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Anti-CD11c antibody, Efalizumab attenuate ventilator-induced lung injury

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Abstract. – BACKGROUND: The pathophysiology of ventilator-induced lung injury (VILI) involves multiple mechanisms including inflammation and inflammatory cells infiltration. The anti-CD11c monoclonal antibody, Efalizumab has been demonstrated to inhibit the T cell activation, migration and adhesion to keratinocytes.

MATERIALS AND METHODS: In this study, we induced lung injury with mechanical ventilation in male Sprague-Dawley rats, the rats were divided into four groups: lung-protective ventilation (LV), injurious ventilation (HV), HV+human IgG control and HV+ Efalizumab groups. Then we detected the lung tissue wet/dry ratio, and the activity of myeloperoxidase (MPO) was determined. The concentration of protein, TNF-a, IL-6, IL-1b and MIP-2 in the BALF were detected by ELISA. The expression ICAM-1 was measured by Realtime PCR.

RESULTS: Compared with the human IgG control treated group, the treatment of Efalizumab attenuate the ventilator-induced lung injury, including the wet/dry ratio and the activity of myeloperoxidase (MPO), meanwhile, the level of pro-inflammatory cytokines, such as TNF-a, IL-6, IL-1b and MIP-2 were decreased in the BALF of Efalizumab-treated group rats compared with the human IgG-treated control group. In addition, the histopathological index of ventilator-induced lung injury was improved after efalizumab treatment, that also reduced the recruitment of inflammatory cells into the lung, such as neutrophils.

CONCLUSIONS: Our data suggested that Efalizumab could protect rat from ventilator-induced lung injury and improve the survival time through the inhibition of intrapulmonary inflammatory response.

Key Words:
Efalizumab, Ventilator-induced lung injury, Intrapulmonary inflammatory.

Introduction

Acute lung injury (ALI) and acute respiratory distress syndrome(ARDS) are characterized by a disturbance of the alveolar-capillary barrier associated with several clinical disorders[1,2]. In addition, mechanical ventilation has been part of basic life support for several decades. Several potential drawbacks and complications have been identified early in the use of mechanical ventilation[3]. During mechanical ventilation, high end-inspiratory lung volume (whether it be because of large tidal volume (VT) and/or high levels of positive end-expiratory pressure) results in a permeability type pulmonary oedema, called ventilator-induced lung injury (VILI). Of these, ventilator-induced lung injury has recently received much attention in both the experimental[4] and the clinical field[5,6].

Recent studies indicate that proinflammatory cytokines and inflammatory responses play an important role in the development of VILI[7]. Neutrophils are an important component of the inflammatory response that characterizes acute lung injury (ALI)[8-10]. The accumulation of activated neutrophils in the lungs is an early step in the pulmonary inflammatory process that leads to lung injury. Moreover, infiltration of activated neutrophils into the lung is an important part of the inflammatory response in acute lung injury. The neutrophils participating in acute lung injury have the ability to migrate to the site of lung injury by crossing endothelial barriers between blood and lung[11-13]. The process of leukocytes recruitment comprises a tightly regulated cascade of adhesive interaction between leukocytes and endothelial cells. Two adhesion molecules are believed to play critical roles in this migration process: lymphocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1). LFA-1 is expressed on memory T cell and ICAM-1 is expressed on vascular endothelial cells at sites of inflammation as well as on keratinocytes in a variety of T cell-mediated disorders[14,15].

Efalizumab (Raptiva, anti-CD11a) is a humanized form of a murine antibody directed against

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CD11a, the subunit of LFA-1. Previous studies suggested that efalizumab inhibits multiple key inflammatory step by binding to CD11a, such as T cell activation[16], T cell trafficking[17] and T cell adhesion[16]. These data suggested that efalizumab might have a therapeutic effect on ventilator-induced lung injury via inhibiting the inflammatory response and the infiltration of inflammatory cells.

In this study, we hypothesised that efalizumab could protect the rat from ventilator-induced lung injury through the suppression of inflammatory response and neutrophils infiltration. So we treated ventilator-induced lung injury rats with efalizumab or human IgG protein via tail vein injection. Our results indicated that the treatment of efalizumab attenuated the lung injury induced by ventilator. Meanwhile, compared with the human IgG-treated group, the administration of efalizumab reduced the infiltration of neutrophils and decreased the MPO activity. In addition, we also found that efalizumab treatment suppressed the expression of pro-inflammatory cytokines in BALF, such as TNF-α, IL-6, IL-1b and MIP-2. In summary, our data suggested that efalizumab has a therapeutic effect of ventilator-induced lung injury.

