

# Tumor Necrosis Factor- $\alpha$ Plays an Initiating Role in Extracorporeal Circulation-induced Acute Lung Injury

Tao Li · Nanfu Luo · Lei Du · Jing Zhou ·  
Jie Zhang · Lina Gong · Nenggang Jiang

Received: 25 September 2012 / Accepted: 24 December 2012 / Published online: 25 January 2013  
© Springer Science+Business Media New York 2013

## Abstract

**Background** Despite advances in critical care, the mortality rate for patients with acute lung injury (ALI) remains high. The aim of this study was to test the hypothesis that tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) plays an initiating role in the onset of extracorporeal circulation (ECC)-induced ALI. **Methods** Eight New Zealand rabbits subjected to 1 h of ECC and 40 min of observation after termination of ECC were used for monitoring pulmonary nociceptor activity. Fifty Sprague-Dawley (SD) rats that received 2 h of ECC and 4 h of rest were used to measure the pulmonary function and inflammatory cytokines release, including total cells, neutrophils, and TNF- $\alpha$  in bronchoalveolar lavage (BAL) and white blood cell (WBC) and neutrophils in blood. An additional 40 SD rats were randomized to pretreatment with inhalation of phosphate buffer solution

(control group), IgG (IgG inh group), or TNF- $\alpha$  antibody (anti-TNF- $\alpha$  inh group) and venous injection of TNF- $\alpha$  antibody (anti-TNF- $\alpha$  iv group). After 2 h of ECC and 4 h of rest, the arterial blood and BAL fluid were collected for measurement of arterial oxygen pressure (PaO<sub>2</sub>) and inflammatory cytokines release. The left-lower-lung tissues of animals were stained with hematoxylin & eosin (H&E). **Results** The results demonstrated that the activities of airway nociceptor and TNF- $\alpha$  release were similarly upregulated at the early stage and in a time-related manner in ECC-induced ALI. Pretreatment with TNF- $\alpha$  antibody inhalation, but not venous injection, improved pulmonary function, inhibited pulmonary inflammation, and attenuated pulmonary histopathological changes after ECC. **Conclusion** We concluded that TNF- $\alpha$  played an important role in the pathogenesis of ALI and acted as an initiating cytokine at the early stage of ECC-induced ALI.

T. Li and N. Luo contributed equally to this work.

T. Li · N. Luo · L. Du · L. Gong  
Department of Anesthesiology and Translational Neuroscience  
Center, West China Hospital, Sichuan University,  
Chengdu 610041, China

L. Du (✉)  
Department of Anesthesiology, West China Hospital,  
Sichuan University, 37 Wainan Guoxue Road,  
Chengdu 610041, Sichuan, China  
e-mail: scudulei@gmail.com

J. Zhou · N. Jiang  
Department of Laboratory Medicine, West China Hospital,  
Sichuan University, Chengdu 610041, China

J. Zhang  
Key Laboratory of Transplant Engineering and Immunology,  
Ministry of Health, West China Hospital, Sichuan University,  
Chengdu 610041, China

**Keywords** TNF- $\alpha$  · Cardiopulmonary bypass ·  
Inflammation · Acute lung injury

## Introduction

Acute lung injury (ALI) is a common cause of mortality in intensive care [1, 2]. Approximately one third of ALIs were induced by noninfectious systemic inflammatory response syndrome (SIRS) from trauma, shock, aspiration, and cardiac surgery with extracorporeal circulation (ECC) [3, 4]. Pathogenesis of ALI includes pulmonary inflammation, which is characterized by neutrophil accumulation in the lungs during the early stages of ALI and lymphocyte-induced lung fibrosis during the late stages. Neutrophils adhere to the injured capillary endothelium and migrate through the interstitium into the air space. In the air space,

cytokines, such as interleukin (IL)-1, IL-6, IL-8, IL-10 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), secreted by alveolar macrophages act locally to stimulate chemotaxis and activate neutrophils [5–7]. Despite the extensive work that has been done to characterize the inflammation that occurs in ALI, the identity of the cellular elements and related inflammatory mediators that initiate or promote pathogenesis are still unknown [8].

