

β -catenin regulates NF- κ B activity and inflammatory cytokine expression in bronchial epithelial cells treated with lipopolysaccharide

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Abstract. In the present study, we demonstrate that lipopolysaccharide (LPS) induces the expression of inflammatory cytokines, including interleukin (IL)-6, IL-8, IL-1 β , tumor necrosis factor (TNF)- α and monocyte chemoattractant protein (MCP)-1 in BEAS-2B human bronchial epithelial cells in a dose- and time-dependent manner. This increase was accompanied by an increased activity of nuclear factor (NF)- κ B. When the expression of β -catenin was analyzed following treatment with LPS, the mRNA level was unaltered; however, the β -catenin protein levels increased with a decrease in phosphorylation at the serine 33/37 residues. Nuclear β -catenin protein levels also increased along with the reporter activity of a β -catenin-responsive TOPFlash vector. To elucidate the regulatory role of β -catenin in the LPS-induced inflammatory response of bronchial epithelial cells, β -catenin production was knocked down using siRNA. Our results revealed that β -catenin protein levels and TOPFlash vector reporter activity were reduced to basal levels by siRNA transfection. In this experimental condition, NF- κ B activity, measured by enzyme-linked immunosorbent assay (ELISA), electrophoretic mobility shift assay (EMSA) and an NF- κ B responsive reporter assay, was reduced to basal levels. Similarly, LPS-induced inflammatory cytokine expression was reduced almost to basal levels following transfection with β -catenin siRNA. These results demonstrate that β -catenin positively regulates NF- κ B activity, as well as the expression

of inflammatory cytokines in the inflammatory response of LPS-treated bronchial epithelial cells.

Introduction

The bronchial epithelium represents the interface between the lungs and air and is known to induce and sustain inflammatory events in respiratory diseases through the production of inflammatory cytokines. Understanding the regulatory mechanisms underlying the bronchial epithelial cell inflammatory response is important for the effective treatment of respiratory diseases, including asthma (1). The human bronchial epithelium is continuously exposed to Gram-negative bacteria of which lipopolysaccharide (LPS) is a glycolipid that constitutes the major portion of the outer membrane (2). High levels of airborne (up to 1 μ g/m³) LPS have been reported in a variety of environments, and LPS as a contaminant in house dust is a factor that increases the severity of asthma (3). LPS also induces the expression of inflammatory cytokines, including interleukin (IL)-8 and IL-6 in bronchial epithelial cells (4). Among the known inflammatory response regulators, nuclear factor (NF)- κ B is the most important, as many genes involved in inflammation have binding sites for NF- κ B in their promoter regions (5).

β -catenin is a member of the WNT/ β -catenin pathway regulating various cellular processes, including proliferation, differentiation and development (6). In our previous study, we reported that a promoter polymorphism of β -catenin that affects its mRNA expression level was significantly associated with the risk of asthma in human subjects, suggesting that β -catenin may be involved in the disease mechanism of asthma (7). It is plausible that β -catenin may modulate the inflammatory response of the bronchial epithelium stimulated by inflammatory inducers. In this study, we investigated whether β -catenin is involved in the regulation of inflammatory cytokine expression, as well as NF- κ B activity in BEAS-2B human bronchial epithelial cells treated with LPS.

Materials and methods

Cell culture. BEAS-2B human bronchial epithelial cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA), and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine

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Abbreviations: IL, interleukin; GSK, glycogen synthase kinase; LPS, lipopolysaccharide; MCP, monocyte chemoattractant protein; PCR, polymerase chain reaction; siRNA, small interfering RNA; TBP, TATA box binding protein; TNF, tumor necrosis factor

Key words: lipopolysaccharide, bronchial epithelial cell, β -catenin, nuclear factor- κ B, cytokine

serum, 100 $\mu\text{g/ml}$ streptomycin and 100 U/ml penicillin at 37°C in 5% CO₂. The cells were treated with 0.001 to 10 $\mu\text{g/ml}$ of LPS (Sigma-Aldrich, St. Louis, MI, USA) to induce an inflammatory response. Untreated cells were used as controls.

