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Molecular mechanisms of lipopolysaccharide-caused induction of *surfactant protein-A* gene expression in human alveolar epithelial A549 cells

Chi-Yuan Chuang[a,b,1], Ta-Liang Chen[a,c,1], Ruei-Ming Chen[d,e,*]

[a] *Graduate Institute of Clinical Medicine, Taipei Medical University, Taipei, Taiwan*
[b] *Department of Pulmonology, Division of Internal Medicine, Ren-Ai Branch, Taipei City Hospital, Taipei, Taiwan*
[c] *Department of Anesthesiology, Taipei Medical University Hospital, Taipei, Taiwan*
[d] *Graduate Institute of Medical Sciences, Taipei Medical University, Taipei, Taiwan*
[e] *Cell Physiology and Molecular Image Research Center and Department of Anesthesiology, Wan-Fang Hospital, Taipei Medical University, Taipei, Taiwan*

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ABSTRACT

Surfactant proteins (SPs) participate in the physiological and pathophysiological regulation of sepsis-induced acute lung injury. Lipopolysaccharide (LPS), a Gram-negative bacterial outer membrane component, is one of the major causes of septic shock. This study was designed to evaluate the effects of LPS on the regulation of *SP-A* and *SP-D* gene expressions in human alveolar epithelial A549 cells. Exposure of A549 cells to LPS increased SP-A mRNA synthesis in concentration and time-dependent manners without affecting SP-D mRNA production. LPS selectively enhanced translocation of transcription factor c-Jun from the cytoplasm to nuclei, but not nuclear factor kappa-B. In parallel, the DNA-binding activity of AP-1 was increased by LPS. Pretreatment of A549 cells with SP600125, an inhibitor of c-Jun N-terminal kinase, decreased c-Jun translocation, and significantly ameliorated LPS-induced SP-A mRNA production. Levels of toll-like receptor (TLR2) mRNA in A549 cells were time-dependently induced following LPS treatment. Application of TLR2 small interference (si)RNA into A549 cells significantly knocked-down the translation of this receptor, and simultaneously alleviated LPS-induced SP-A synthesis. Taken together, this study has shown that LPS selectively induces *SP-A* gene expression possibly through TLR2-mediated activation of c-Jun in human alveolar epithelial A549 cells.

1. Introduction

Gram-negative septicemia, a complication from acute pulmonary infection, can lead to organ dysfunction or hypoperfusion abnormalities (Angus et al., 2001; Cazzola et al., 2004). Lipopolysaccharide (LPS), a Gram-negative bacterial outer membrane component, has been reported as one of the major causes of septic shock (Raetz et al., 1991; Welbourn and Yong, 1992). During pulmonary infection, surfactant proteins (SPs) participate in the physiological and pathophysiological regulation of sepsis-induced acute lung injury (Mendelson, 2000). In human lung, the LPS-induced acute pulmonary inflammation causes rapid changes in the composition of the surfactant pool and the resident cell population (Mendelson, 2000; Rooney, 2001). Pulmonary alveolar type II epithelial cells, located in the corners of the alveoli, have highly specialized functions for synthesizing, secreting, and reutilizing surfactants (Rooney, 2001). The critical function of pulmonary surfactants, a complex surface-active lipoprotein, is to reduce surface tension at the alveolar air–liquid interface, thereby preventing alveolar collapse upon expiration and allowing for normal breathing (Clements and King, 1976). Alterations in the levels of surfactant components in the lungs during inflammation are quite complex because surfactant lipids, SP-A, and SP-D appear to be regulated through host's defense mechanisms (McIntosh et al., 1996; Crouch and Wright, 2001; LeVine and Whitsett, 2001).

There are four SPs, known as SP-A, -B, -C, and -D. SP-A and -D are hydrophilic and participate in pulmonary host defense (Crouch, 1998; McCormack and Whitsett, 2002). By comparison, SP-B and SP-C are hydrophobic and contribute to the adsorption of surfactant lipids onto the surface film that lines the alveolus (McCormack and Whitsett, 2002). Previous studies have shown that transgenic mice with targeted disruptions of the *SP-A* or *SP-D* genes have increased susceptibility to infection from viral and bacterial pathogens (LeVine et al., 2000; Crouch and Wright, 2001). Levels of SP-A and SP-D can be modulated by pathogens. In acute respiratory distress syndrome associated with either Gram-positive or -negative pneumonia, inflammatory mediator-induced lung dam-

* Corresponding author at: Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, 250 Wu-Xing St., Taipei 110, Taiwan.
Tel.: +886 2 27361661x3222; fax: +886 2 86621119.
E-mail address: rmchen@tmu.edu.tw (R.-M. Chen).
[1] These authors contributed equally to this work.