Finding out the initiating cytokines of ECC-induced ALI is of great clinical relevance and believed to be beneficial in the early diagnosis of ALI and in improving the prognosis of the patient. Among those mediators of inflammation, TNF- $\alpha$  is a prospective candidate. TNF- $\alpha$  is a member of a group of cytokines that are involved in SIRS and stimulate the acute phase reaction. Previous animal and clinical studies have discovered the pathogenic mediator role of TNF- $\alpha$  in pulmonary inflammation and identified the therapeutic effect of monoclonal TNF- $\alpha$  antibody [9, 10]. Our latest study also suggested that ALI is accompanied by an increase in TNF- $\alpha$  release and neutrophil adhesion [11]. In this study we postulated that TNF- $\alpha$  might play an important role in pathogenesis of ALI and act as an initiating cytokine at the early stage of ECC-induced ALI.

## Material and Methods

This study was approved by the Institutional Animal Care and Use Committee of Sichuan University, and all animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

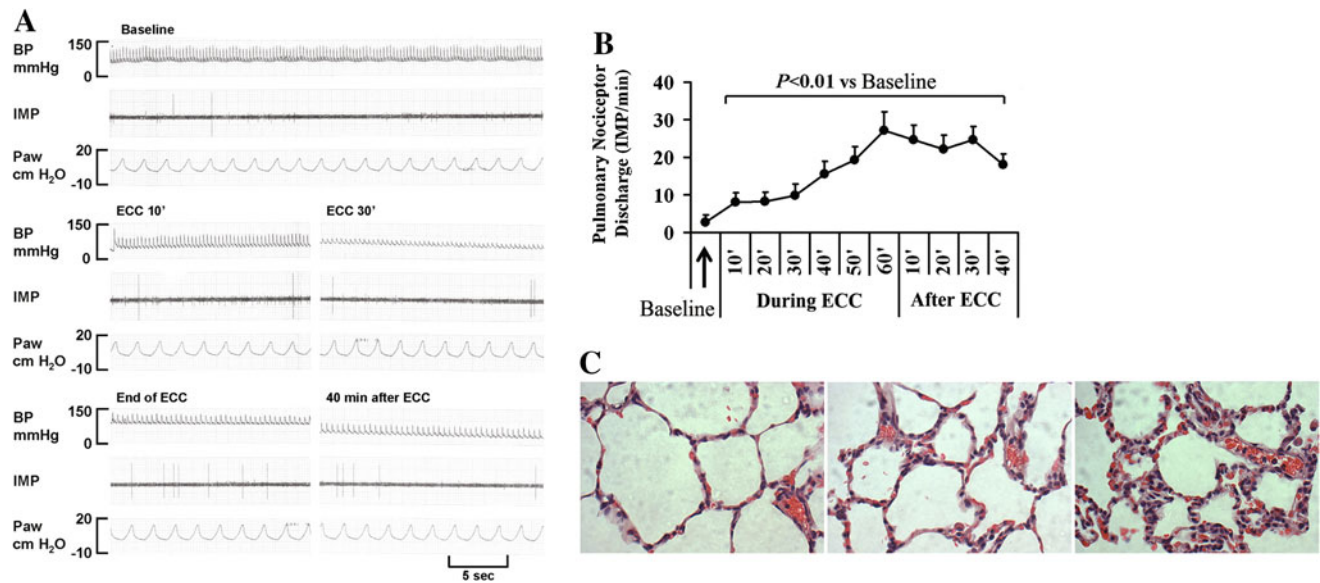
### Recording Technique and Identification of Airway Nociceptor

Eight male adult New Zealand rabbits, weighing 2.5–3.0 kg, were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). A midline incision was made to expose the trachea and vagus nerve. The trachea was cannulated low in the neck and the lungs were mechanically ventilated with a Harvard ventilator model 683 (Harvard Apparatus, Holliston, MA). Positive end expiratory pressure (PEEP) was maintained by placing the expiratory outlet under 3–4 cmH<sub>2</sub>O. Airway pressure ( $P_{aw}$ ) was monitored at the tracheal tube with a Statham pressure transducer P23 (Gulton-Statham Products, Rochester, NY). The chest was opened widely in the midline to allow location of the receptive field. The values of  $P_{aw}$  and afferent activities were recorded by a thermorecorder (Astro-Med Dash IV; Astro-Med, Inc., West Warwick, RI). Single-unit activities were

