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Novel transcriptional regulation of the schlafen-2 gene in macrophages in response to TLR-triggered stimulation

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Abstract

Schlafen-2 (slfn-2) is a member of slfn family, regulators of T cell development and its expression is altered during infection by microbial pathogens. However, the molecular mechanism involved in slfn expression is still to be determined. In this study, we isolated slfn-2 as a LPS-induced differentially expressed genes (DEGs) in RAW 264.7 cells and examined expression and regulation of slfn-2 in CpG-DNA-treated and LPS-treated macrophages. We defined a transcriptional start site in the slfn-2 gene. To examine the promoter organization of the slfn-2 gene, we cloned a ~1.8 kb region upstream of the transcription start site. Sequence analysis indicates consensus sites for AP-1 and NF-κB. Comprehensive mutant analyses, ELISA-based transcription factor activation assay, and ChIP assays reveal that functional interaction of AP-1 and NF-κB with the promoter element is necessary for the Toll-like receptor (TLR)-mediated slfn-2 gene expression by CpG-DNA and LPS treatment in macrophages. In summary, we identified a slfn-2 promoter for the first time and demonstrated that CpG-DNA and LPS triggers slfn-2 gene expression by activating NF-κB and AP-1 pathways in macrophages.
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1. Introduction

The immediate recognition of microbial pathogens and their products in the first line of host defense is mediated by an innate immune system that uses conserved pattern recognition receptors to distinguish the pathogen-associated molecular pattern (PAMP) signatures of microbial pathogen components (Janeway and Medzhitov, 1998; Aderem and Ulevitch, 2000). Macrophages mediate a key function of innate immune responses, and can be induced to express a variety of genes involved in immune and inflammatory responses. Macrophages are also involved in phagocytosis and determining whether an acquired immune response is required following pathogenic infection (Aderem, 2001; Dobrovolskaia and Vogel, 2002).

A wide variety of microbial pathogens components (PAMP) including LPS, bacterial DNA, lipoteichoic acid, and peptidoglycan are capable of stimulating innate immune responses (Aderem and Ulevitch, 2000; Akira et al., 2006). The recognition of PAMP by macrophages is mediated by Toll-like receptors (TLRs). Detection of LPS by macrophages is mediated through TLR4, a transmembrane receptor that shares a high degree of homology with TLR9 (Hoshino et al., 1999). Macrophages recognize synthetic oligodeoxynucleotides (CpG-DNA) and bacterial DNA containing unmethylated CpG dinucleotides in

Abbreviations: PAMP, pathogen-associated molecular pattern; CpG-DNA, DNA containing immunostimulatory CpG motifs; IRAK, IL-1R-associated kinase; TRAF, TNF receptor-associated kinase; IKK; IκB kinase; DEG, differentially expressed gene; ACP, annealing control primer; slfn-2, schlafen-2; SAPK, stress-activated protein kinase; MEK, MAPK/ERK kinase

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the context of particular base sequences (CpG motifs) (Krieg et al., 1995; Stacey et al., 1996; Lee et al., 2006a). Immune activation by CpG-ODN depends on TLR9, which determines the specificity of CpG motifs (Hemmi et al., 2000).

Exposure of macrophages to PAMP such as CpG-DNA or LPS results in activation of the myeloid differentiation protein (MyD88)/IL-1R-associated kinase (IRAK) pathway, which is thought to be a downstream effector of TLR9 and TLR4. Activation of the MyD88/IRAK pathway stimulates the transcription factors such as NF-κB and AP-1, and these transcription factors in turn enhance the transcriptional upregulation of genes downstream of the transcription factor binding motifs, which include cytokines (Krieg, 2002; Kwon and Kim, 2003; Lee et al., 2004a), cell adhesion and migration-associated molecules (Krieg, 2002; Rhee et al., 2007), and cell surface molecules (Krieg, 2002; Lee et al., 2006b).

Schlafen-2 (slfn-2) is one of the hundreds of genes whose expression is altered during infection by microbial pathogens (Geserick et al., 2004). Although some light has been shed on the relationship between slfn gene and immune system (Eskra et al., 2003; Schurr et al., 2005; Smith et al., 2006), the manner in which slfn gene expression is regulated in immune cells has yet to be investigated. In the present study, we isolated slfn-2 as a LPS-induced differentially expressed genes (DEGs) in RAW 264.7 cells and examined expression and regulation of slfn-2 in CpG-DNA-treated and LPS-treated macrophages. We identified for the first time a slfn-2 promoter and demonstrated that CpG-DNA and LPS triggers slfn-2 gene expression by activating NF-κB and AP-1 pathways in macrophages.