Real-time reverse transcription polymerase chain reaction (PCR) of inflammatory cytokines. The cells were cultured in 12-well plates and total RNA was extracted using an RNeasy kit (Qiagen, Hilden, Germany). Total RNA was reverse transcribed using a cDNA Reverse Transcription kit (Applied Biosystems Inc., Foster City, CA, USA). Briefly, the reaction was performed in a final volume of 20 μl that included 100 mM dNTP, random primers, MultiScribe™ Reverse Transcriptase, RNase inhibitor and 1 μg total RNA. The reaction mixtures were heated at 25°C for 10 min, 37°C for 120 min and 85°C for 5 sec. Real-time PCR was performed using a StepOne PCR System (Applied Biosystems, Inc.) in triplicate in a final volume of 20 μl that included TaqMan gene expression master mix, an optimized concentration of each primer, 250 nM TaqMan probe and 2.0 μl cDNA reaction mixture. The reaction mixtures were pre-heated at 95°C for 10 min to activate the enzyme, and then subjected to 40 cycles of melting at 95°C for 15 sec and annealing/extension at 60°C for 1 min. The real-time PCR efficiencies were approximately 100%. The assay-on-demand gene expression products (Applied Biosystems, Inc.) were used to evaluate the mRNA expression levels of IL-6 (Hs00174131_m1), IL-8 (Hs99999034_m1), IL-1 β (Hs01555410_m1), tumor necrosis factor (TNF)- α (Hs01113624_g1), monocyte chemoattractant protein (MCP)-1 (Hs00234140_m1), β -catenin (Hs00170025_m1) and 18S rRNA (Hs99999901_s1). The 18S rRNA was used as an internal control. For each sample, the mRNA levels were normalized against the 18S rRNA level and the ratios of normalized mRNA to the untreated control sample were determined using the comparative Ct method, as previously described (8).

Enzyme-linked immunosorbent assay (ELISA) to measure NF- κ B DNA binding activity. Nuclear protein extracts were prepared from the LPS-treated cells using a nuclear extract kit (Active Motif, Carlsbad, CA, USA) and analyzed to determine the protein concentration using a BCA protein assay kit (Pierce, Rockford, IL, USA). The binding activity of NF- κ B to its target DNA sequence (5'-GGGACTTTCC-3') was measured using a TransAM NF- κ B ELISA kit (Active Motif). Briefly, 10 μg of protein in the nuclear protein extracts were added to 96-well plate wells coated with oligonucleotide containing the target DNA sequence. Following incubation and washing, an anti-NF- κ B antibody was added to the wells followed by a horseradish peroxidase-conjugated secondary antibody.

Electrophoretic mobility shift assay (EMSA) to determine NF- κ B activity. Primer sets containing an NF- κ B target sequence consisting of a forward primer, 5'-AGTTGAGGGGACTTTCCAGGC-3' and a complementary reverse primer, 5'-GCCTGGGAAAGTCCCCTCAACT-3' were biotin-labeled at their 5' end. The forward and reverse primers were annealed by heating at 95°C for 5 min and cooled slowly to room temperature. Subsequently, 10 ng of annealed primer, 8 μg of protein in the nuclear protein extract, and 1 μg of poly d(I-C) were incubated at 15°C for 30 min in a final volume of 10 μl . For competition experiments, 660 ng of unlabeled NF- κ B

probe was added. The reaction mixtures were separated by 6% non-denaturing polyacrylamide gel electrophoresis at 120 V in Tris-Borate-EDTA buffer, and electrotransferred to a nylon membrane at 300 mA for 30 min. The location of the primer-protein complexes was visualized by incubating the membrane with horseradish peroxidase-conjugated streptavidin followed by enhanced chemiluminescence detection.

Reporter assay for NF- κ B and β -catenin. For NF- κ B reporter assay, the cells were co-transfected with a pGL 4.32 vector (Promega, Madison, WI, USA) containing the NF- κ B response element linked to a firefly luciferase reporter gene and a 1:50 ratio of pGL 4.17 vector (Promega) containing *Renilla* luciferase reporter gene. Cells were harvested 24 h after transfection, and luciferase activity was measured using a Dual-Luciferase Reporter Assay System (Promega). For each assay, firefly luciferase activity was normalized to the *Renilla* luciferase activity to control for variations in transfection efficiency.

For the β -catenin reporter assay, the cells were co-transfected with a TOPFlash vector (Millipore, Billerica, MA, USA) containing the β -catenin response element linked to a firefly luciferase reporter gene and a 1:50 ratio of pGL 4.17 vector containing *Renilla* luciferase reporter gene. Luciferase activity was measured as described above.

Western blot analysis of total β -catenin. The cells were lysed with ice-cold RIPA buffer containing 25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS and protease inhibitor cocktail (Sigma-Aldrich). Total cell lysates were obtained after removing the insoluble materials by centrifugation at 20,000 \times g for 20 min at 4°C. The protein concentrations were determined using a BCA protein assay kit (Pierce), and 50 μg protein were separated by 12% polyacrylamide gel electrophoresis and electrotransferred onto nitrocellulose membranes at 150 mA for 1.5 h. The membranes were then blocked for 3 h at room temperature with phosphate-buffered saline containing 5% skim milk and 0.1% Tween-20 and incubated with a 1:1,000-dilution of anti- β -catenin antibody (BD Biosciences, San Jose, CA, USA) overnight at 4°C, and subsequently incubated with a 1:1,000-dilution of horseradish peroxidase-conjugated secondary antibody (Cell Signaling, Beverly, MA, USA) for 2 h at room temperature. Peroxidase activity was visualized using an ECL kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Anti- β -actin antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was used as the loading control for total cell lysates.