recorded as described previously [12, 13]. Briefly, the vagus nerve (either right or left) of the rabbit was separated from the carotid sheath, placed on a dissecting platform, and covered with mineral oil. A small afferent bundle was cut from the vagus nerve. This bundle was dissected into thin filaments using two pairs of fine forceps. The filaments were further divided and placed on electrodes to record action potentials. The electrodes were connected to a high-impedance probe (Grass Model HIP 511; Grass Technologies, an Astro-Med subsidiary), from which the output was fed into an amplifier (Grass P511). After suitable amplification, action potentials from a single unit of the vagal sensory receptors were displayed on an oscilloscope and a loudspeaker. In addition, a voltage analog of impulse frequency was produced by a rate meter (Frederick Haer, Brunswick, ME) at a bandwidth of 0.1 s. The receptive field was located by identifying the most sensitive point on the lung surface using a glass rod with a 0.5-mm round tip. Airway nociceptors, including high-threshold A $\delta$  fiber receptors (HTARs) and C fiber receptors (CFRs), can be activated by a variety of inflammatory mediators and may serve as biosensors to monitor inflammation in the lung [3]. These biosensors were identified by their discharge patterns and confirmed by verifying their receptive fields in the lung and their conduction velocities (above 1.6 m/s for HTARs and less than 1.5 m/s for CFRs). Unit activity was expressed as impulses per minute (imp/min).

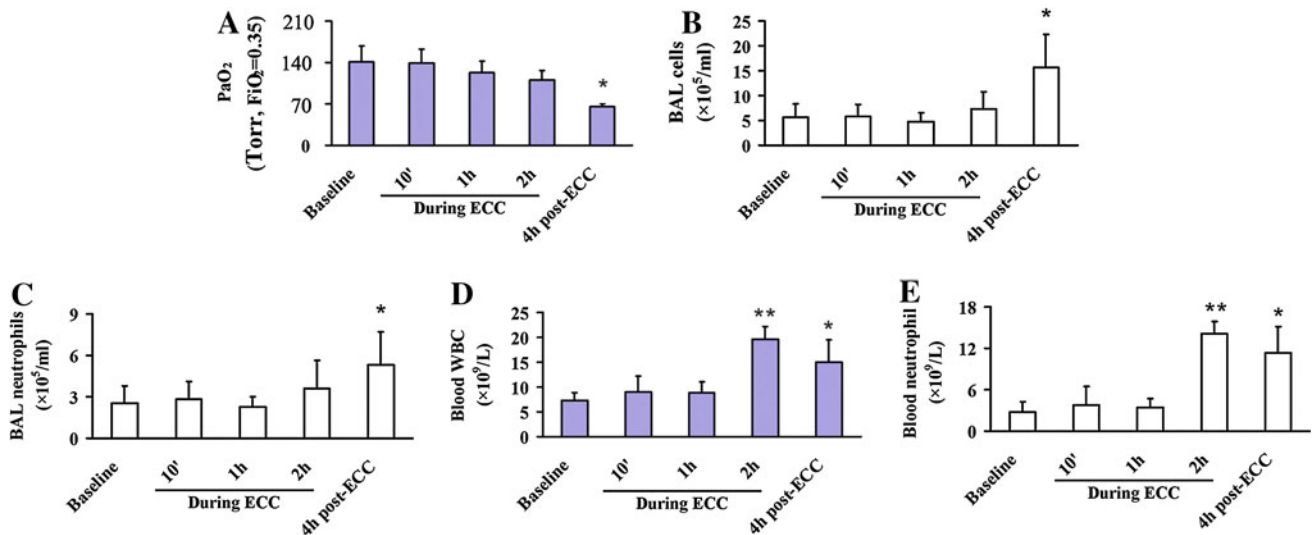
### Acute Lung Injury Model Induced by ECC

Male adult Sprague-Dawley (SD) rats, weighing 225–250 g, were anesthetized with 3 % intraperitoneal pentobarbital injection (40 mg/kg), then the ECC-induced ALI model was established as described previously [14]. Briefly, the trachea was intubated and ventilation was controlled mechanically at a respiratory rate (RR) of 65 bpm and a tidal volume ( $V_t$ ) of 7 ml/kg. After heparinization (3 mg/kg), the right common carotid artery was cannulated with a 20G retention catheter and connected with a three-way joint for measurement of blood pressure (BP). The skin was cut from the inguinal groin, and the femoral vein was cannulated with a 24G retention catheter and also connected with a three-way joint to maintain anesthesia. The two joints then were connected with a roller pump (Stöckert II, Munich, Germany) and a 1/16-in. tube, which was primed with 7 % Voluven® (Fresenius, Bad Homburg, Germany). ECC was established by a circuit from the right common carotid artery to the femoral vein, and the rate of blood flow was kept at one fourth that of cardiac output. The establishment of the rabbit ECC model was similar to that for the rat, except that the right common carotid artery and femoral vein were cannulated with 16G retention catheters and connected with a 1/8-in. tube.