Materials and Methods

Animals

Eighty specific pathogen-free male Sprague-Dawley rats weighing 240-290g were purchased from the SLRC Laboratory (ShangHai, China). Rats were housed in pathogen-free laboratory for 72h with free access to water and food. The rats were randomized into four groups: lung-protective ventilation group (LV) (n=20), injurious ventilation group (HV) (n=20), HV+human IgG-treated group (n=20) and HV+efalizumab-treated group (n=20). Rats were sacrificed at indicated time points after ALI induction and different administration.

Mechanical Ventilation

Rats received efalizumab (2.5 mg/kg, dissolved in phosphate buffered saline (PBS)) or human IgG protein (2.5 mg/kg) via tail vein injection. Thirty minutes later, rats were anesthetized by intraperitoneal injection of thiopental (37 mg/kg). Thiopental was chosen as the anesthetic agent because it is slowly metabolized in rodents. The dose that we used ensures a profound anesthesia for at least 4 h. A tracheostomy was performed, and each animal was injected with succinylcholine (5 mg/kg) via the dorsal penile vein, after which the animal was ventilated with a rodent volume ventilator (Harvard Apparatus, Ealing, Les Ulis, France). Two ventilation modalities were used, for 2h each, as follows: (1) health control, with low VT ventilation (7 ml/kg VT and 3 cm H_2O positive end-expiratory pressure [PEEP], 40 breaths/min) (LV group) and (2) an injurious strategy, using a high VT and no PEEP (42 ml/kg VT, zero end-expiratory volume [ZEEP], 40 breaths/min) (HV group). Then rats were killed by an intravenous injection of thiopental at the end of the mechanical ventilation period, the thorax was opened, and the blood was sampled by cardiac puncture. Simultaneously, three BAL procedures were performed, each with 2 ml of normal saline. The retrieved fluid and the blood were centrifuged (2,000 g, for 10 min), and the supernatant and plasma were stored for further processing. The survival after mechanical ventilation was assessed and the cumulative survival curve was depicted using the Kaplan-Meier method.

Histopathologic Analysis

Following sacrifice, the whole left lower lobe of the lung was fixed in a 4% formaldehyde neutral buffer solution for 24 hours, dehydrated in a graded ethanol series, embedded in paraffin, and sliced at 5 m. Paraffin sections were stained with hematoxylin-eosin (HE) for histopathological analysis.

Lung Wet/Dry Weight Ratio

The superior lobe of the right lung was cleansed and weighed to obtain the wet weight, and was then placed in an oven at 80°C for 48h for measurement of the dry weight. The ratio of the wet weight to dry weight was calculated to assess the tissue edema. The experiment was repeated three times and results were shown with the mean value.

Btonchoalveolar Lavage (BAL) Examination

The trachea was exposed and cannulated with a catheter. The left lung was lavaged for 4 times with sterile phosphate buffered saline (PBS) in a volume of 0.5 ml/wash. The fluid recovered after lavage was greater than 90% on average. The BAL fluid (BALF) was centrifuged at 2000 rpm for 10 min at 4°C, and the supernatant was stored at –80°C for cytokine and protein analysis, while the cell pellet was resuspended in PBS for counting the neutrophils.

ELISA

Detection of TNF-α, IL-6, IL-1b and MIP-2 amount with ELISA according to the manufacturer's protocol. The experiment was repeated three times and results were shown with the mean value.

Neutrophil Counts

BALF was done on different treated groups rats to obtain total cell count and percentage of neutrophils as well as myeloperoxidase (MPO) activity. The activity of MPO was assessed using previously described, standard methods[18]. The experiment was repeated three times and results were shown with the mean value.