2. Materials and methods

2.1. Reagents

Phosphorothioate backbone-modified oligodeoxynucleotides were purchased from GenoTech (Daejeon, Korea). The CpG-DNA 1826 consisted of 20 bases that contained two CpG motifs (underlined): TCCATGACGTTCCTGACGTT. The non-CpG-DNA 2041 (CTGGTCTTTCTGGTTTTTTTCTGG) served as a negative control. The *Escherischia coli* LPS (Sigma, St. Louis, MO, USA) was suspended in sterile water and added to the cells. IKK-2 inhibitor BMS-345541 and stress-activated protein kinase (SAPK)/Jun *N*-terminal kinase (JNK) inhibitor SP 600125 were purchased from Calbiochem (San Diego, CA, USA). MAPK/ERK kinase (MEK) inhibitor PD 98059 and p38 inhibitor PD 169316 were purchased from A.G. Scientific, Inc. (San Diego, CA, USA). When the inhibitors were used, the cells were preincubated with SP 600125 for 10 min and with BMS-345541, PD 98059, or PD 169316 for 1 h before stimulation with LPS or CpG-DNA.

2.2. Cell culture

The murine macrophage cell line RAW 264.7 was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin.

2.3. Isolation of primary mouse macrophage from peritoneal cavity

Female BALB/c mice were obtained from Central Lab. Animal Inc. (Seoul, Korea). Animal studies were approved by the institutional animal care and use committee of the Hallym University. Resident peritoneal macrophages were collected from the peritoneal cavity of healthy male C57BL/6 mice by washing with chilled PBS. The macrophages were stimulated with 100 ng/ml of LPS or 3 μg/ml of CpG-DNA 1826 for the indicated time periods.

2.4. Differentially displayed reverse transcription polymerase chain reaction (DDRT-PCR)

To analyze genes that are specifically expressed in inflammation triggered by LPS, we screened LPS-induced differentially expressed genes in RAW 264.7 cells using a differential display PCR method based on annealing control primers (ACPs). After RAW 264.7 cells were treated with LPS (100 ng/ml) or CpG-DNA 1826 (3 μg/ml) for the indicated periods, total RNA was extracted with a total RNA isolation Kit (Active Motif, Carlsbad, CA, USA) according to the manufacturer's instructions. For the first-strand cDNA synthesis and amplification of differentially expressed cDNA, isolated total RNA was reverse-transcribed using dT-ACP1 (5′-CTGTGAATGCTGCGACTACGATXXXXX$(T)_{18}$-3′) primer of a GeneFishing DEG kit (Seegene, Korea) according to the manufacturer's instructions (Hwang et al., 2003). Briefly, 3 μg of total RNA was reverse-transcribed in the first-strand buffer containing 10 μM dT-ACP1 primer, 200 U M-MLV reverse transcriptase, 2 mM dNTP, and 20 U RNase inhibitor. The reaction was conducted at 42 °C for 90 min. After first-strand synthesis, 1 μl of the first-strand cDNA (∼50 ng) was subjected to the standard PCR reaction for 30 cycles by using dT-ACP2 (5′-CTGTGAATGCTGCGACTACGATXXXXX$(T)_{15}$-3′) primer and arbitrary ACP primers. The differentially expressed bands were extracted from the agarose gel and the products were cloned into a TA cloning vector (Promega, Madison, WI, USA). In order to identify the insert DNA, isolated plasmids were sequenced and complete sequences were analyzed by BLAST program at NCBI GenBank.

2.5. RT-PCR analysis

After cells were treated with LPS (100 ng/ml), CpG-DNA 1826 (3 μg/ml) and/or pathway-specific inhibitors for the indicated periods, we performed RT-PCR analysis as described elsewhere (Lee et al., 2006b). One microliter of the cDNA mixture was subjected to the standard PCR reaction for 25 cycles using the following primer sets: slfn-2, 5′-CTCACCTCAGAAAACAGGAGAATGC-3′ (sense), 5′-CAGAAGTGAGTGACAGGCAGCTG-3′ (anti-sense); mouse GAPDH, 5′-ATGGTGAAGGTCGGTGTGAACG-3′ (sense),

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