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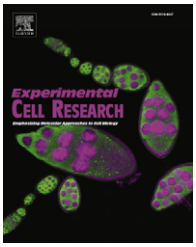

Research Article

Involvement of JNK and NF-κB pathways in lipopolysaccharide (LPS)-induced BAG3 expression in human monocytic cells

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ABSTRACT

Lipopolysaccharide (LPS) is an outer-membrane glycolipid component of Gram-negative bacteria known for its fervent ability to activate monocytic cells and for its potent proinflammatory capabilities. Bcl-2-associated athanogene 3 (BAG3) is a survival protein that has been shown to be stimulated during cell response to stressful conditions, such as exposure to high temperature, heavy metals, proteasome inhibition, and human immunodeficiency virus 1 (HIV-1) infection. In addition, BAG3 regulates replication of Varicella–Zoster Virus (VZV) and Herpes Simplex Virus (HSV) replication, suggesting that BAG3 could participate in the host response to infection. In the current study, we found that LPS increased the expression of BAG3 in a dose- and time-dependent manner. Actinomycin D completely blocked the LPS-induced BAG3 accumulation, as well as LPS activated the proximal promoter of BAG3 gene, supported that the induction by LPS occurred at the level of gene transcription. LPS-induced BAG3 expression was blocked by JNK or NF-κB inhibition, suggesting that JNK and NF-κB pathways participated in BAG3 induction by LPS. In addition, we also found that induction of BAG3 was implicated in monocytic cell adhesion to extracellular matrix induced by LPS. Overall, the data support that BAG3 is induced by LPS via JNK and NF-κB-dependent signals, and involved in monocytic cell-extracellular matrix interaction, suggesting that BAG3 may have a role in the host response to LPS stimulation.

Introduction

Lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria, which makes it prime target for recognition by the immune system. Exposure of host cells to LPS can induce an inflammatory response. Upon stimulation, LPS binding to the CD14-Toll-like receptor 4 (TLR4) complex triggers dimerization and structural changes in the receptor that led to activate downstream signaling. Signaling from TLR4 is transduced through nuclear factor-κB (NF-κB) pathway and a series of mitogen-activated protein kinases (MAPKs), such as extracellular signal regulated kinase (ERK)1/2, p38, stress-activated protein kinase/Jun N-terminal kinase (SAPK/JNK) [1,2]. Subsequently, those signal pathways finally lead to activation of transcription factors, which in turn initiates the transcription of a range of pro- and anti-inflammatory molecules [3,4]. The host defense response to

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LPS includes expression of a variety of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). Thus, LPS may be important in a number of inflammation diseases and is commonly used as a model for inflammation.

BAG3 belongs to the family of co-chaperones proteins that share the Bcl-2 associated athanogene (BAG) domain [5]. Expression of BAG3 has been reported to be stimulated to protect cells from some stressful stimuli, such as heat shock, heavy metal exposure, and some chemotherapeutic drugs [6–11], consistent with a role in anti-stress responses. Upon HIV-1 infection, BAG3 expression is reported to be elevated in human lymphocytes and fetal microglial cells [12,13]. In addition, BAG3 positively regulates Varicella–Zoster Virus (VZV) as well as Herpes Simplex Virus (HSV) replication [14,15], suggesting that induction of BAG3 may be a part of the host cell response to infection.

Monocytes/macrophages infiltrate most inflammatory tissues and are crucial contributors to inflammatory reactions because of their ability to secrete cytokines, present antigens, phagocytize foreign particles and release proteolytic enzymes and oxygen radicals. Immune cell attachment to the extracellular matrix and migration into the affected tissues is an important aspect of the inflammatory response against LPS exposure [16,17]. In addition, accumulating evidences have supported the regulatory role of BAG3 in cell adhesion to the extracellular matrix [18–20]. In the current study, we demonstrated for the first time that LPS induced BAG3 expression at the transcriptional level via JNK and NF-κB pathways in human monocytes. In addition, using siRNA to suppress BAG3 induction, we found that BAG3 was implicated in monocytic cell adhesion to fibronectin, a matrix glycoprotein highly expressed in injured tissues [16,21,22].