The Measure of Protein Concentration in Lung BALF

The concentration of protein in the BALF was measured using Bradford reagent (Bio-Rad Protein Assay kit, Hercules, CA, USA). Briefly, 160 μl of each standard and sample solution was pipeted into separate microtiter plate wells, and 40 μl of the dye reagent was added to each well and mixed thoroughly. The mixture was incubated at room temperature for at least 5 min before measurement of the OD at 595 nm. Comparison to a standard curve provided a relative measurement of the protein concentration. The experiment was repeated three times and results were shown with the mean value.

Myeloperoxidase (MPO) Activity Assay

MPO activity in the homogenized lung tissue was measured as described by Gray et al[19]. The MPO concentration was detected using a MPO ELISA kit (Bluegene, Shangai, China). Briefly, the lung tissue were homogenized and centrifuged at 15000 rpm for 20 min at 4°C. The supernatants and standard sample were added into a microtiter plate (100 μl/well) precoated with a murine anti-MPO mAb. After incubation for 1h at 37°C, the plate was washed for 6 times followed by addition of the substrate and stop solution, and the optical density (OD) at 450 nm was measured using a microplate reader. All the sample were assayed in triplicate.

RNA isolation and Real-time PCR

Total RNAs were isolated from Rat lung tissues by TRIzol reagent, and reverse transcriptions were performed by Takara RNA PCR kit (Takara, Dalian, China) following the manufacturer's instructions. In order to quantify the transcripts of the interest genes, real-time PCR was performed using a SYBR Green Premix Ex Taq (Takara, Utsu, Shiga, Japan) on the ABI7900 fast real-time detection system. The primer sequences used are available upon request. The experiment was repeated three times and results were shown with the mean value.

Statistical Analysis

All the data were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) and expressed as Means±SD. Significant differences were assessed by one-way analysis of variance (ANOVA) followed by Fisher protected least significant difference test. A probability p value < 0.05 was considered to indicate a statistical significance.

Results

The Treatment of Efalizumab Attenuated Ventilator-Induced Morphologic Lesions in Lung Tissue

Previous studies demonstrated that efalizumab inhibits multiple inflammatory response by binding to CD11a, such as T cell activation[16], T cell trafficking[17] and T cell migration[16]. So we hypothesized that efalizumab could alleviate the ventilator-induced lung injury by inhibiting the inflammatory responses. Then, we administrated ventilator-induced ALI rats with efalizumab via tail vein injection, the human IgG protein as the control. As the data showed in Figure 1, we observed that lung specimens from HV group with human IgG-treated (Figure 1C) or vehicle-treated (Figure 1B) animals displayed significant histological abnormalities, including infiltration of leukocytes into the interstitial spaces, hemorrhage, and marked swelling of the alveolar walls. In addition, we found that the treatment of efalizumab (Figure 1D) improved the histology of the lung compared with the human IgG-treated control group (Figure 1C) and not treated HV group (Figure 1D). These results indicated that efalizumab could alleviate ventilator-induced morphologic lesions in lung tissue.

Effect of Efalizumab Treatment on Ventilator-Induced Lung Edema Index

To quantitatively analyze the effect of efalizumab on the degree of ventilator-induced lung edema index, the right upper lobe of the lungs was measured in each animal. Our result showed