Western blot analysis of nuclear β -catenin. The cells were harvested and then nuclear fractions were collected using a nuclear extract kit (Active Motif). Protein concentrations in each fraction were determined using a BCA protein assay kit (Pierce). Protein (15 μg) was separated using a 12% polyacrylamide gel electrophoresis and analyzed by western blot analysis using an anti- β -catenin antibody followed by horseradish peroxidase-conjugated anti-mouse secondary antibody. Anti-TATA box binding protein (TBP) was used as the loading control for nuclear protein extracts.

Transfection with small interfering RNA (siRNA). The cells were seeded in 12-well plates at a density of 5 \times 10⁵ cells/well

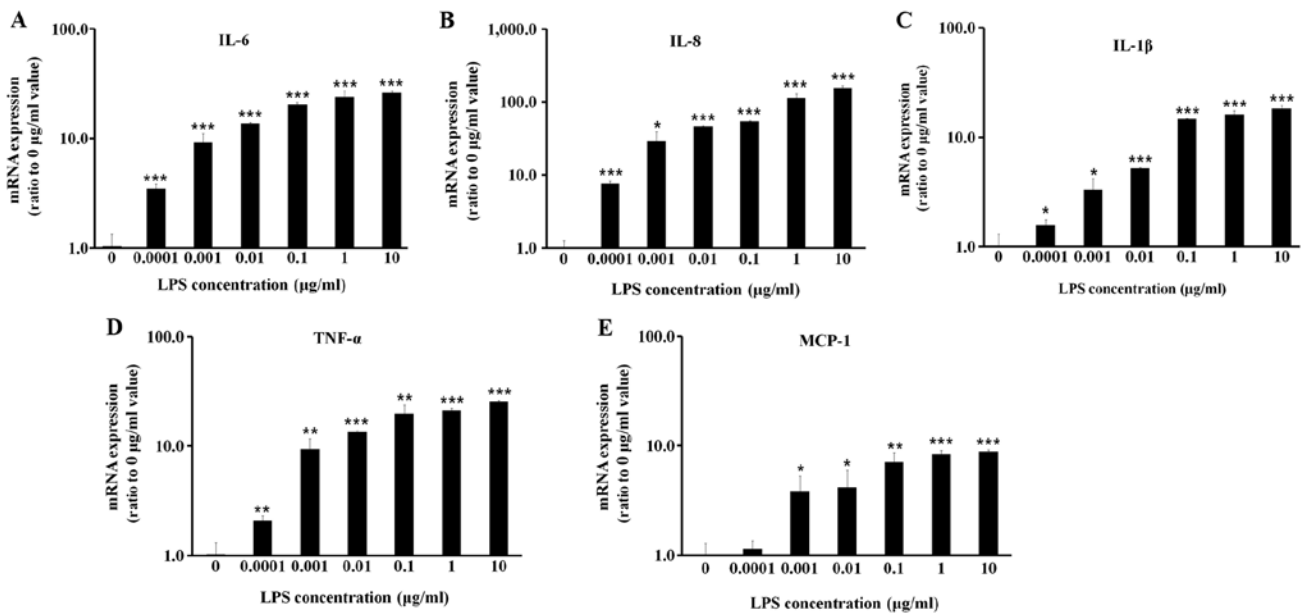


Figure 1. Effects of various lipopolysaccharide (LPS) concentrations on inflammatory cytokine expression. BEAS-2B human bronchial epithelial cells were treated with LPS at various concentrations ranging from 0.0001 to 10 $\mu\text{g/ml}$ for 3 h and total RNA was extracted for the quantification of inflammatory cytokine mRNA expression levels, including (A) interleukin (IL)-6, (B) IL-8, (C) IL-1 β , (D) tumor necrosis factor (TNF)- α and (E) monocyte chemoattractant protein (MCP)-1. *P<0.05, **P<0.01, ***P<0.001 compared with the untreated control marked as 0.

and then transfected with 50 nM of control siRNA or β -catenin siRNA (Santa Cruz Biotechnology, Inc.) using Lipofectamine RNAiMAX transfection reagent (Invitrogen, Carlsbad, CA, USA). After 18 h, the cells were treated with LPS (0.001 to 10 $\mu\text{g/ml}$) to induce an inflammatory response. Untreated cells were used as controls.

Statistical analysis. All data are expressed as the means \pm standard deviation from at least 3 replicate experiments. Statistically significant differences between the treated and untreated samples were detected using unpaired t-tests. A P-value of <0.05 was considered statistically significant. All analyses were performed using SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA).

Results

LPS induces inflammatory cytokine expression in BEAS-2B human bronchial epithelial cells in a dose- and time-dependent manner. When the BEAS-2B human bronchial epithelial cells were treated with various concentrations of LPS (0.001 to 10 $\mu\text{g/ml}$), the expression levels of inflammatory cytokines, including IL-6, IL-8, IL-1 β , TNF- α and MCP-1 increased in a dose-dependent manner (Fig. 1). It was found that LPS induced significantly high levels of inflammatory cytokine expression at a dose of 0.1 $\mu\text{g/ml}$, and all subsequent experiments were conducted at this LPS concentration. Subsequently, when the cells were treated with 0.1 $\mu\text{g/ml}$ LPS for various periods of time, the inflammatory cytokine expression began to increase 0.5 to 1 h following treatment with LPS and subsequently increased in a time-dependent manner for up to 3 h. TNF- α , however, showed its maximum expression 2 h following treatment with LPS, and decreased 3 h following treatment with LPS (Fig. 2).

