Mucin1 relieves acute lung injury by inhibiting inflammation and oxidative stress

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ABSTRACT

Acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is a kind of diffuse inflammatory injury caused by various factors, characterized by respiratory distress and progressive hypoxemia. It is a common clinical critical illness. The aim of this study was to investigate the effect and mechanism of the *Mucin1 (MUC1)* gene and its recombinant protein on lipopolysaccharide (LPS)-induced ALI/ARDS. We cultured human alveolar epithelial cell line (BEAS-2B) and used MUC1 overexpression lentivirus to detect the effect of *MUC1* gene on BEAS-2B cells. In addition, we used LPS to induce ALI/ARDS in C57/BL6 mice and use hematoxylin and eosin (H&E) staining to verify the effect of their modeling. Recombinant MUC1 protein was injected subcutaneously into mice. We examined the effect of MUC1 on ALI/ARDS in mice by detecting the expression of inflammatory factors and oxidative stress molecules in mouse lung tissue, bronchoalveolar lavage fluid (BALF) and serum. Overexpression of MUC1 effectively ameliorated LPS-induced damage to BEAS-2B cells. Results of H&E staining indicate that LPS successfully induced ALI/ARDS in mice and MUC1 attenuated lung injury. MUC1 also reduced the expression of inflammatory factors (IL-1β, TNF-α, IL-6 and IL-8) and oxidative stress levels in mice. In addition, LPS results in an increase in the activity of the TLR4/NF-κB signaling pathway in mice, whereas MUC1 decreased the expression of the TLR4/NF-κB signaling pathway. MUC1 inhibited the activity of TLR4/NF-κB signaling pathway and reduced the level of inflammation and oxidative stress in lung tissue of ALI mice.

Key words: Mucin1; acute lung injury; inflammation; oxidative stress; TLR4/NF-κB.

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Introduction

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), are a clinical syndrome characterized by respiratory distress and progressive hypoxemia, which is caused by various factors such as pulmonary capillary endothelial cells and alveolar epithelial cells injury, resulting in impaired alveolar capillary barrier, pulmonary inflammatory cell infiltration, diffuse alveolar and pulmonary interstitial edema.[1] ARDS patients have a PaO_2/FiO_2 ratio of less than 300 mm Hg according to the Berlin definition.[1] Although the diagnosis and treatment of ALI/ARDS has made some progress, due to its many pathogenic factors, the pathogenesis is very complicated. At present, there are still no effective treatment measures, which makes the mortality rate of the disease still as high as 40%, seriously affecting the prognosis of critically ill patients.[2] Therefore, ALI/ARDS has always been a hot and difficult point in critical medical research.

The pathogenesis of ALI/ARDS is mainly related to two factors, one is the factors that cause the accumulation of a large number of proteins and neutrophils in the pulmonary interstitial and alveolar, and the other is the factor that causes the obstruction of clearance of pulmonary edema fluid and inflammatory cells.[3] The protein-rich edema fluid in ALI/ARDS is associated with a large number of neutrophils, naked epithelial cells, and pro-inflammatory cytokines including cytokines, proteases, and oxidants.[4] Therefore, reducing excessive inflammatory response is the key to alleviating ALI/ARDS.

Mucin1 (MUC1) is a high molecular weight, highly glycosylated transmembrane protein encoded by the *MUC1* gene. It is also the first protein found in the mucin family and is widely distributed in epithelial tissues. Under normal circumstances, MUC1 is mainly expressed in the glandular surface of epithelial cells in various tissues and organs such as breast, pancreas, digestive tract, respiratory tract and reproductive tract. Early studies have shown that the main biological functions of MUC1 mucin are lubrication, cell protection, adhesion maintenance, and cell recognition.[5] With the deepening of the research on MUC1, it was found that MUC1 is a multifunctional molecule. MUC1 regulates cell-cell interactions, mediates signal transduction between cells, and participates in immune regulation.[6] Elevation of MUC1 were detected from the patients of various pulmonary diseases such as interstitial lung disease, pneumonia, asthma and COPD.[7] Besides, plasma Mucin1 levels in early ARDS had a good predictive value for patients' prognosis.[7] Wang *et al.*[8] found that knockdown of endogenous MUC1 can lead to aggravation of sepsis-induced ALI. In addition, MUC1 also mediates the lung protection of Paclitaxel.[9] However, whether exogenous MUC1 supplementation can improve ALI is unclear.

In this study, we established a mouse ALI/ARDS model using lipopolysaccharide (LPS) and administered recombinant mouse MUCl protein, and cultured human alveolar epithelial cell line (BEAS-2B) to study the effect of MUC1 on ALI/ARDS and its mechanism.