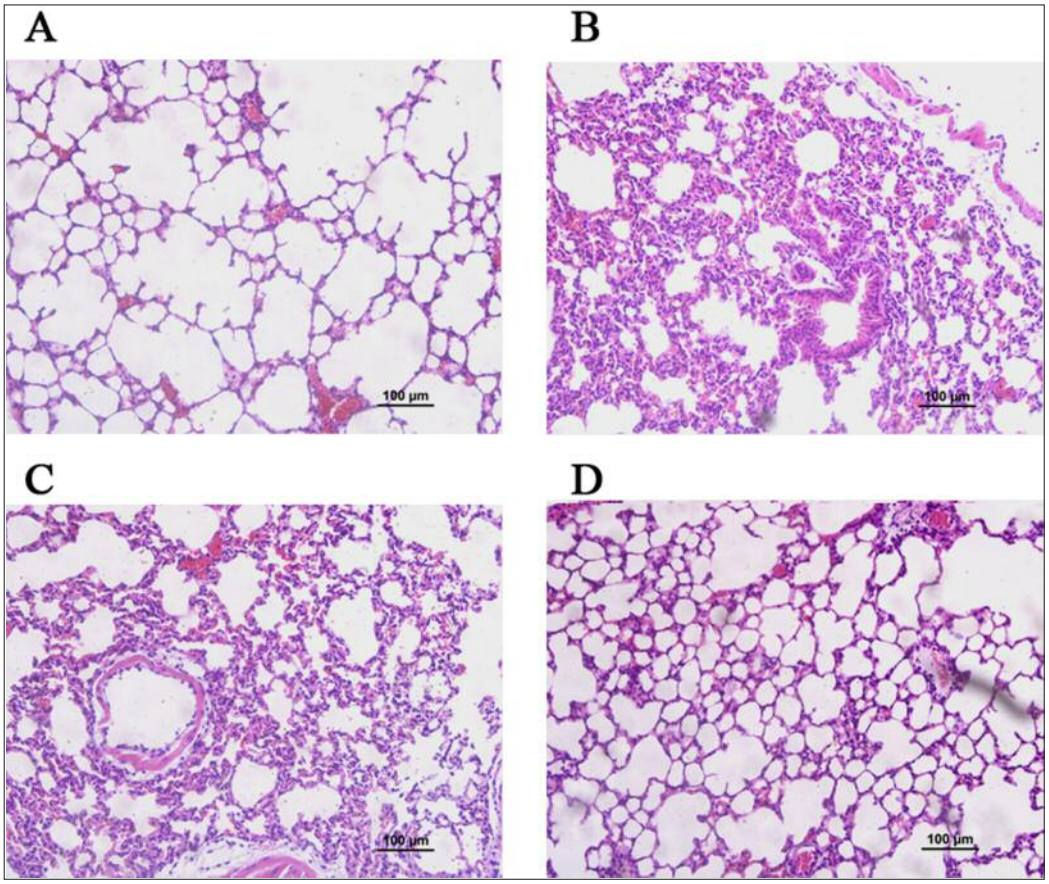

Figure 1. Histopathological index of injured rats lungs. The H&E-stained lung sections following the different treatments in ***(A)*** LV group (healthy control); ***(B)*** HV group (vehicle-treated group); ***(C)*** human IgG-treated control group and ***(D)*** efalizumab-treated group.

that the efalizumab-treated ALI rats had a significantly lower wet/dry ratio compared to the human IgG-treated control group and vehicle-treated group (Figure 2). This data demonstrated that efalizumab could decrease lung edema caused by ventilator-induced lung injury.

The Treatment of Efalizumab Reduced the Activity of MPO and the Infiltration of Neutrophils Caused by Ventilator-Induced Lung Injury

To further investigate the effect of efalizumab on the inflammatory cells infiltration, we detected the level of the activities of MPO, a reliable marker of neutrophil infiltration. We found that the treatment of efalizumab reduced the activity of MPO compared with the human IgG-treated group and the vehicle-treated HV group (Figure 3A). Then, we measured the neutrophils count in the BALF, the result was consistent with the data

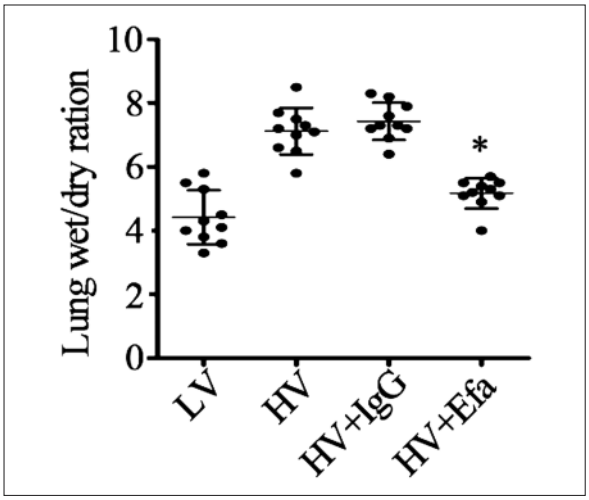

Figure 2. The wer/dry ratio of different treated group of rats. The Wet/dry weight ration of lung tissues from rats with indicated treatment was measured. Data are expressed as mean ± SEM of the values of 10 rat of each group. $p < 0.05$ compared with the IgG-treated control group (HV+IgG) or vehicle-treated group (HV).