LPS induces NF- κ B activity in BEAS-2B human bronchial epithelial cells. The activity of NF- κ B, the major inflammatory transcription factor, was examined in the cells treated with 0.1 $\mu\text{g/ml}$ LPS for various periods of time. The binding activity of NF- κ B to its target DNA sequence was investigated by both ELISA (Fig. 3A) and EMSA (Fig. 3B) experiments. The results from the ELISA and EMSA experiments were similar in that NF- κ B was found to bind its target DNA sequence following treatment with 0.5 h LPS (Fig. 3A and B). In the EMSA experiments, the shifted electrophoretic mobility patterns of the labeled NF- κ B probe were inhibited by excess amounts of unlabeled NF- κ B probe, showing that the results were not mediated by non-specific binding (Fig. 3B, lanes 3-7 vs. lane 8). The NF- κ B reporter assay was conducted using a pGL4.32 vector, which has a luciferase gene linked to an NF- κ B response element. The results indicated that NF- κ B-driven luciferase expression increased at approximately 1 h following treatment with LPS (Fig. 3C).

LPS upregulates the level of β -catenin protein in total cell lysates and nuclear extracts. When the cells were treated with 0.1 $\mu\text{g/ml}$ LPS for various periods of time, the mRNA level of β -catenin was not significantly altered (Fig. 4A). However, the β -catenin protein level in the total cell lysates was upregulated 0.5 h following treatment with LPS, which was accompanied by the reduced phosphorylation of β -catenin at serine 33/37 residues (Fig. 4B). The nuclear β -catenin protein level was also upregulated 0.5 h following treatment with LPS (Fig. 4C). A reporter assay was conducted using a TOPFlash vector, which has a luciferase gene linked to a β -catenin response element (Fig. 4D). The results revealed that LPS induced β -catenin-driven luciferase expression.

Knockdown of β -catenin results in a decrease in NF- κ B activity in LPS-treated cells. In the knockdown experiments, the cells

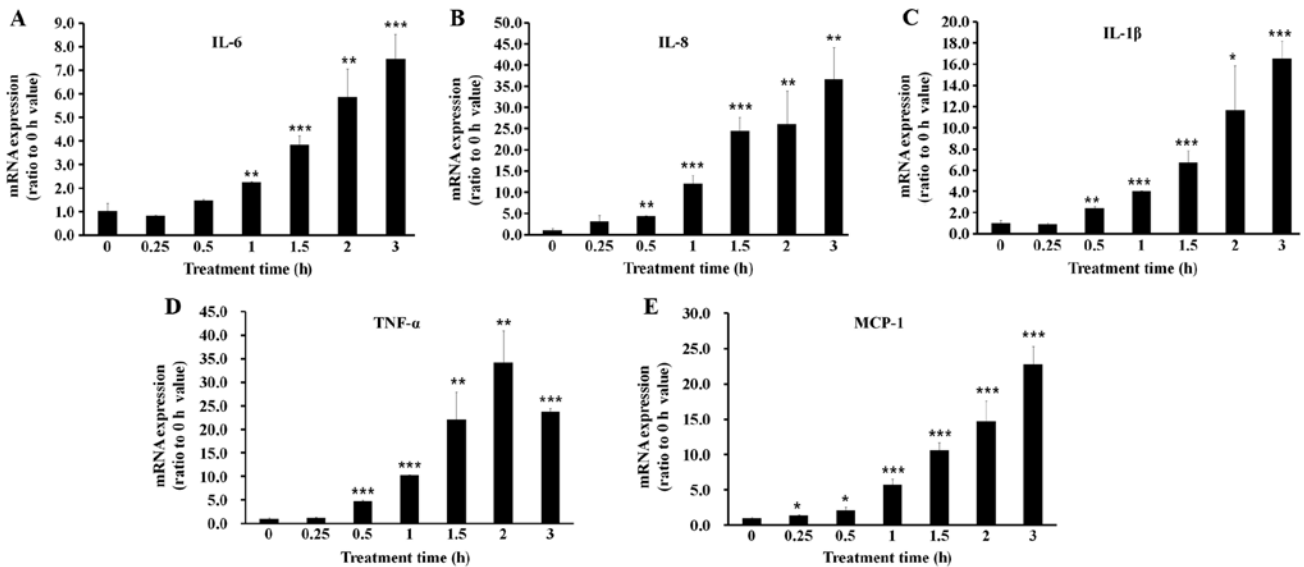


Figure 2. Time course of lipopolysaccharide (LPS)-induced inflammatory cytokine expression. BEAS-2B cells were treated with 0.1 μ g/ml LPS for various periods of time and total RNA was extracted for the quantification of inflammatory cytokine mRNA expression levels, including (A) interleukin (IL)-6, (B) IL-8, (C) IL-1 β , (D) tumor necrosis factor (TNF)- α and (E) monocyte chemoattractant protein (MCP)-1. * P <0.05, ** P <0.01, *** P <0.001 compared with the untreated control marked as 0.

