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B cell activating factor (BAFF) gene promoter activity depends upon co-activator, p300

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Abstract

B-cell activating factor (BAFF) plays a critical role for mature B cell generation and maintenance. We have previously described that mouse BAFF (mBAFF) transcript expression was increased by toll-like receptor 4 (TLR4) agonist, lipopolysaccharide (LPS), through reactive oxygen species (ROS) production and NF-κB activation. Here, we investigated whether mBAFF expression could be regulated by promoter activation through the cooperation of NF-κB and p300, co-activator to various transcription factors. We cloned mBAFF promoter into luciferase-expressing pGL3-basic vector and computer-analyzed its NF-κB binding motif. Due to the existence of NF-κB binding motifs, activity in 2.0 kb mBAFF promoter was higher than that in 1.0 or 0.5 kb mBAFF promoter. When Raw 264.7 murine macrophages were stimulated with LPS, 2.0 kb mBAFF promoter activity was increased time dependently. Serum deprivation (0.5% FBS) producing ROS and exogenous H_2O_2 treatment also enhanced mBAFF promoter activity, which was reduced by *N*-acetyl-L-cysteine (NAC), a well-known ROS scavenger. LPS and serum-starved ROS production increased NF-κB activation. mBAFF promoter activity was augmented by co-transfection with p65 and/or co-activator, p300. It was inhibited by dominant negative (DN) p300. Binding of p300 to BAFF promoter was detected by chromatin immunoprecipitation (ChIP) assay. Data suggest that mBAFF expression could be regulated by promoter activation through NF-κB activation, which might be dependent on the cooperation with co-activator, p300.

Keywords: BAFF promoter; NF-κB; ROS; p300; LPS

Introduction

B-cell activating factor (BAFF) plays a role in B cell maturation and maintenance. BAFF is produced by macrophages or dendritic cells (DCs) upon stimulation with lipopolysaccharide (LPS) or interferon-γ. Its biological role is mediated by the specific receptors, B-cell maturation antigen (BCMA), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and BAFF receptor (BAFF-R) (Mackay et al., 2003; Mak and Yeh, 2002). BAFF is

Abbreviations: BAFF, B cell activating factor belongs to TNF-α family; FBS, fetal bovine serum; LPS, lipopolysaccharide; NAC, *N*-acetyl-L-cysteine; NF-κB, nuclear factor kappaB; ROS, reactive oxygen species; TLR, toll-like receptor; mBAFF, mouse BAFF

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associated with autoimmune disorders and induces rheumatic arthritis in a transgenic mouse model (Mackay et al., 1999; Schneider et al., 1999). In addition, BAFF induces class-switch DNA recombination to produce IgG, IgA, and IgE (He et al., 2003; Litinskiy et al., 2002), which is in relation to allergic disorder, such as asthma (Corrigan, 2004; Kang et al., 2006; Ostergaard, 1985; Rackeman, 1947). BAFF also plays a role in myeloma and lymphoma cell survival, growth, and adhesion (Fu et al., 2006; He et al., 2004; Tai et al., 2006).

BAFF expression was in part regulated by ROS generated by LPS, TLR4 agonist, stimulation (Moon et al., 2006). That was supported by NF-κB activation as judged by reduced IκB degradation and increased nuclear translocation of p65/RelA with LPS stimulation, serum deprivation and Prx II deletion. Data suggest that TLR4-mediated BAFF expression was increased by ROS and it was inhibited by Prx II controlling ROS production (Moon et al., 2006). Other reports showed that human BAFF gene was regulated by NF-κB activation in human non-Hodgkin cell lymphoma and multiple myeloma (Fu et al., 2006; Tai et al., 2006). The activity of many inducible transcription factors is regulated through their interaction with cellular co-activators such as CBP (CREB-binding protein)/p300 (Janknecht and Hunter, 1996a, b). Co-activator molecules are believed to link enhancer-bound transcription factors with the general transcriptional machinery, and CBP/p300 has been linked to transcriptional regulation by several transcription factors including NF-κB (Gerritsen et al., 1997; Perkins et al., 1997). However, it is unknown whether BAFF promoter activation could be regulated by cooperation with p300, co-activator.

Here, we investigated whether promoter activation was involved in the transcriptional up-regulation of mouse BAFF (mBAFF) gene expression and whether BAFF promoter activation could be regulated by p65, an NF-κB family protein, through cooperation with p300. To analyze the molecular mechanisms on mBAFF gene expression through promoter activation, we have, in a first step, cloned and analyzed the promoter activity of mBAFF gene. NF-κB binding sites exist in mBAFF promoter. Our data showed that p65-mediated mBAFF promoter activation was dependent on the presence of co-activator, p300. It suggests that BAFF expression could be up-regulated by the cooperation of transcription factor, p65 and co-activator, p300.

Materials and methods

Reagents

Bacterial LPS from *Escherichia coli* 055:B5 (Sigma; L2880) was resuspended in serum-free medium at a concentration of 1 mg/ml and stored at −20 °C. *N*-actylcysteine (NAC) was purchased from Sigma Chemical Company (St. Louis, MO). Except where indicated, all other materials are obtained from the Sigma chemical company (St. Louis, MO). Plasmids of p300 and dominant negative (DN) p300 was kindly provided by Dr. Jeong-Hyung Lee, Kangwon National University, Korea.

Cloning BAFF promoter

The mBAFF promoter (2.0 kb) upstream sequence (AF119383) from the starting codon (ATG) was obtained from Genebank database (AC 138397). Primers were designed from the sequence; forward primer including a *Sac*I site (gga gct caa tact aat agg aca ggg) and reverse primer including a *Xho*I site (ctc gag tcc acg agg agc act t); 2.0 kb upstream sequence was amplified from C57BL/6 mouse chromosomal DNA by PCR. It was cloned into the site between *Sac*I and *Xho*I of pGL3 plasmid that contains firefly luciferase (pGL3-mBAFF-Luc). The shorter size (1.0 and 0.5 kb) of mBAFF promoter from starting codon was also cloned into the same vector. Each product was sequenced and matched to Genebank data.

Cell cultures

Raw264.7 murine macrophages were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (Gibco), 2 mM L-glutamine, 100 U/mL penicillin, and 100 Units/mL streptomycin (Sigma Chemical Company, St. Louis, MO). For the measurement of promoter activity, 2×10^5 cells were plated on 6-well culture dishes (Corning) 24 h prior to transfection of pGL3-mBAFF-Luc. Cultures were treated with either LPS or H_2O_2 for an appropriate time of 18 h after the transfection.

Measurement of mBAFF promoter activity

Raw264.7 cells were transfected with pGL3-mBAFF-Luc and pcDNA-lacZ for monitoring transfection efficiency by β-galactosidase assay. Luciferase activity was determined by incubating cell extracts with luciferase substrate (Promega). Luminescence was measured using a luminometer (Perkin Elmer, Wellesely, MA). Luciferase units of experimental vector were normalized to the control vector in each sample.

RT-PCR

Spleen cell suspension was prepared by the above method. RNA was isolated from cells using TRIZOL (Invitrogen, Carlsbad, CA). cDNA was synthesized