Materials and Methods

Animals and grouping

Eight-weeks-old C56/BL6 male mice (18~22 g) were used in this study. All mice were housed in a day-night automatic replacement and constant temperature and humidity Specific Pathogen Free (SPF) environment. Mice were divided into 3 groups: control group, ALI group and ALI+MUC1 group. Mice in the ALI and ALI+MUC1 groups were constructed in the ALI/ARDS model and mice in the ALI+MUC1 group were injected subcutaneously daily with recombinant mouse MUC1 protein (100 μg/kg) (Baize, Shanghai, China) daily one week prior to modeling.[10] The mouse ALI/ARDS modeling method is as follows: After anesthetizing the mice with sodium pentobarbital (50 mg/kg), we used tape to secure the mice in the supine position on the operating table. After wiping the neck skin of the mouse with an alcohol cotton ball, we use scissors to gently cut the neck skin and bluntly separate the neck muscles from both sides. After seeing the trachea, we injected LPS (5 mg/kg) (Sigma, St. Louis, MO, USA) into the trachea using a microsyringe. Then we held the mouse in the hand and gently shake it to evenly distribute the LPS in both lungs. If the wet rales can be heard in the lungs of mice, it means that LPS has successfully entered the lungs of mice.[11] After 24 h, we sacrificed the mice and took mouse serum and lung tissue.

Cell culture and treatment

We cultured BEAS-2B cells (American Type Culture Collection, ATCC; Manassas, VA, USA) using Dulbecco's modified eagle medium (DMEM) medium (Gibco, Rockville, MD, USA). Fetal bovine serum (FBS) (Gibco) was added to DMEM medium and configured to 10%. All cell experiments were performed in a sterile super clean bench. LPS (1 μg/ml, 24 h) was used to induce cell damage. LPS can lead to a decrease in cell proliferation and an increase in the level of apoptosis, thereby establishing LPS-induced alveolar epithelial cell damage *in vitro*.[12]

Lentivirus transfection

Lenti-NC and Lenti-MUC1 were constructed with Shanghai Jima Biotechnology Co., Ltd. (Shanghai, China). After passage of BEAS-2B cells into 24-well plates and the number of cells reached 2×10^5/well. We mixed the lentiviral suspension and medium and added 6 μg/ml of polybrene (Invitrogen, Carlsbad, CA, USA). After 24 h of culture, we replaced the medium containing the lentivirus with fresh medium. The transfection efficiency of lentivirus was determined by detecting RNA expression.

Western blot analysis

We passage BEAS-2B cells into 6-well plates. Radioimmunoprecipitation assay (RIPA) lysate (Invitrogen, Carlsbad, CA, USA) is used to lyse cells and extract proteins. The bicinchoninic acid (BCA) method (Beyotime, Shanghai, China) was used to detect protein concentration. We configured 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel according to the instructions. The protein (30 μg) was then added to each well of the electrophoresis gel for SDS-PAGE. We then transferred the protein band to the polyvinylidene fluoride (PVDF) membrane through transfer (Roche, Basel, Switzerland). After washing the PVDF membrane with phosphate buffered saline-tween (PBST), we blocked it with 5% skim milk and incubated it with primary antibody dilution (MUC1, rabbit, 1:3000, Abcam, Cambridge, MA, USA; TLR4, 1:2000, rabbit, Abcam; NF-κB p65, 1:3000, rabbit, Abcam; β-actin, rabbit, 1:3000, Abcam) for 4°C overnight. We then incubated the PVDF membrane for 2 h at room temperature using secondary antibody dilution (Goat anti-rabbit, 1:3000, Abcam). Finally, we detected protein bands by electro-chemi-luminescence (ECL).

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

After obtaining mouse lung tissue, we washed lung tissue with phosphate buffered saline (PBS) and ground to powder at low temperature. Then we dissolved the powder in TRIzol (Invitrogen). After detecting the RNA concentration using a spectrophotometer, we configured a reverse transcription system based on the RNA

concentration and reversed the mRNA to complementary deoxyribose nucleic acid (cDNA) according to the manufacturer's instructions (10 min at 55°C; 10 min at 80°C; 30 min at 4°C). The SYBR Green kit (Invitrogen) was used to amplify cDNA. Cycling mode included UDG activation for 2 min at 50°C, Dual-Lock™ DNA polymerase for 2 min at 95°C and 30 cycles (denature for 3 sec at 95°C and anneal for 30 s at 60°C). The primers for amplifying the cDNA are as follows: MUC1 sense, AGGTCGATGCACGTCGAGTGCA, MUC1 anti-sense, GGTTACAGCGATGCAGCTACGT; TLR4 sense, CAGTACGTCATCGATCGAGTCA, TLR4 anti-sense, ACCGTCGATAGTCACGTACGT; NF-κB p65 sense, AGTCTGCAGTCAGTACGTCGAT, NF-κB p65 anti-sense, ACGTTGCAGTGACGTGTACAC; GAPDH sense, ATGGCAGTCGTACCGATGCA, GAPDH anti-sense, GGCTAAAATACGCGCTACGT. The relative expression level of RNA is represented by $2^{-\Delta\Delta CT}$. The extraction and detection of RNA in cells is similar to tissue.

Wet/dry weight ratio of lung tissue

After obtaining the lung tissue of the mouse, we gently wiped the blood on the surface of the lung tissue with gauze and measured the weight of the lung tissue at this time, which is wet weight. We then placed the lung tissue in a 55°C incubator for 48 h. After the lung tissue of the mice was dried, we measured the weight of the lung tissue and recorded it as dry weight. Wet/dry weight ratio (W/D) represents the severity of pulmonary edema in mice.