Materials and methods

Cell culture

Human monocytic leukemia U937 and THP-1 cells were maintained in RPMI 1640 (Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum in a humidified incubator with 5% CO_2.

Isolation of human monocytes

For the purification of monocytes, whole blood was collected from healthy donors. First, peripheral blood mononuclear cells (PBMC) were obtained by density centrifugation using Ficoll. Briefly, 10 ml of peripheral blood was centrifuged at 400×*g* for 7min at room temperature and the plasma phase was removed. The remaining sample was diluted with an equal volume of phosphate buffered saline (PBS) and overlaid on 12.5 ml Ficoll–Paque™ solution (Amersham Biosciences, Freiburg, Germany). The subsequent Ficoll gradient centrifugation (800×*g*, 25min, without brake) was performed at room temperature. The PBMC cells collected from the interface were washed three times with HBSS, and allowed to aggregate in the presence of fetal calf serum (FCS). T lymphocytes were removed by resetting with neuraminidase-treated sheep erythrocytes, followed by Ficoll–Paque separation. After further purification by resetting technique and density centrifugation, recovery of highly purified monocytes (85–90%), as assessed by FCAS analysis, was obtained. Human monocytes were resuspended in RPMI1640 medium supplemented with penicillin–streptomycin.

Reagents

Actinomycin D and lipopolysaccharide (LPS) from *Escherichia coli* 055:B5 were obtained from Sigma (Saint Louis, MO). Wortmannin, PD98059, SB203580 and SP600125 were purchased from Calbiochem (La Jolla, CA).

RNA isolation and real-time reverse transcription polymerase chain reaction (RT-PCR)

RNA isolation and real-time RT-PCR was performed as previously reported [23]. For BAG3, the forward primer was 5′-ATGCAGCGATTCCGAACTGAG-3′ and the reverse was 5′-AGGATGAGCAGTCAGAGGCAG-3′, the amplicon size is 191 base pair (bp). For β-actin, the forward primer was 5′-GAGACCTTCAACACCCCAGCC-3′ and the reverse was 5′-GGATCTTCATGAGGTAGTCAG-3′, the amplicon size is 205 bp. Results were normalized against those of β-actin.

Luciferase assay

The 5′-flanking region of human BAG3 genomic DNA between −1556 and +5 (+1 represents the translation start site) and serial 5′-deleted BAG3 promoter fragments was constructed as previously reported [11]. The luciferase activity was determined using the Dual-Luciferase® Reporter Assay System (Promega, Madison, WI), according to the manufacturer's instructions. All transfection experiments were repeated for three times in triplicate. Firefly (*Photinus pyralis*) luciferase activities normalized by *Renilla* (*Renilla reniformis*) activities are presented as fold induction relative to the normalized firefly luciferase activity in cells transfected with the pGL4 empty vector only, which was taken as 1.0.

Transfection of dominant negative mutants of IκB-α or JNK1

Dominant negative mutants of pCMV-IκB-α vector and empty vector were purchased from Clontech (Mountain View, CA). The dominant negative JNK1 (DN-JNK1) vector was generated as previously reported [24]. U937 cells were transfected by a FuGene HD transfection reagent (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

Chromosomal immunoprecipitation (ChIP) assay

ChIP assays were performed as previously reported using a kit from Upstate Biotechonology Inc. (Lake Placid, NY) according to the supplied protocol [11]. Real-time PCR was performed using primers specific for the BAG3 sequence between −233 and +5 to generate a 238 bp amplification product. A standard curve was prepared using serial dilutions of the pBAG3/-233 promoter construct. The amount of BAG3 promoter that was present in the immunoprecipitation and input fractions was calculated from the standard curve. The input represents 1% of the material used in the immunoprecipitation assay. The immunoprecipitation/input ratio of the vehicle-treated sample was considered as 100%, and the immunoprecipitation/input ratio of the LPS-treated sample was expressed as a percentage of the untreated.

Cell adhesion assay

Cell adhesion was analyzed using Fibronectin Cell Adhesion Assay (ScienCell™ Research Laboratories) according to the supplied