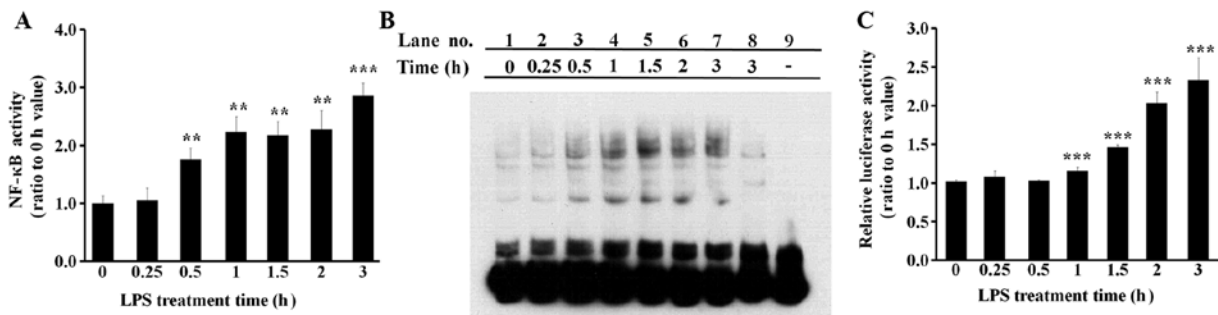


Figure 3. NF- κ B activity during the lipopolysaccharide (LPS)-induced inflammatory response. BEAS-2B cells were treated with 0.1 μ g/ml LPS for various periods of time and NF- κ B activity was measured. (A) Target DNA binding activity of NF- κ B measured by ELISA. (B) For EMSA, nuclear protein extracts were incubated with biotin-labeled NF- κ B probe alone (lanes 1-7) or in combination with 66-fold unlabeled NF- κ B probe (lane 8). Lane 9 contains biotin-labeled NF- κ B probe only. (C) Luciferase reporter assays were conducted after transfection with a pGL 4.32 vector containing the NF- κ B response element. ** P <0.01, *** P <0.001 compared with the untreated control marked as 0.

were transfected with control siRNA or β -catenin siRNA and treated with LPS in order to induce NF- κ B activity. Both the β -catenin protein level and luciferase expression by a β -catenin responsive promoter, which were upregulated following treatment with LPS, were decreased by β -catenin siRNA transfection compared with control siRNA transfection [Fig. 5A (lane 3 vs. lane 4) and B], clearly showing that the siRNA-mediated knockdown of β -catenin expression had occurred.

In this experimental condition, NF- κ B activity was measured by ELISA (Fig. 5C), EMSA (Fig. 5D) and reporter assays (Fig. 5E). The target DNA binding activity of NF- κ B measured by ELISA was significantly decreased by β -catenin siRNA compared with control siRNA in the LPS-treated cells (Fig. 5C), and the EMSA results showed identical patterns (Fig. 5D, lane 3 vs. lane 4). The shifted electrophoretic mobility patterns of the labeled NF- κ B probe were inhibited by excess unlabeled NF- κ B probe, showing that the results were not mediated by non-specific binding (Fig. 5D, lanes 5-8). Luciferase expression mediated by a promoter containing

the NF- κ B target sequence was also significantly decreased by β -catenin siRNA compared with control siRNA in the LPS-treated cells (Fig. 5E).

Knockdown of β -catenin results in decreased inflammatory cytokine expression in LPS-treated cells. The cells were transfected with control siRNA or β -catenin siRNA and treated with LPS for the induction of inflammatory cytokine expression. The experimental results demonstrated that the expression levels of IL-6, IL-8, IL-1 β , TNF- α and MCP-1, which were induced by treatment with LPS, were all significantly decreased by β -catenin siRNA transfection compared with control siRNA transfection in the LPS-treated cells (Fig. 6).