Preparation of bronchoalveolar lavage fluid (BALF)

After obtaining the lung tissue of the mouse, we gently wiped the lung tissue with gauze. We then pipet 0.6 mL of sterile PBS containing 500 μM ethylenediaminetetraacetic acid (EDTA), then slowly inject into the lung tissue and pump back the syringe to obtain the lavage fluid. A lung tissue was lavaged three times.

ELISA

After obtaining bronchoalveolar lavage fluid (BALF) or serum from mice, we centrifuged BALF or serum to remove impurities. We then used IL-1β, TNF-α, IL-6, IL-8 and MUC1 ELISA kits (Lianke, Hangzhou, China) to detect the concentration of inflammatory markers. We can calculate the concentrations of several inflammatory factors in the sample to be tested based on the concentration curve produced by the standard.

Myeloperoxidase, malondialdehyde, catalase and glutathione peroxidase activity assay

After obtaining mouse lung tissue, we weighed the same quality lung tissue and ground it to powder in PBS at low temperature. Myeloperoxidase (MPO), malondialdehyde (MDA), catalase (CAT) and glutathione peroxidase (GSH-Px) kits (Lianke, Hangzhou, China) were used to detect the expression of oxidative stress-related molecules in lung tissue. The concentration of these biomarkers can be calculated according to the concentration curve made by the standard.

Hematoxylin & Eosin staining

After obtaining mouse lung tissue, we fixed lung tissue with 4% paraformaldehyde for 24 h. Then we immersed the lung tissue once in gradient alcohol for dehydration. The lung tissue was then immersed once in xylene for 30 min and paraffin for 1 h. Then we used an embedding machine to make lung tissue wax blocks. A microtome was used to make paraffin sections. Paraffin sections were deparaffinized and hydrated and placed in hematoxylin stain for 5 min. Then we rinsed the paraffin sections with running water for 3 min. Paraffin sections were placed in hydrochloric acid for 3 s and immediately rinsed into water for washing. Then we placed the paraffin sections in the iridescence solution for 3 min. Finally, we dehydrated and sealed the paraffin sections.

Immunohistochemical staining

After dewaxing and hydrating the paraffin sections, we placed the sections in citrate buffer and heated to 95°C for 20 min. After the paraffin sections were naturally dried, we incubated the lung tissue with 3% H_2O_2 for 15 min. We then incubated the lung tissue with 10% goat serum for 30 min. We configured primary antibody dilution (IL-1β, 1:300, rabbit, Abcam; TNF-α, 1:100, rabbit, Abcam, SOD_1, 1:300, rabbit, Abcam; SOD_2, 1:500, rabbit, Abcam; TLR4, 1:100, rabbit, Abcam; NF-κB p65, 1:300, rabbit, Abcam) to incubate the lung tissue at 4°C overnight. In the next day, after washing the sections with PBS, we incubated the lung tissue for 1 h using the universal secondary antibody dilution in the immunohistochemical kit (Keygen, Nanjing, China). For the negative control, we used PBS instead of the primary antibody. For the positive control, we used liver tissue sections containing the targets. Then we used 3,3'-diaminobenzidine to develop color and observed and recorded the staining results using a microscope. For each tissue section, we randomly selected 5 high-power fields and calculated the percentage of positive cells in the same type of cells.

Reactive oxygen species level detection

After the cells were passaged to a 6-well plate and treated accordingly, we collected the cells with trypsin and washed the cells with PBS. After removing the supernatant by centrifugation, we resuspended the cells in 1 mL of PBS and added the fluorescent probe DCFH-DA (Keygen, Nanjing, China). Then we used a FACSCalibur flow cytometer (Ex/Em is 488/530 nm, 10^5 events) to measure the fluorescence intensity.

Statistical analysis

Experimental data were shown as mean ± standard deviation. Comparison between multiple groups was done using One-way ANOVA test followed by *post-hoc* test (Least Significant Difference). Statistical Product and Service Solutions (SPSS) 21.0 statistical software and Graphpad prism 7.0 software (La Jolla, CA, USA) were used for data analysis and chart production in this study. $P<0.05$ indicates that the difference was statistically significant.

Results

Overexpression of MUC1 attenuates LPS-induced damage of BEAS-2B cells *in vitro*

We used MUC1 overexpression lentivirus to increase MUC1 expression in BEAS-2B cells and detected transfection efficiency by Western blot (Figure 1A) and RT-PCR (Figure 1B). The expression of inflammatory factors in the cell supernatant was examined by ELISA (Figure 1C), and the results showed that MUC1 effectively inhibited the pro-inflammatory effects of LPS and decreased the expression of IL-1β, TNF-α, IL-6 and IL-8. In addition, we also examined the activity of MDA (Figure 1D) and CAT (Figure 1E) in BEAS-2B cells, and the results showed that MUC1 can inhibit MDA and promote CAT activity. Reactive oxygen species (ROS) can cause oxidative damage to the body. We measured the level of ROS in BEAS-2B cells by flow cytometry (Figure 1F) and the results showed that MUC1 can effectively eliminate ROS in BEAS-2B cells.