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Table 1
Effects of lipopolysaccharide on viability of A549 cells.

LPS (ng/ml)	Cell viability, OD values at 550 nm			
	1 h	6 h	16 h	24 h
0	0.62 ± 0.05	0.79 ± 0.05	0.58 ± ± 0.03	0.73 ± 0.03
1	0.59 ± 0.07	0.84 ± 0.02	0.60 ± 0.02	0.71 ± 0.02
10	0.60 ± 0.07	0.82 ± 0.02	0.63 ± 0.03	0.83 ± 0.05
100	0.55 ± 0.15	0.75 ± 0.02	0.60 ± 0.02	0.78 ± 0.01
1000	0.72 ± 0.08	073 ± 0.03	0.57 ± 0.03	0.54 ± 0.01*

A549 cells were exposed to 1 ng/ml, 10 ng/ml, 100 ng/ml, and 1000 ng/ml lipopolysaccharide (LPS) for 1 h, 6 h, 16 h, and 24 h, respectively. Cell viability was analyzed using a colorimetric method. Each value represents mean ± SEM for $n = 6$.
* Values significantly differ from the respective control, $p < 0.05$.

age reduces the amounts of SP-A in the bronchiolar lavage fluid (Gunther et al., 1996, 2001). Regulation of *SP* gene expression by pathogens is involved via signal-transducing events. Toll-like receptor 2 (TLR2), which is a type I transmembrane protein with extracellular domains comprised largely of leucine-rich repeats and intracellular signaling domains, can be expressed in alveolar epithelial type II cells and macrophages (Droemann et al., 2003). TLR2 can transduce pathogen-triggered signals to regulate certain inflammation-related gene expressions through the activation of transcription factors (Ransone and Verma, 1990; Shaulian and Karin, 2002). Activator protein-1 (AP-1) and nuclear factor-kappa B (NFκB) are two typical transcription factors that have responded to TLR2-mediated signals and participated in the regulation of cell proliferation, differentiation, and death (Angel and Karin, 1991; Jochum et al., 2001; Shaulian and Karin, 2002).

SP-A is known to play a central role in surfactant homeostasis and function (Blau et al., 1994). George et al. (2003) reported that repeat exposure to inhaled endotoxin increased the whole lung *SP-A* gene expression, whereas SP-A protein levels in lung lavage fluid decreased (George et al., 2003). Pulmonary alveolar epithelial type II cells are the major sources of SP-A production and secretion (Rooney, 2001). However, the effects of LPS on the regulation of *SPs* gene expressions in alveolar type II epithelial cells are not well known. Therefore, this study was designed to evaluate the effects of LPS on the regulation of *SP-A* and *SP-D* gene expressions and its possible mechanisms using human lung carcinoma type II epithelium-like A549 cells as the experimental model.

2. Materials and methods

2.1. Cell culture and drug treatment

Human lung carcinoma type II epithelium-like A549 cells, purchased from the American Type Culture Collection (Rockville, MD, USA), were grown in DMEM/Ham's F-12 culture medium (Sigma Chemical, St. Louis, MO, USA) with 10% (v/v) heat-inactivated fetal calf serum, 100 U/ml penicillin, 2 mM L-glutamine, and 100 μg/ml streptomycin in 75 cm^2 culture flasks at 37 °C in a humidified atmosphere 5% CO_2. LPS, purchased from Sigma, was dissolved in dimethyl sulfoxide (DMSO) and sonicated to disperse large LPS aggregates as described (Kitchens et al., 2001). The concentration of DMSO in the medium was kept to less than 0.1% to avoid toxicity of this solvent to A549 cells. SP600125, an inhibitor of c-Jun N-terminal kinase, was purchased from Sigma, dissolved in DMSO, and pretreated for 1 h before LPS administration. Control cells received DMSO only.