Discussion

Our interest in β -catenin as a modulator of bronchial inflammation was prompted by a previous study reporting that a genetic polymorphism of β -catenin was significantly asso-

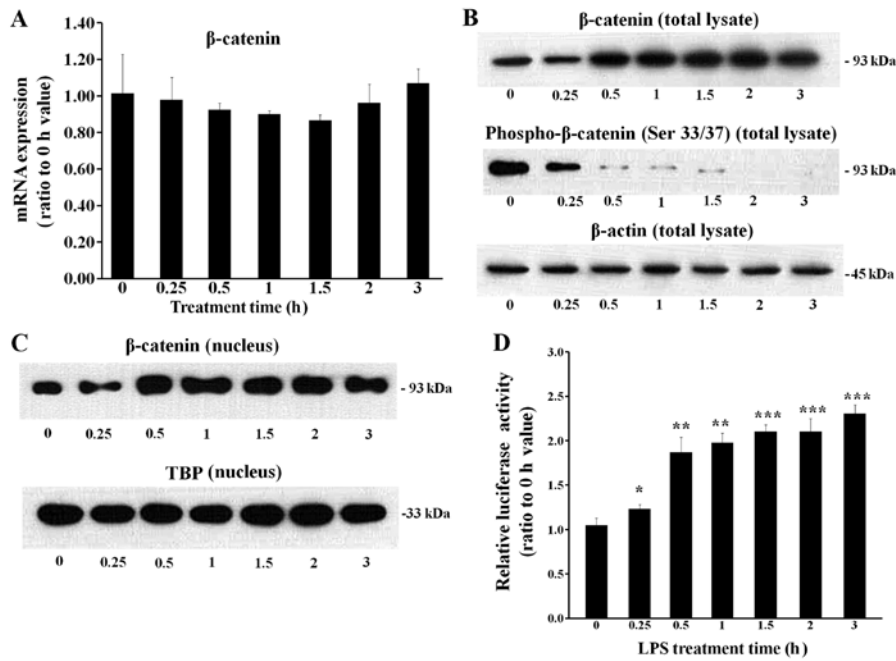


Figure 4. Modulation of β -catenin during the lipopolysaccharide (LPS)-induced inflammatory response. (A) BEAS-2B cells were treated with $0.1 \mu\text{g/ml}$ LPS for various periods of time and β -catenin mRNA expression level was quantified by real-time PCR. (B) Protein levels of β -catenin and phosphorylated β -catenin (at serine 33/37 residues) were analyzed by western blot analysis of total cell lysates. β -actin was used as the endogenous control. (C) Nuclear β -catenin was analyzed by western blot analysis of nuclear extracts. TATA box binding protein (TBP) was used as the endogenous control. (D) Luciferase reporter assays were conducted after transfection of a TOPFlash vector containing β -catenin response element. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the untreated control marked as 0.

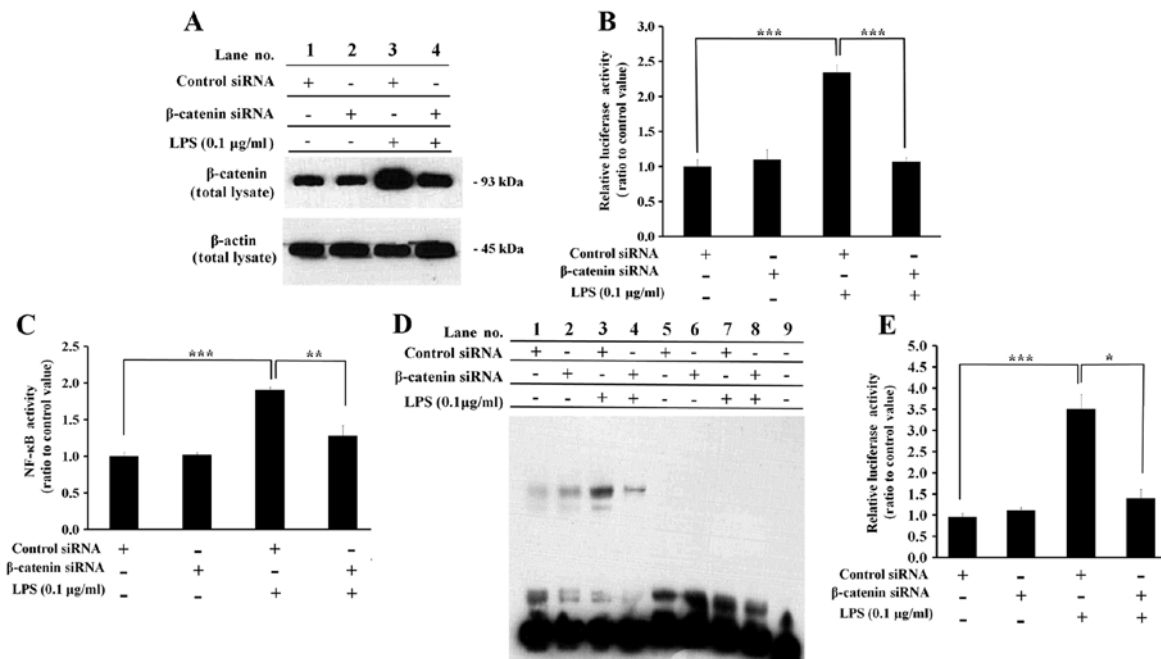


Figure 5. Effects of β -catenin knockdown on NF- κ B activity in the lipopolysaccharide (LPS)-induced inflammatory response. (A) BEAS-2B cells were transfected with control siRNA or β -catenin siRNA for 18 h, and stimulated with 0 or $0.1 \mu\text{g/ml}$ LPS for 3 h. Total cell lysates were analyzed for β -catenin protein levels with β -actin as the endogenous control. (B) Luciferase reporter assays were conducted after transfection of a TOP flash vector containing the β -catenin response element. (C) In the same experimental condition, NF- κ B activity was measured by ELISA. (D) For EMSA, nuclear protein extracts were incubated with biotin-labeled NF- κ B probe alone (lanes 1-4) or in combination with 66-fold unlabeled NF- κ B probe (lanes 5-8). Lane 9 contains biotin-labeled NF- κ B probe only. (E) Luciferase reporter assays were conducted after transfection of a pGL 4.32 vector containing the NF- κ B response element. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