Recombinant mouse MUC1 protein attenuates LPS-induced ALI/ARDS and reduces inflammation levels

We used LPS to construct septic ALI/ARDS. We observed the modeling effect by H&E staining (Figure 2A) and found that LPS successfully induced mouse ALI/ARDS and the lung tissue of the mouse showed significant swelling and inflammatory tissue infiltration, while the lung tissue of MUC1-treated mice was significantly improved. MUC1 reduced the total cells amount (Figure 2B) and neutrophils amount (Figure 2C) in the BALF. MUC1 also significantly reduced swelling of mouse lung tissue and reduced W/D (Figure 2D). We detected the serum level of MUC1 in the three groups of mice and found that the serum level of MUC1 in the mice with ALI was significantly higher than that in the control group (Figure 2E). We examined the levels of inflammatory factors in BALF and serum of mice by ELISA (Figure 2F) and showed that MUC1 can reduce IL-1β, TNF-α, IL-6 and IL-8 in mice. In addition, IHC staining (Figure 2G) results also indicated that MUC1 reduced the expression of IL-1β and TNF-α.

Recombinant mouse MUC1 protein reduces oxidative stress level in mouse lung tissue

To further investigate the protective effect of MUC1 on the lung, we examined the effect of MUC1 on oxidative stress in lung tissue. The results of IHC staining (Figure 3A) showed that the expression of SOD_1 and SOD_2 in lung tissue of LPS-induced mice was significantly decreased while MUC1 could increase the expression of SOD_1 and SOD_2. In addition, we examined the activities of MPO (Figure 3B), MDA (Figure 3C), CAT (Figure 3D) and GSH-Px (Figure 3E) in mouse lung tissue and results showed that MUC1 can decrease the activity of MPO and MDA and increase the activity of CAT and GSH-Px.

Recombinant mouse MUC1 inhibits TLR4/NF-κB signaling pathway in mouse lung tissue

The TLR4/NF-κB signaling pathway is an important pathway mediating LPS-induced ALI/ARDS. Therefore, we examined the effect of MUC1 on the TLR4/NF-κB signaling pathway. The results of IHC staining (Figure 4A) and Western blot (Figure 4B) indicated that MUC1 significantly reduced the expression of TLR4 and NF-κB p65. In addition, the results of RT-PCR (Figure 4C, 4D)