**Fig. 1** The activity of airway nociceptor was increased at the early stage of extracorporeal circulation-induced ALI. **a** Representative nociceptor activity recorded from the cervical vagus nerve. **b** The time course of pulmonary nociceptor activity throughout the study. **c** Representative photomicrographs of hematoxylin & eosin-stained

left lung tissue sections (×40) harvested at the end of study. Values are expressed as mean ± SD (n = 8). ALI, acute lung injury; BP, blood pressure; IMP, impulses (sensory activity); Paw, airway pressure



**Fig. 2** **a** Arterial oxygen pressure. **b, c** Counts of total cells and neutrophils in bronchoalveolar lavage fluid. **d, e** Counts of white blood cell and neutrophils in blood throughout the study. Values are presented as mean ± SD (n = 10). \*P < 0.01, \*\*P < 0.001 vs. baseline

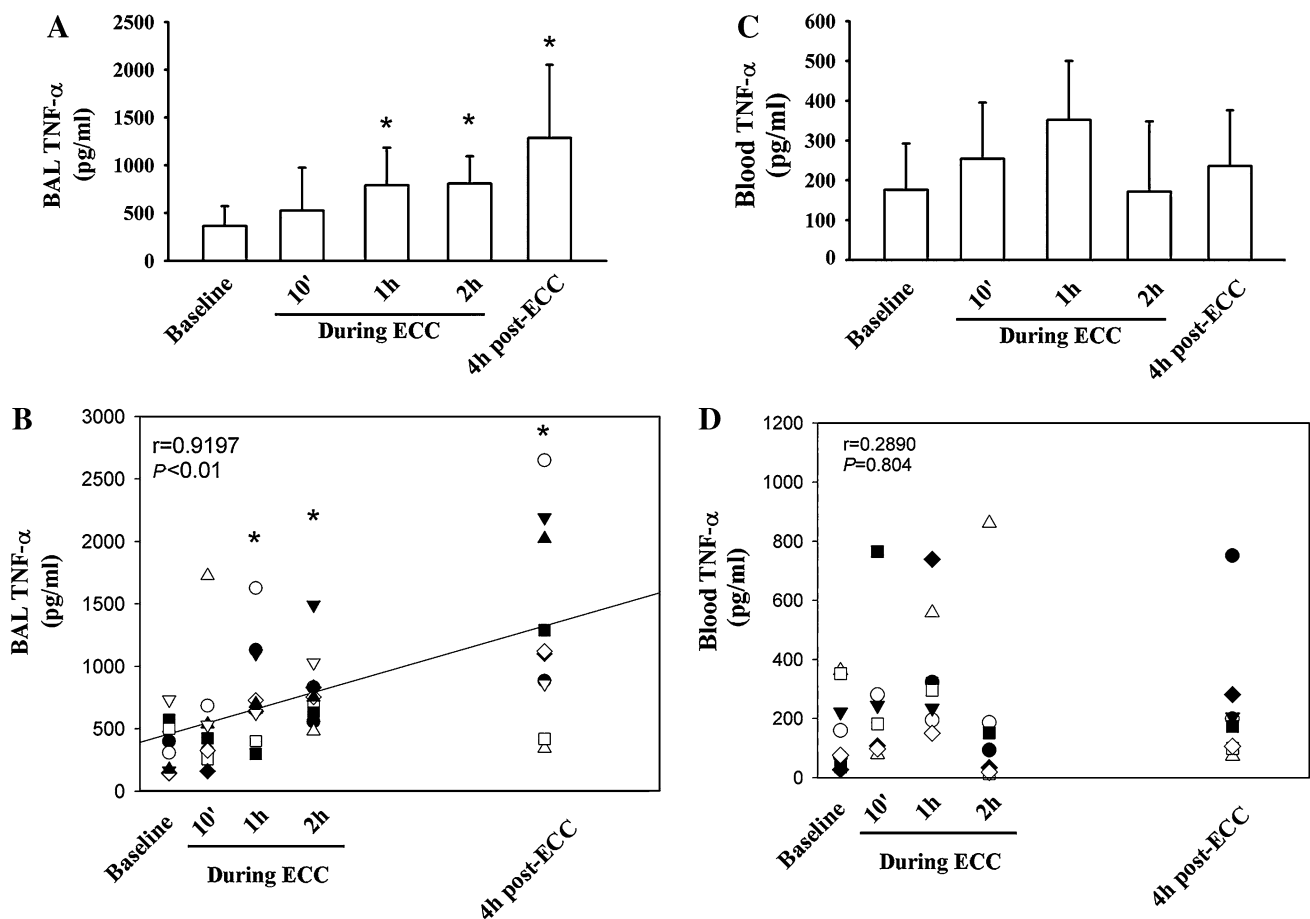
#### Determination of Inflammatory Cytokines Releases Related to ECC