ciated with the risk of asthma in human subjects (7). Until now, however, the role of β -catenin has not been elucidated

in the inflammatory response of bronchial epithelial cells. In the present study, we demonstrate that β -catenin plays a

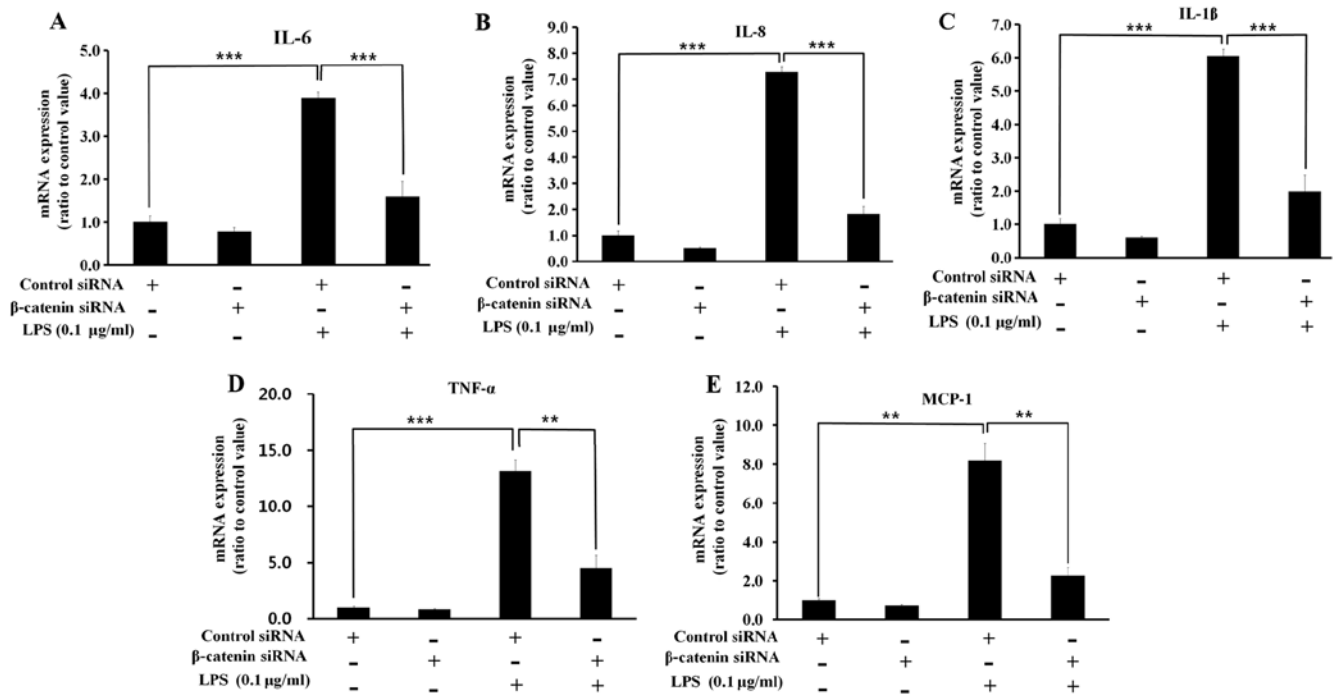


Figure 6. Effects of β -catenin knockdown on inflammatory cytokine expression in the LPS-induced inflammatory response. BEAS-2B cells were transfected with control siRNA or β -catenin siRNA for 18 h and stimulated with 0 or 0.1 μ g/ml LPS for 3 h. Total RNA was extracted to measure inflammatory cytokine mRNA expression levels including (A) interleukin (IL)-6, (B) IL-8, (C) IL-1 β , (D) tumor necrosis factor (TNF)- α and (E) monocyte chemoattractant protein (MCP)-1 by real-time PCR. Data are shown as the ratio to the value of control cells which were treated with 0 μ g/ml LPS after control siRNA transfection. ** P <0.01, *** P <0.001.

role in the regulation of the inflammatory response of human bronchial epithelial cells treated with LPS.