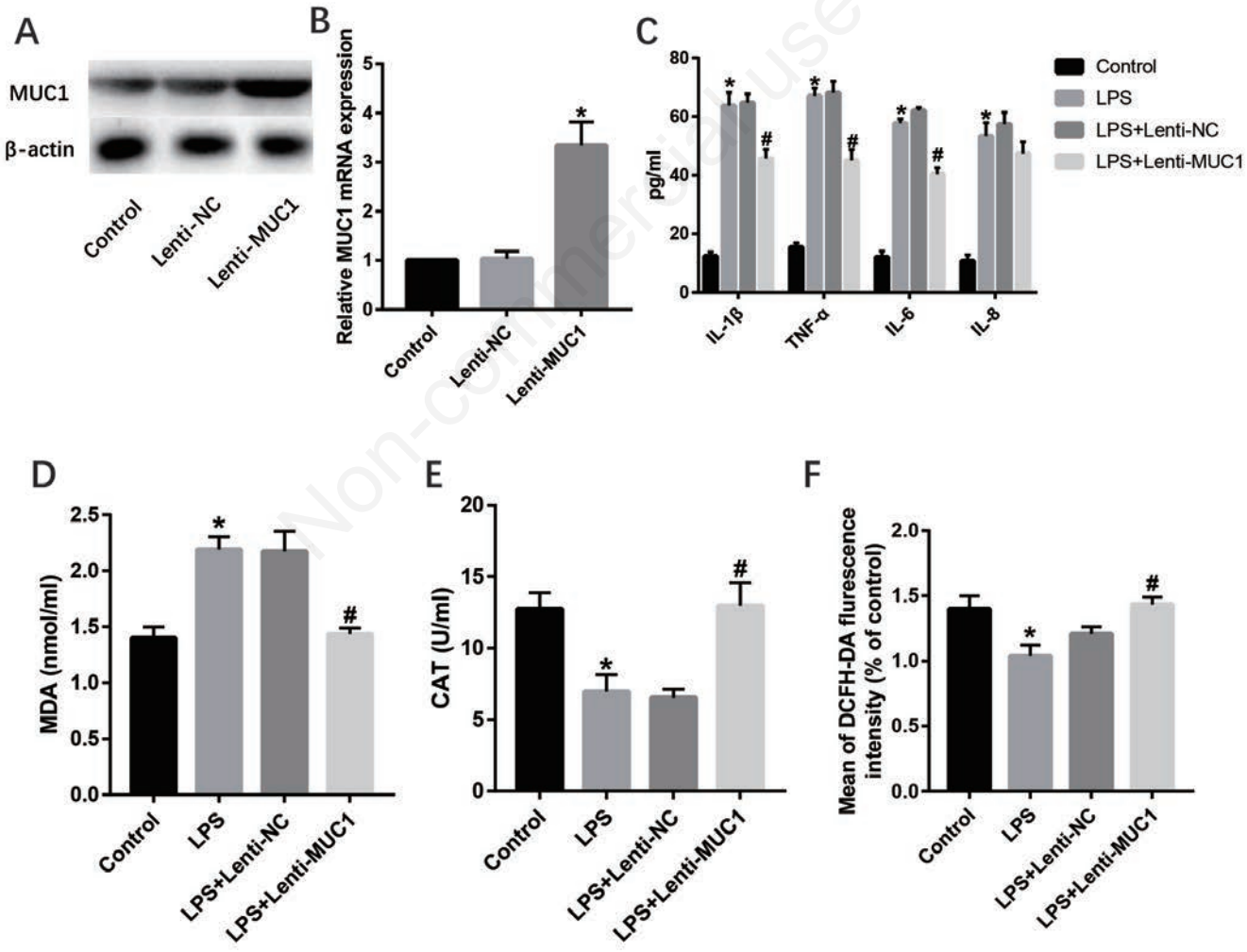

Figure 1. Overexpression of MUC1 attenuates LPS-induced damage of BEAS-2B cells *in vitro*. A,B) Western blot and RT-PCR results of MUC1; C) ELISA results of IL-1β, TNF-α, IL-6 and IL-8; D,E) MDA and CAT activity in BEAS-2B cells; F) ROS level in BEAS-2B cells. *p<0.05 *vs* the control group; #p<0.05 *vs* the LPS+Lenti-NC group.

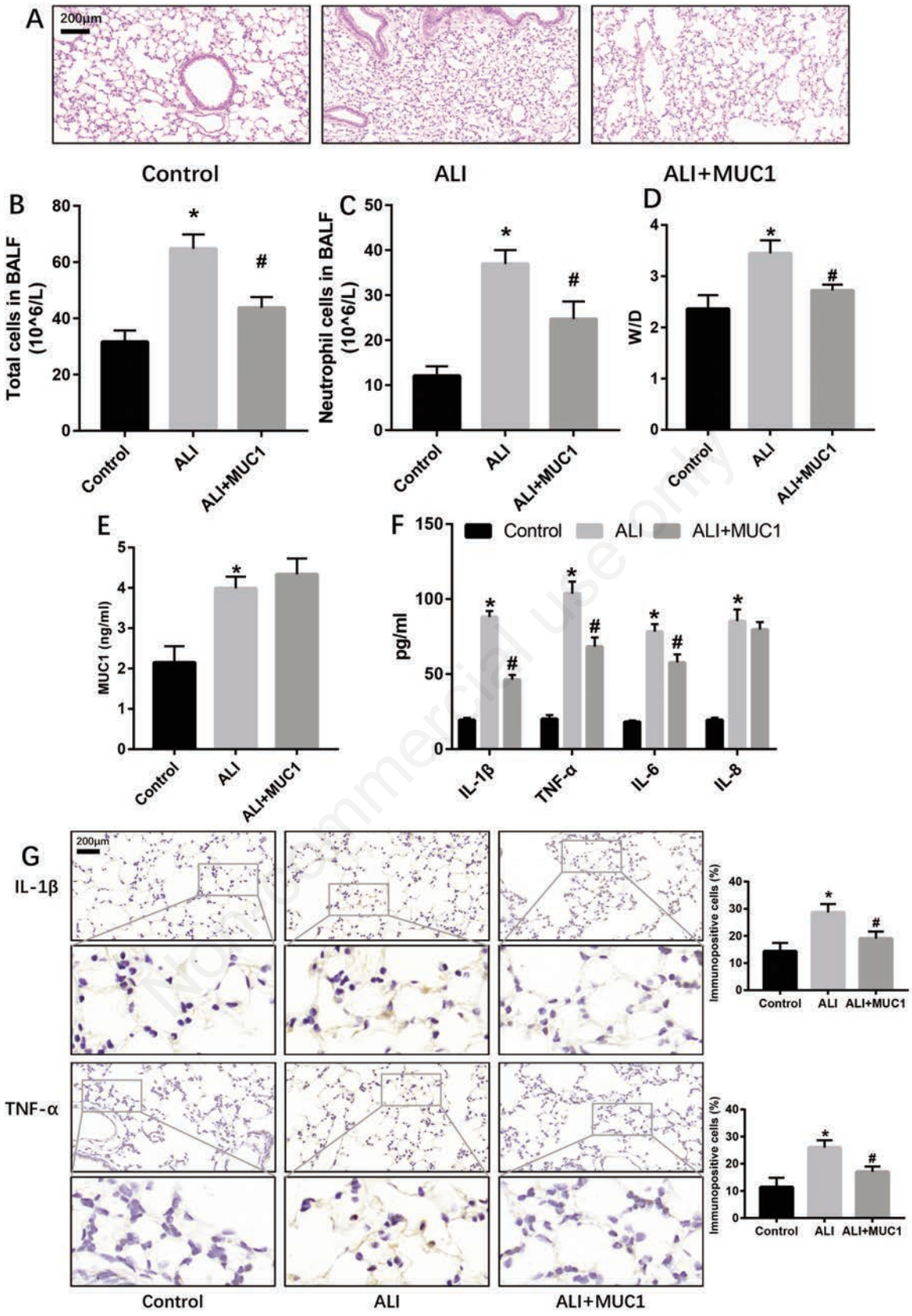

Figure 2. Recombinant mouse MUC1 protein attenuates LPS-induced ALI/ARDS and reduces inflammation levels. A) H&E staining of mice lung tissue (magnification: 200×); B) Number of total cells in BALF; C) Number of neutrophil in BALF; D) W/D of mice lung tissue; E) MUC1 level of mice serum; F) ELISA results of IL-1β, TNF-α, IL-6 and IL-8; G) IHC staining results of IL-1β and TNF-α (magnification: 200×). *p<0.05 *vs* the control group; #p<0.05 *vs* the ALI group.

were similar to those of IHC staining, indicating that MUC1 can inhibit the activity of the TLR4/NF-κB signaling pathway.