In order to investigate the pulmonary inflammatory cytokines releases related to ECC, 50 male adult SD rats were employed. The blood and BAL samples of these animals were obtained at baseline, 10 min, 1 h, and 2 h during ECC, and 4 h after termination of ECC (n = 10 at each time point). The white blood cell (WBC) and neutrophil counts in the blood and total cell and neutrophils count in

the BAL fluid were measured by an Auto Hematology Analyzer (BC-3000 Plus, Mindray, Shenzhen, China). The TNF-α level in the BAL fluid was determined by a commercial ELISA kit (Bender MedSystems, Vienna, Austria).

#### Pretreatment with TNF-α Antibody Inhalation

In an additional experiment, 40 male adult SD rats, weighing 225–250 g, were pretreated with inhalation of phosphate buffer solution (control group, n = 10), IgG (IgG inh group,



**Fig. 3** The release (a) and linear regression analysis (b) of TNF- $\alpha$  in bronchoalveolar lavage fluid. The level (c) and linear regression analysis (d) of TNF- $\alpha$  in blood. Values are presented as mean  $\pm$  SD ( $n = 10$ ). \* $P < 0.01$  vs. baseline

$n = 10$ ), or TNF- $\alpha$  antibody (anti-TNF- $\alpha$  inh group,  $n = 10$ ) and venous injection of TNF- $\alpha$  antibody (anti-TNF- $\alpha$  iv group,  $n = 10$ ). After a 10-min washout, the ECC model was established as described above. After 2 h of ECC and 4 h of rest, the arterial blood and BAL fluid were collected for measurement of PaO<sub>2</sub> and inflammatory cytokines releases.

#### Histological Examination

The left-lower-lung tissues of rats or rabbits were placed in 10 % neutral formalin and embedded in paraffin, then sectioned into 5- $\mu$ m intervals and stained with hematoxylin & eosin (H&E). The results were assessed for alveolar inflammatory infiltration and interstitial edema in a blinded fashion by a pathologist.

#### Statistical Analysis

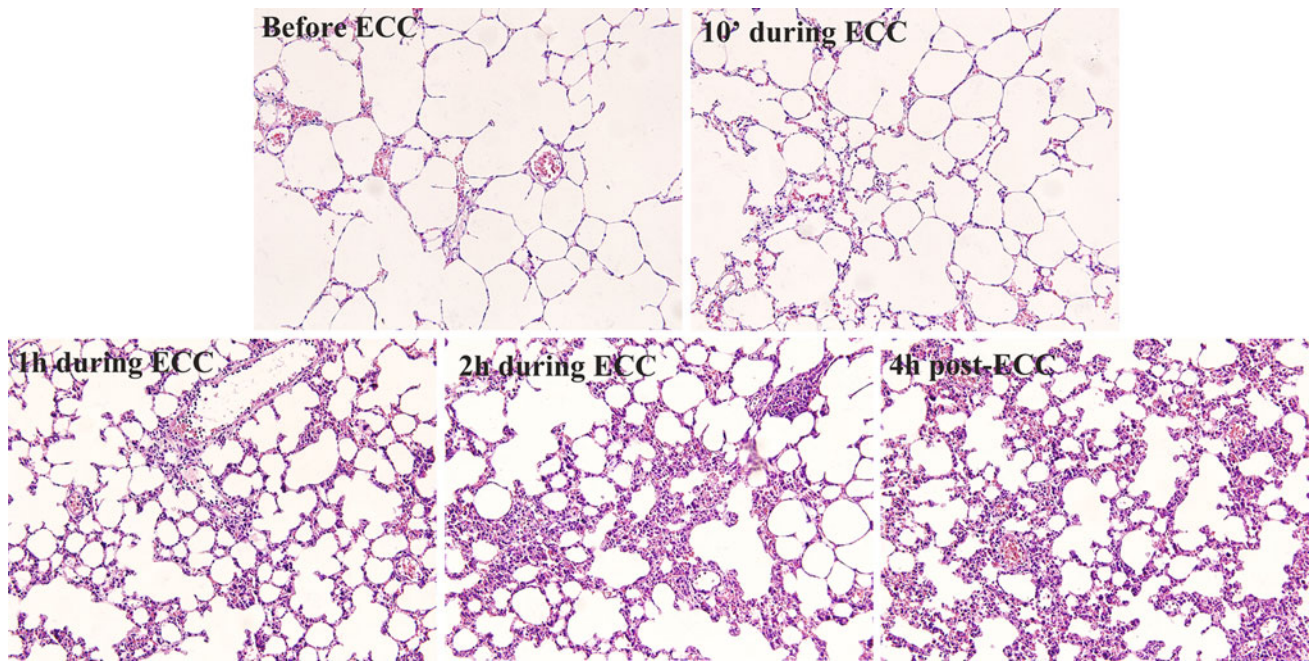
All values are presented as mean  $\pm$  SD. The result of airway nociceptor activity was analyzed by a two-sided