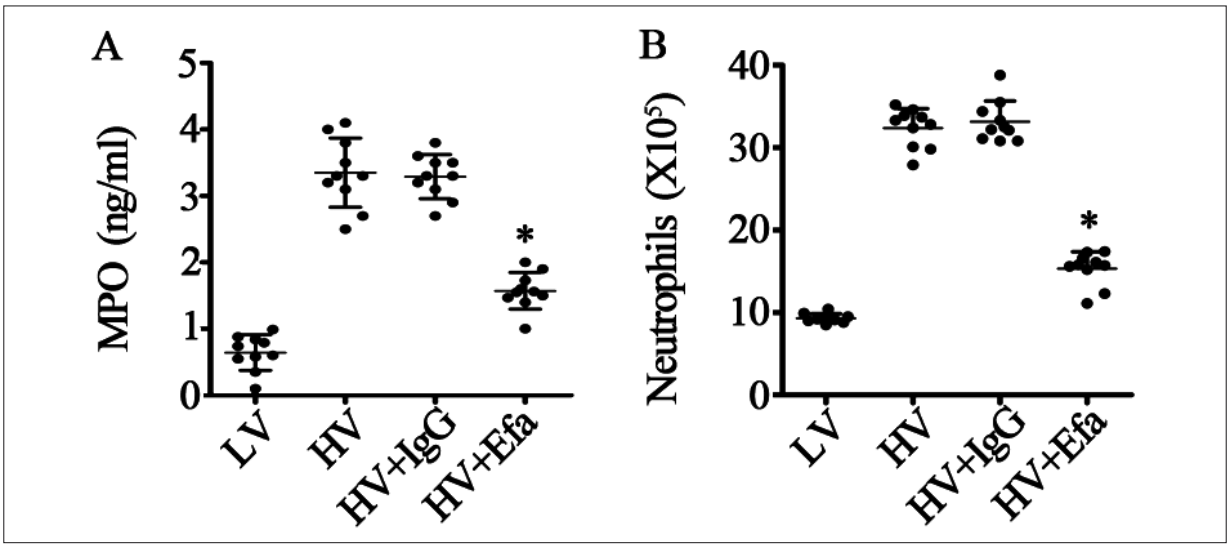

Figure 3. Treatment with efalizumab reduced MPO activity and neutrophils infiltration caused by ventilator-induced lung injury. The myeloperoxidase (MPO) activity ***(A)*** and the neutrophils count ***(B)*** in lung tissues from rats with indicated treatment was measured. Data are expressed as mean ± SEM of the values of 10 rat of each group. Data are expressed as mean ± SEM of the values of 10 rat of each group. *$p < 0.05$ compared with the IgG-treated control group (HV+IgG) or vehicle-treated group (HV).

of the activity of MPO (Figure 3B). These results suggested that efalizumab could inbibit the infiltration of neutrophils into lung tissue.

Treatment with Efalizumab Downregulated the Expression of ICAM-1 and the Protein Concentration in Injury Lung

Previous studies indicated that ICAM-1 was involved in intrapulmonary recruitment of leukocytes. So, we detected the expression of ICAM-1 in different treated ALI rats. we found that the expression of ICAM-1 was upregulated in the ventilator-induced lung injury rats. Compared with the IgG-treated control and the vehicle-treated HV group, the expression of ICAM-1 was downregulated in the efalizumab-treated group (Figure 4A).

To assess the degree of lung injury, we then measured the ventilator-induced protein leakage