Discussion

The mechanism of lung epithelial cell damage is mainly related to neutrophils and their products. Normally neutrophils can pass through the alveolar epithelium without causing an increase in lung epithelial permeability. However, in pathological conditions, migration of a large number of neutrophils can lead to epithelial damage.[13] In addition, neutrophil activation by chemokines and other pro-inflammatory stimuli plays an important role in lung epithelial cell damage during migration to the alveoli. Existing studies have shown that once neutrophils pass through the epithelium and migrate to the alveoli, they adhere to the alveolar surface, phagocytizing and killing the bacteria. Under physiological conditions, neutrophils can cross the space beside the cells and reseal the epithelial cell junctions, thereby maintaining a complete epithelial barrier and alveolar dryness.[14] However, under pathological conditions, a large number of activated neutrophils induce the tight junctional lysis and the apoptosis and necrosis of type I and type II alveolar epithelial cells by releasing toxic mediators. These toxic mediators mainly include proteases, matrix metalloproteinases, cationic peptides and ROS, which are important in increasing epithelial permeability and injuring alveolar epithelial cells. In addition, neutrophils and other cell-derived oxidants also have an indirect pro-inflammatory effect and can also directly cause lung epithelial cell damage through apoptotic or necrotic pathways. Therefore, although the pathogenesis of ALI/ARDS has made some progress, its pathogenesis is very complicated and involves many links.[15] It is currently believed that neutrophils play an