2.2. Assay of cell viability

Cell viability was determined using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously (Cherng et al., 2008). Briefly, A549 cells (1×10^4 cells/well) were seeded overnight in 96-well tissue

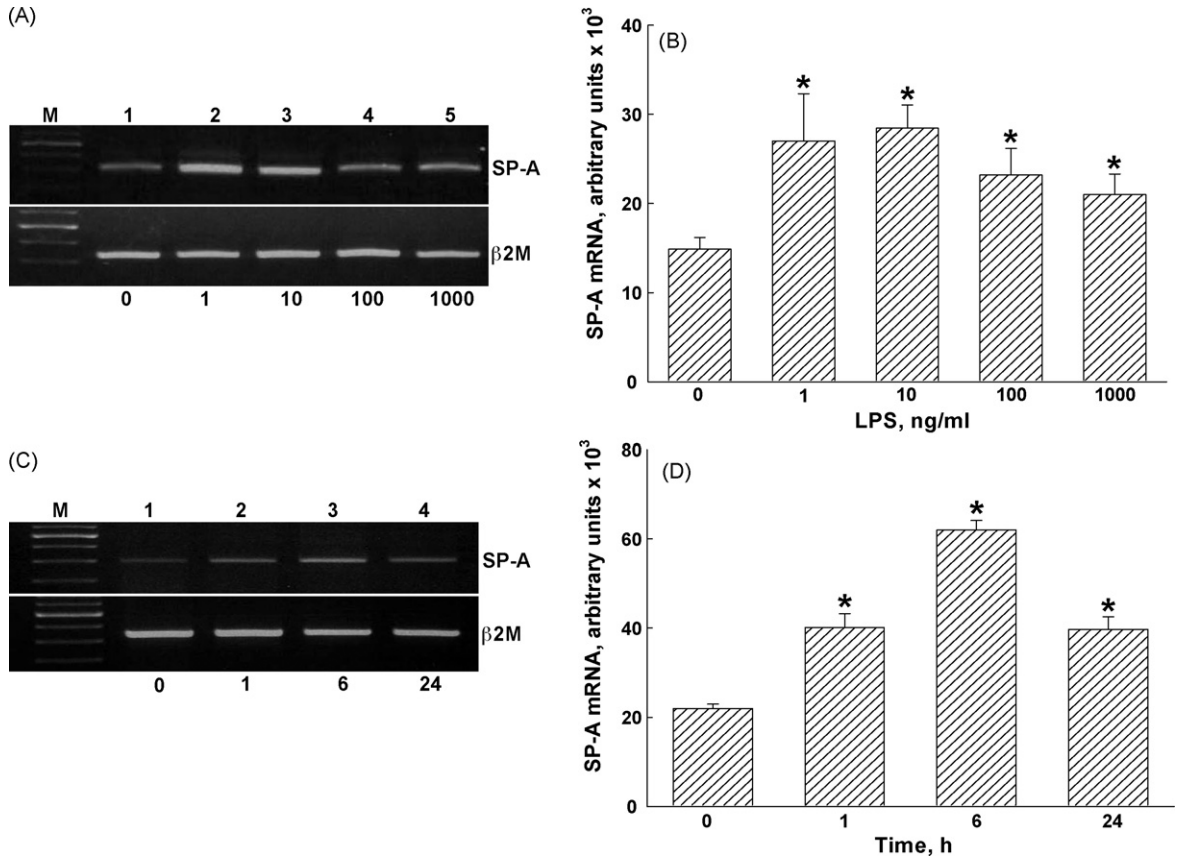

Fig. 1. Effects of lipopolysaccharide (LPS) on surfactant protein-A (SP-A) mRNA production in A549 cells. Exposure of A549 cells to 1 μg/ml, 10 μg/ml, 100 μg/ml, and 1000 μg/ml of LPS for 6 h (A and B), or to 1 μg/ml of LPS for 1 h, 6 h, and 24 h (C and D). Total RNA was prepared for RT-PCR analysis of SP-A mRNA (A and C, *top panels*). Amounts of β2M mRNA were quantified as the internal controls (A and C, *bottom panels*). These DNA bands were quantified and statistically analyzed (B and D). Each value represents the mean ± SEM for $n = 6$. The symbol, *, indicates that a value significantly ($p < 0.05$) differed from the control groups. M, DNA 100 bp marker.