. Activation of p38 MAPK and NF-κB in macrophages infected with IBDV

Activation of p38 MAPK and NF-κB in SM infected with IBDV was measured by immunocytochemistry method. In brief, endogenous peroxidases were blocked for 15 min with methanol–H_2O_2, and after several washes unspecific-binding sites were blocked for 1 h with normal horse serum. Samples were then incubated overnight in a humidified chamber at 4 °C with the primary antibody against p38 MAPK (Cell Signaling tech, MA) and NF-κB (Santa Cruz, CA) diluted 1:200. This was followed by incubation with biotinylated antibody for 1 h, and the avidin–biotin complex (ABC Kit, Vector Laboratories) for 1 h. The reaction was developed with 0.05% diaminobenzidine and 0.03% H_2O_2. Negative controls were carried out by omission of the primary antibody in the overnight incubation.