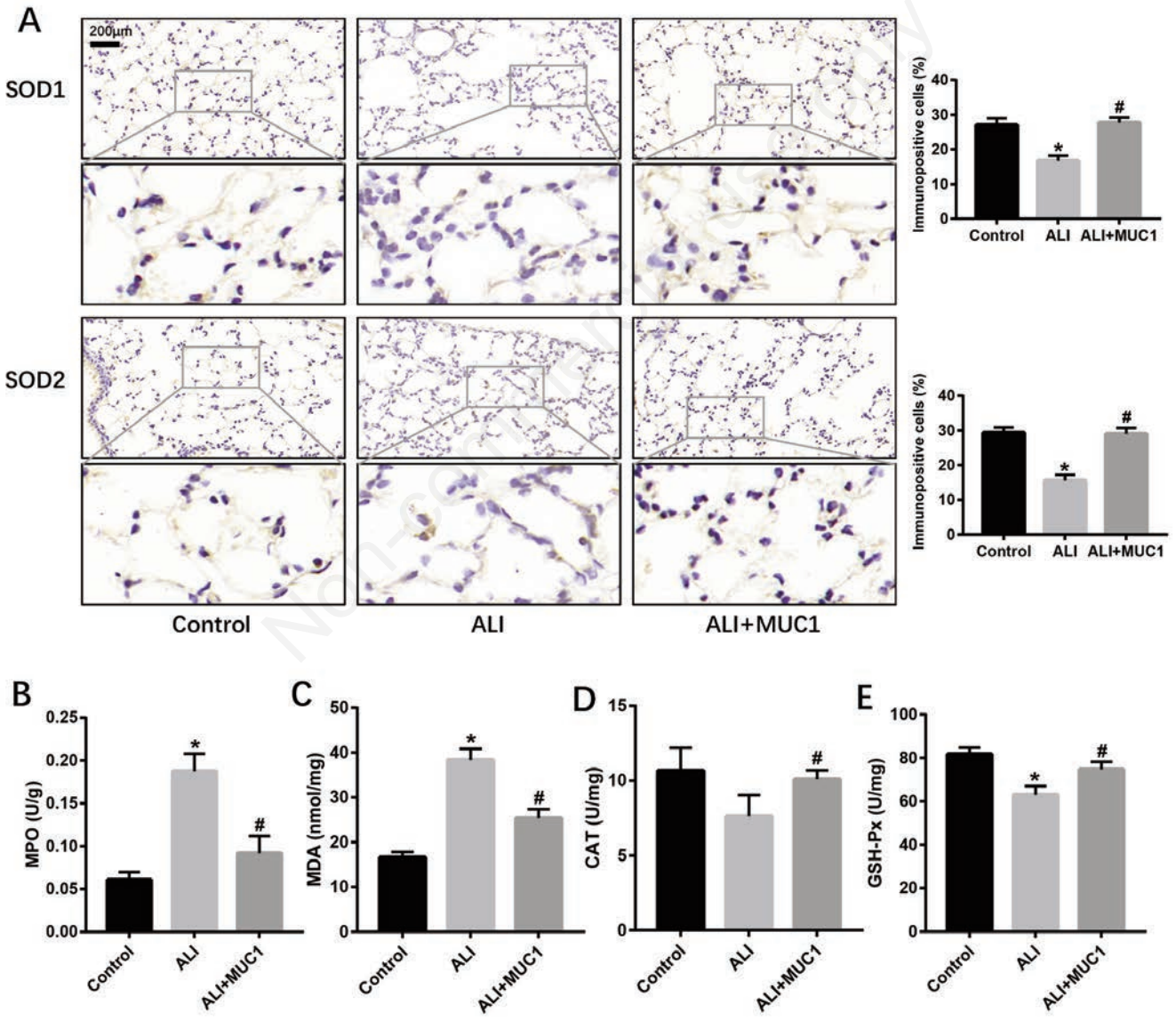

Figure 3. Recombinant mouse MUC1 protein reduces oxidative stress level in mouse lung tissue. A) IHC staining results of SOD_1 and SOD_2 in mice lung tissue (magnification: 200×); B-E) MPO, MDA, CAT and GSH-Px activity in mice lung tissue. *p<0.05 *vs* the control group; #p<0.05 *vs* the ALI group.

important role in the pathogenesis of ALI/ARDS by releasing a large amount of inflammatory mediators and ROS.[16] Our study found that MUC1 can effectively inhibit LPS-induced inflammation in mouse lung tissue. IL-1β, IL-6, IL-8 and TNF-α are abundantly expressed in LPS-induced mice. After treatment of mice with MUC1, the inflammatory factors in the lung tissue, BALF and serum of the mice were significantly reduced. In addition, the level of oxidative stress in mouse lung tissue was also significantly improved. These results indicate that MUCl can alleviate ALI/ARDS by reducing inflammation levels and oxidative stress levels in mice.

MUC1 can mediate the role of multiple pathways in different diseases. MUC1 was found to mediate the c-Jun N-terminal kinase/activator protein 1 pathway to promote the invasion and metastasis of liver cancer cells.[17] In addition, MUC1 has also been found to regulate the progression of colon cancer by adjusting the activity of the Phosphatidylinositol 3-Kinase/Protein Kinase B (PI3K/AKT) signaling pathway.[18] A study found that MUC1 knockdown could influence NF-κB signal.[19] It indicated that MUC1 may have a regulatory effect on NF-κB. Our results found that the regulation of TLR4/NF-κB signaling pathway may be the key to alleviating ALI/ARDS by MUC1. LPS, as a cell wall component of pathogenic microorganisms, is also the main cause of sepsis-induced ALI/ARDS. LPS can be specifically recognized by the cell membrane receptor TLR4.[20] In recent years, tubular epithelial cells, heart, intestinal epithelial cells, and airway epithelial cells have also been found to express TLR4. The structure of TLR4 is divided into three regions: extracellular, transmembrane and intracellular domain.[21] The extracellular domain is a leucine rich repeat (LRR) that mediates the recognition of pathogen-associated molecular patterns. The intracellular domain is a highly conserved sequence that shares homology with the intracellular domain of the