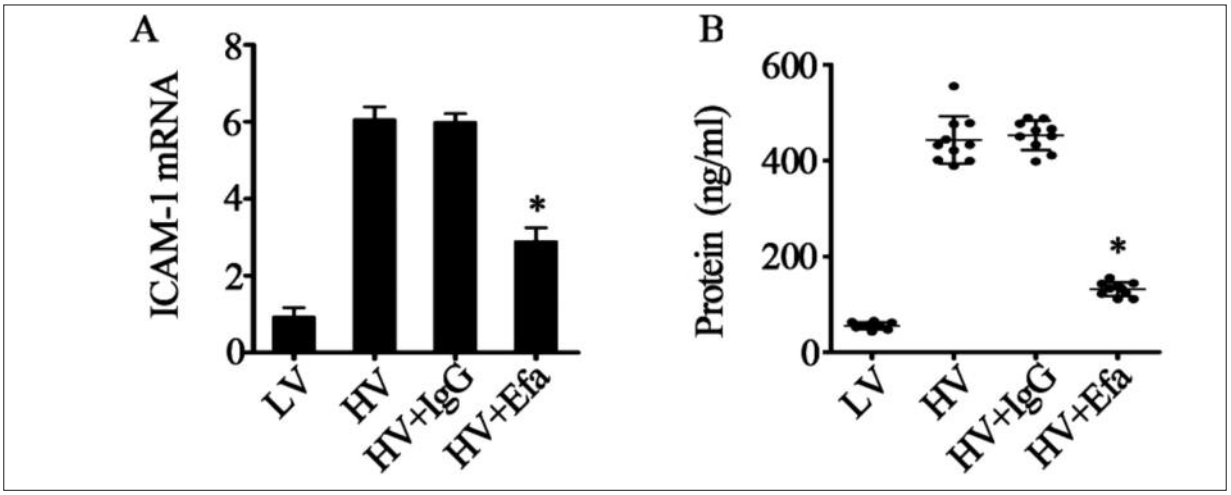

Figure 4. Treatment with efalizumab downregulated the expression of ICAM-1 and the protein concentration in injury lung. ***(A)*** The mRNA levels of ICAM-1 in lung tissues from rats with indicated treatment was measured by Realtime PCR. ***(B)*** The protein concentration in lung tissue from rats with indicated treatment was measured. Data are expressed as mean ± SEM of the values of 10 rat of each group. *$p < 0.05$ compared with the IgG-treated control group (HV+IgG) or vehicle-treated group (HV).

in BALF. As shown in Figure 4B, the concentration of protein in the efalizumab-treated rats was decreased compared to the IgG-treated control group (Figure 4B).

Treatment with Efalizumab Suppresses TNF-α, IL-1β, IL-6 and MIP-2 Elevation Caused by Ventilator-Induced Lung Injury

The activation and the secretion of pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1β and MIP-2 in the homogenized lung, is considered to play a critical role in the pathogenesis of lung injury. To further analysis the function of efalizumab on inflammatory responses in ventilator-induced lung injury, we detected the level of some pro-inflammatory cytokines in the BALF. We found that the administration of efalizumab decreased the concentration of inflammatory cytokines in the rats BALF, such as TNF-α (Figure 5A), IL-6 (Figure 5B), IL-1β (Figure 5C) and MIP-2 (Figure 5D), compared with the IgG-treated control group.