Our results revealed that LPS induced inflammatory cytokine expression in bronchial epithelial cells in a dose- and time-dependent manner (Figs. 1 and 2), which was accompanied by the induction of NF- κ B activity. NF- κ B activity was measured using 3 different methods, ELISA, EMSA, and a reporter assay (Fig. 3). When the expression of β -catenin was analyzed in the LPS-treated bronchial epithelial cells, the mRNA levels were not altered (Fig. 4A). However, the β -catenin protein levels in the total cell lysates were increased at 0.5 h following treatment with LPS with a simultaneous reduction in the phosphorylation level at the serine 33/37 residues (Fig. 4B). Nuclear β -catenin levels were also increased 0.5 h following treatment with LPS (Fig. 4C). β -catenin is a member of the WNT/ β -catenin pathway that has been reported to regulate cellular processes, including proliferation, differentiation and development (6). The level of β -catenin is post-translationally regulated in the WNT/ β -catenin pathway. In its inactive state, β -catenin protein is degraded by a destruction complex composed of AXIN, glycogen synthase kinase (GSK)3 β and adenomatous polyposis coli (APC). GSK3 β phosphorylates β -catenin at serine 33 and 37 residues creating a binding site for E3 ubiquitin for ubiquitination and proteolytic degradation. When the WNT/ β -catenin pathway is activated, the AXIN-GSK3 β -APC complex is disrupted and GSK3 β is inactivated, resulting in the dephosphorylation and stabilization of β -catenin followed by its nuclear translocation (9,10). The experimental results presented in this study clearly indicate that LPS induced the dephosphorylation, stabilization and nuclear translocation of β -catenin, as well as the reporter activity of the

β -catenin-responsive TOPFlash vector in the bronchial epithelial cells (Fig. 4).

To elucidate the role of β -catenin in the LPS-induced inflammatory response of bronchial epithelial cells, β -catenin was knocked down using siRNA. The results revealed that the β -catenin protein level, as well as its activity as a transcriptional activator as measured by the β -catenin-responsive TOPFlash vector reporter activity, was reduced to basal levels by β -catenin siRNA transfection (Fig. 5A and B). In this experimental condition, NF- κ B activity was measured using 3 different methods, ELISA, EMSA and reporter assays; as shown by all 3 methods, its activity was reduced to basal levels (Fig. 5C-E). Similarly, LPS-induced inflammatory cytokine expression was reduced to almost basal levels by β -catenin siRNA transfection (Fig. 6). These experimental data clearly demonstrate that β -catenin is involved in the activation of NF- κ B, as well as in the induction of inflammatory cytokine expression in the inflammatory response of LPS-treated bronchial epithelial cells.

A number of studies have reported a major role for NF- κ B in the inflammation of bronchial epithelial cells stimulated by toxic and pathogenic agents, including cigarette smoke extract, diesel exhaust particles, wood dust, respiratory syncytial virus, rhinoviruses, *Bordetella pertussis* and house dust mites (11-16). The exaggerated activation of NF- κ B has been found in bronchial epithelial cells with a mutation characteristic of cystic fibrosis, a genetic disease characterized by chronic airway inflammation (17). Erythromycin, which improves the clinical symptoms of patients with bronchiolitis, has been reported to suppress the activation of NF- κ B and IL-8 production in human bronchial epithelial cells (18). All the above studies

have suggested that the regulation of NF- κ B activity has crucial importance for the effective treatment of respiratory diseases involving bronchial inflammation.

Until now, β -catenin has been mainly associated with cancer (19), and mutations in the β -catenin gene have been commonly observed in endometrioid ovarian cancer, hepatoblastoma, Wilms' kidney tumors and some colorectal cancers (20). Considering the role of β -catenin in various cellular processes of differentiation and development, however, β -catenin may also be involved in chronic inflammatory diseases (6). Our previous study reported that genetic polymorphisms of β -catenin are associated with asthma (7); however, further studies are required to elucidate the role of β -catenin in the regulation of inflammation. A few studies have reported controversial roles of β -catenin as a regulator of inflammation. Duan *et al* reported that β -catenin negatively regulated the inflammatory response induced by pathogenic Gram-negative bacteria. They reported that *Salmonella typhimurium* stimulated the degradation of β -catenin and increased the expression levels of IL-6 and TNF- α in a mouse model and in colonic epithelial cells (21). On the contrary, Kim *et al* reported that β -catenin positively regulated the inflammatory response to LPS. They reported that LPS induced β -catenin accumulation and nuclear translocation followed by the induction of NADPH oxidase in RAW 264.7 macrophages and murine bone-marrow-derived macrophages (22). We have previously demonstrated that β -catenin positively regulates inflammatory cytokine expression in THP-1 human monocytic cells stimulated by the Der p 1 house dust mite allergen (23). Despite discrepancies, these data suggest a role of β -catenin in the regulation of the inflammatory response.

In this study, we provide clear experimental evidence that β -catenin positively regulates NF- κ B activity, as well as inflammatory cytokine expression in bronchial epithelial cells treated with LPS. The results of this study suggest that β -catenin may be a target for the modulation of bronchial inflammation, even though further studies are required to elucidate the molecular mechanisms involved and to provide clinical evidence.

Acknowledgements

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