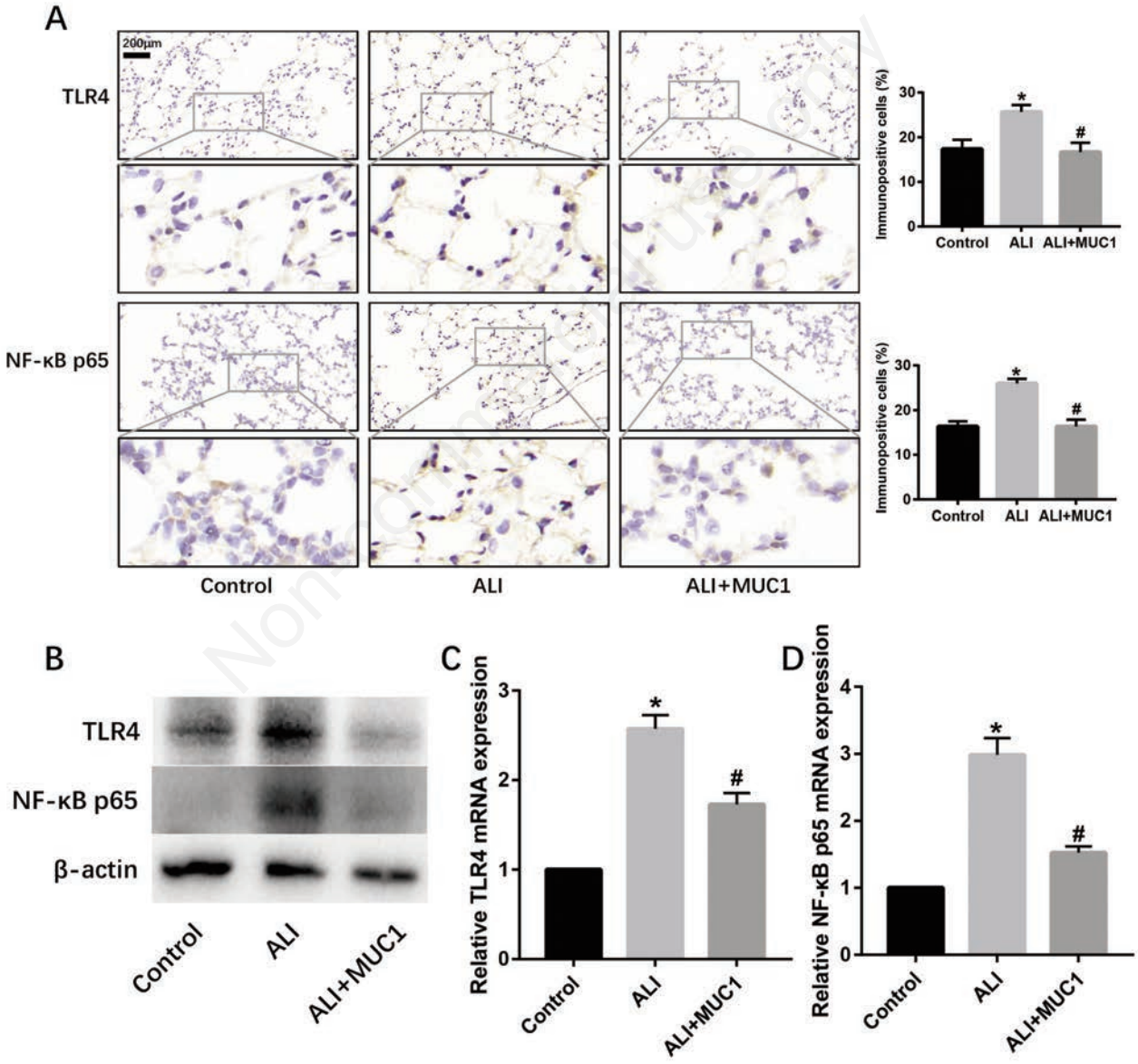

Figure 4. Recombinant mouse MUC1 inhibits TLR4/NF-κB signaling pathway in mouse lung tissue. A) IHC staining results of TLR4 and NF-κB p65 (magnification: 200×); B) Western blot results of TLR4 and NF-κB p65; (C, D) RT-PCR results of TLR4 and NF-κB p65. *p<0.05 *vs* the control group; #p<0.05 *vs* the ALI group.

IL-1 receptor, also known as the Toll/IL-1 receptor (TIR) region. Like other TLRs, TLR4 plays a role in the induction of the innate immune response and subsequent adaptive immune responses. The signal transduction pathway activated by the binding of LPS to TLR4 is mainly dependent on the signal transduction pathway of MyD88, which can initiate the AP-1 pathway and NF-κB pathway. When TLR4 binds to its specific ligand, it promotes its own dimerization (which can be a homodimer or a heterodimer) and transmits signals and stimulates downstream effects through its TIR region. First, the TIR domain interacts with the TIR domain of carboxyl-terminal of the MyD88. The activated MyD88 interacts with the amino-terminal death domain of serine/threonine protein kinase 4 (IRAK-4) through its amino-terminal death domain, and recruits IRAK-4 into the TLR4 signaling complex to promote IRAK-4 mediated autophosphorylation of IRAK-1 and then interact with TNF-receptor-associated factor 6 (TRAF6). The activated TRAP6 forms a complex with TGF-β-activated kinase (TAK1) and TAK 1 binding protein (TAB) to activate NF-κB-inducible kinase (NIK). The activated NIK further phosphorylates the IKKβ enzyme complex, and the activated IKKβ enzyme complex acts on the inhibitor of NF-κB, IκBα, which phosphorylates the two serine sites of IκBα, resulting in ubiquitination and degradation of IκBα.[22] In addition, NF-κB is released from the complex of IκBα/NF-κB and migrates into the nucleus, resulting in the transcription, translation, and ultimately the release of inflammatory factors.[23] A study has shown that silencing of TLR4 can reduce the number of inflammatory cells in BALF, thereby reducing inflammatory damage.[24] In addition, the inhibition of TLR4 can reduce the oxidative damage of lung epithelial cells by reducing MPO.[25] Inhibition of TLR4 has also been found to regulate Nrf2 (a transcription factor that resists oxidative stress) to reduce the damage to human endometrial epithelial cells.[26] Our study found that the activity of TLR4/NF-κB signaling pathway was significantly increased in lung tissue of LPS-induced mice, and the expression of TLR4 and NF-κB p65 was significantly increased, indicating that TLR4/NF-κB signaling pathway is involved in LPS-induced ALI/ARDS. However, MUC1 effectively reduced the expression of TLR4 and NF-κB p65. This may be the mechanism by which MUC1 mitigates ALI/ARDS.

To sum up, MUC1 has shown a good therapeutic effect on ALI/ARDS, showing its good application prospects. Overexpression of MUC1 in BEAS-2B cells effectively improved LPS-induced damage *in vitro*. In addition, recombinant mouse MUCl protein reduced inflammation levels and oxidative stress levels in mice. The TLR4/NF-κB signaling pathway mediates LPS-induced ALI/ARDS, and the lung protective effect of MUC1 may be related to its inhibition of TLR4/NF-κB signaling pathway. To our knowledge, this is the first study to investigate the role of exogenous MUC1 in ALI/ARDS. We believe this study can provide new targets for the clinical treatment of ALI/ARDS.

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