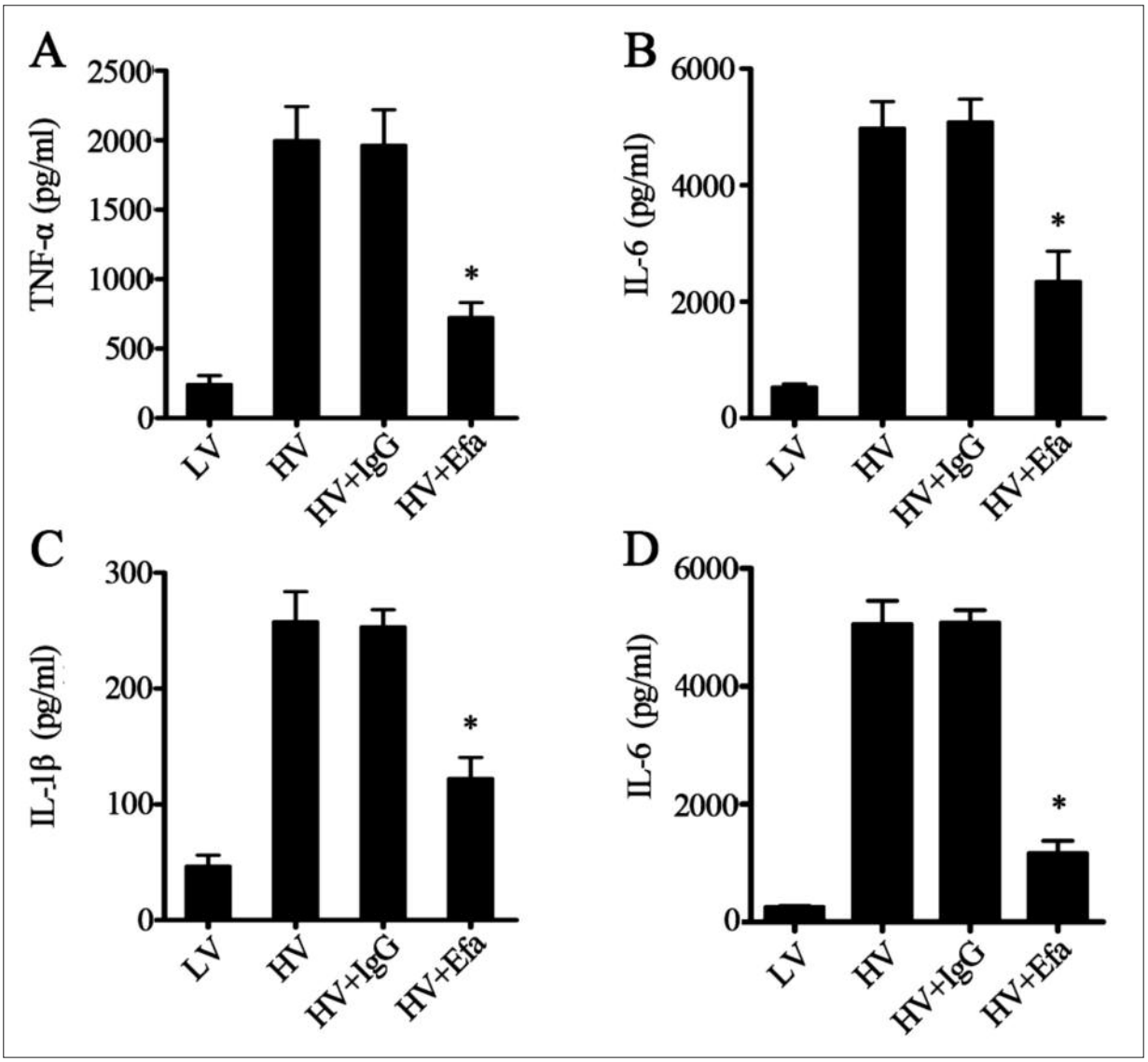

Figure 5. The production of proinfammatory cytokines in BALF of ventilator-induced lung injury rats. ***A,*** The concentration of TNF-α in BALF of rats with indicated treatment was monitored by ELISA. ***B,*** The concentration of IL-6 in BALF of rats with indicated treatment was monitored by ELISA. ***C,*** The concentration of IL-1β in BALF of rats with indicated treatment was monitored by ELISA. ***D,*** The concentration of MIP-2 in BALF of rats with indicated treatment was monitored by ELISA. Data are expressed as mean ± SEM of the values of 10 rat of each group. $*p < 0.05$ compared with the IgG-treated control group (HV+IgG) or vehicle-treated group (HV).

Treatment with Efalizumab Improves Survival After Ventilator-Induced Lung Injury

To assess a long-term beneficial effect of efalizumab in our model of ventilator-induced lung injury, the survival rate between different treated rats was compared. As shown in Figure 6, the survival was significantly improved in efalizumab-treated group rats in comparison with the human IgG-treated control group rats and vehicle-treated group rats (Figure 6).

Discussion

Activation of inflammatory mediators is considered to play a major role in the pathogenesis of injurious ventilation[20]. In these process, the infiltration of pathogenic lymphocytes into the lung tissue is one of the critical steps. Previous studies suggested that interaction of cell adhesion molecules (CAM) play central roles in mediating immune and inflammatory responses. Leukocyte function-associated antigen (LFA-1, $\alpha_L\beta_2$, and CD11a/CD18) and very late antigen (VLA-4, $\alpha_4\beta_1$, and CD49d/CD29) are members of integrin-type CAM that are predominantly involved in leukocyte trafficking and extravasation. LFA-1 is exclusively expressed on leukocytes and interacts with its ligands ICAM-1, -2, and -3 to promote a variety of homotypic and heterotypic cell adhesion events required for normal and pathologic function of immune system. Recently, the inhibition of LFA-1 by peptides, small molecules, and its monoclonal antibody as an adhesion-based therapeutic strategies for inflammation and autoimmune diseases has been shown.