Student  $t$ -test. Multiple group data were analyzed by one-way ANOVA followed by Bonferroni's correction for post-hoc  $t$ -test (SPSS 13.0 software; SPSS, Inc., Chicago, IL).  $P$  values  $< 0.05$  were considered statistically significant.

#### Results

##### ECC-induced ALI Increased the Activity of Airway Nociceptor

As shown in Fig. 1a and b, under basal conditions, airway nociceptors discharged sporadically at a very low frequency ( $2.6 \pm 0.9$  imp/min). After 10 min of ECC, the frequency significantly increased to  $8.1 \pm 1.4$  imp/min ( $P < 0.01$  vs. baseline). This increase was sustained until the end of 60 min of ECC ( $27 \pm 6.0$  imp/min,  $P = 0.001$  vs. baseline). During the 40-min observation after termination of ECC, the activities of the nociceptors were still at a relatively high level ( $1827$  imp/min,  $P < 0.01$  vs. baseline). Data of H&E staining indicated that the neutrophil



**Fig. 4** Representative photomicrographs of hematoxylin & eosin-stained left lung tissue sections ( $\times 20$ ) harvested at baseline, during 10 min, 1 h, and 2 h of extracorporeal circulation, and 4 h after termination of extracorporeal circulation

infiltration and the thickness of the alveolar membrane were greatly increased after ECC, suggesting that a clear ALI was induced by ECC (Fig. 1c).