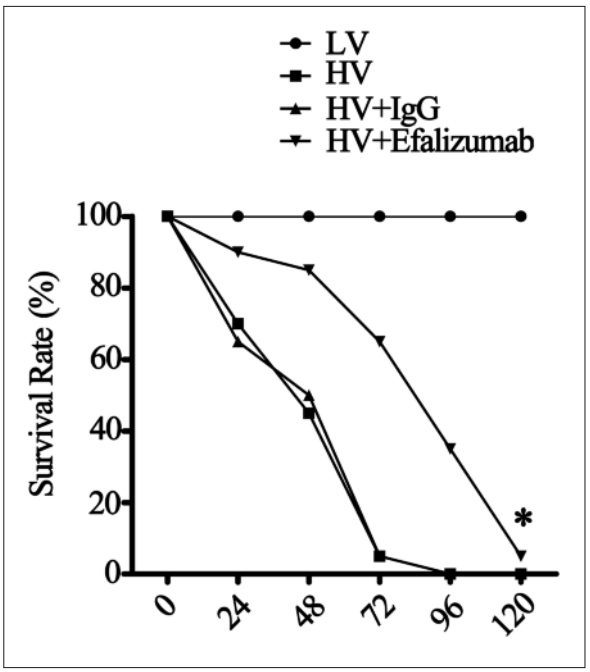

Figure 6. Treatment with efalizumab delayed the death of rats with ventilator-induced lung injury. The Kaplan-Meier survival curves of rats (n=10) with indicated treatment were monitored. *$p < 0.05$ compared with the IgG-treated control group (HV+IgG) or vehicle-treated group (HV).

Efalizumab, a T-cell-targeted, recombinant, humanized, monoclonal IgG1 antibody[21,22], it could inhibit T-cell activation, migration and reactivation[23,24] and reduces the chemotactic properties of monocytes and neutrophils and downregulates VLA-4[25]. So we hypothesized that efalizumab could attenuate the ventilator-induced lung injury through inhibiting the inflammatory responses.

In our study, we found that the treatment of efalizumab improved the histology of lung compared with the human IgG-treated control group (Figure 1C) and no treated HV group (Figure 1D). This results suggested that efalizumab have the therapeutic effect on ventilator-induced lung injury. Efalizumab might be used as a drug for the treatment of ventilator-induced lung injury. In addition, we analysized the effect of efalizumab on the degree of ventilator-induced lung injury edema index; we found that the data was consistent with the results of histology (Figure 2).

As we know, neutrophils rolling along the endothelium may initiate a cascade of cellular interactions, resulting in endothelial damage and subsequent development of multiple organ damage[12]. Our experimental data showed that as a marker of neutrophil influx, the MPO activity was decreased in the efalizumab-treated rats compared to the human IgG-treated group rats (Figure 3A). Meanwhile, we measured the cell count of neutrophils infiltration into the lung tissue. Indeed, the treatment of efalizumab could inhibit the infiltration of neutrophils into target lung tissue. Above data indicated that efalizumab could suppress the infiltration of neutrophils into lung tissue in the ventilator-induced lung injury. Moreover, the expression of ICAM in lung tissue (Figure 4A) and the concentration of protein in lung BALF (Figure 4B) was decreased in efalizumab-treated group compared with the human IgG-treated group and vehicle-treated HV group.

In addition, the inflammatory responses play an important role in the development and pathogenesis of ventilator-induced lung injury. So we detected the production of pro-inflammatory cytokines in the lung BALF; we found that the administration of efalizumab reduced the expression of inflammatory cytokines expression, such as TNF-α (Figure 5A), IL-6 (Figure 5B), IL-1β (Figure 5C) and MIP-2 (Figure 5D). This suggested that efalizumab could suppress the inflammatory responses through inhibiting the inflammatory cells infiltration.

Conclusions

Our study described the therapeutic effect of efalizumab on the ventialtor-induced lung injury. As a T cell targeted monoclonal antibody, efalizumab treatment significantly alleviated the ventilator-induced lung injury and prolonged the survival time of injurious ventilation treated rats (Figure 6). Efalizumab might used as a drug for the treatment of ventilator-induced lung injury by inhibiting the infiltration of neutrophils into lung tissue and the inflammatory responses in the target lung tissue.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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