#### ECC Reduced $PO_2$ and Increased Inflammatory Cytokines Releases

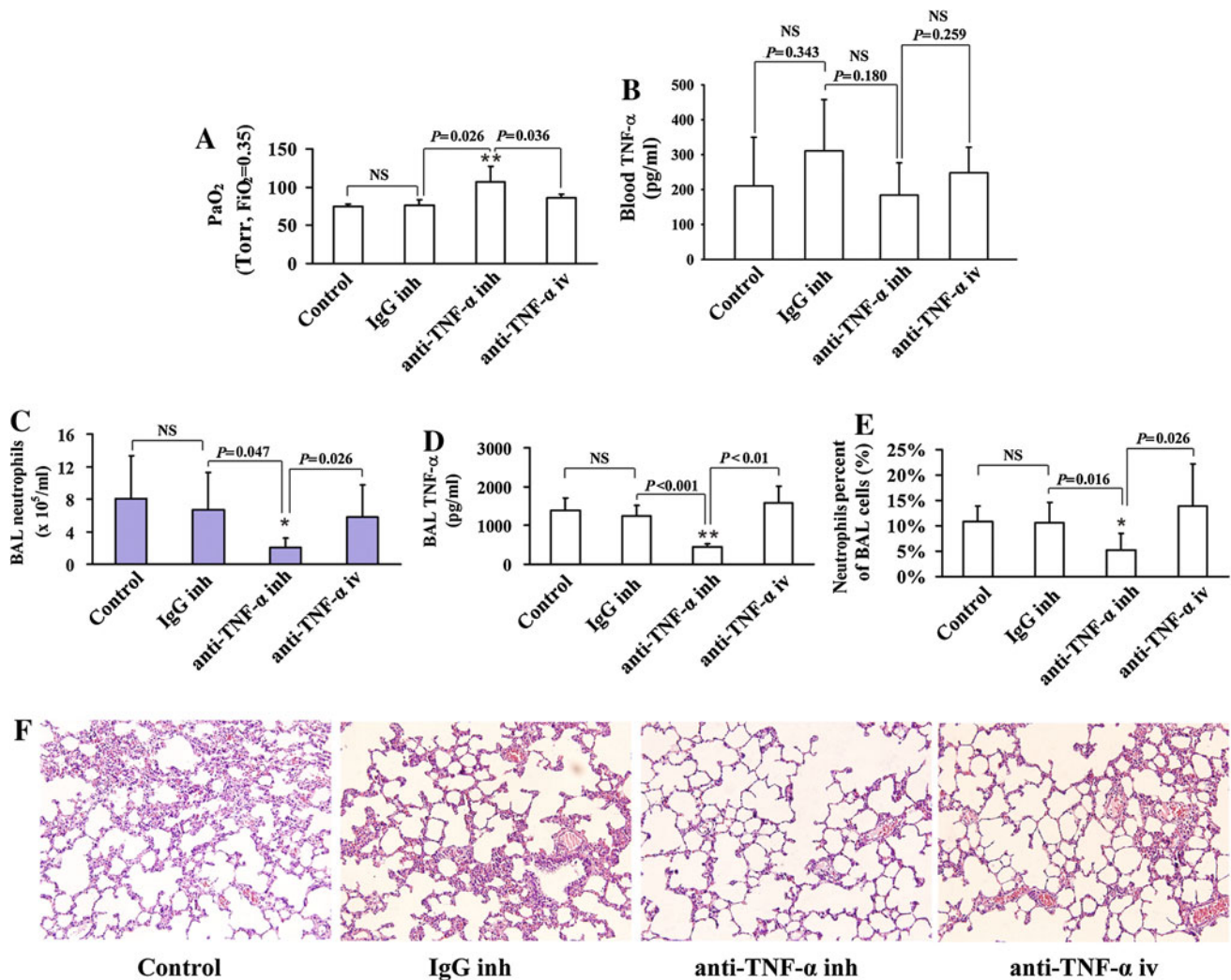
At baseline and during 2 h of ECC, there were no significant differences in  $PaO_2$  and in the counts of total cells and neutrophils in the BAL fluid. Four hours after termination of ECC, the  $PaO_2$  was greatly reduced ( $P < 0.01$ ) and the total cells count was nearly 2-fold higher ( $P < 0.01$ ) and the neutrophils count was nearly 1.5-fold higher ( $P < 0.01$ ) when compared with the baseline (Fig. 2a–c). Prior to these changes, significant increases of WBC and neutrophil counts in the blood were observed at the end of 2 h of ECC (Fig. 2d and e). More quickly, the  $TNF-\alpha$  level in the BAL started to increase after 10 min of ECC and exhibited a marked increase during the remaining time points (all  $P < 0.01$ , Fig. 3a). The result of linear regression analysis indicated that the release of  $TNF-\alpha$  was increased in a time-related manner ( $r = 0.9197$ , Fig. 3b). In contrast, the  $TNF-\alpha$  level in the blood was similar throughout the study, indicating that the  $TNF-\alpha$  in the BAL, but not in the blood, was probably involved in the pathogenesis of ECC-induced ALI (Fig. 3c and d). Typical pulmonary inflammation induced by ECC was further confirmed by increases of neutrophil infiltration and thickness of alveolar membrane (Fig. 4).

#### Inhalation of $TNF-\alpha$ Antibody Improved Pulmonary Function and Inhibited Pulmonary Inflammation after ECC

After 2 h of ECC and 4 h of rest, neither inhalation of IgG nor intravenous injection of  $TNF-\alpha$  antibody improved the  $PaO_2$  after ECC (Fig. 5a), while inhalation of  $TNF-\alpha$  antibody significantly increased the  $PaO_2$  compared with the control group ( $P < 0.001$ ) and the anti- $TNF-\alpha$  iv group ( $P = 0.036$ ). The levels of  $TNF-\alpha$  in the blood were comparable among groups (Fig. 5b). However, the release of  $TNF-\alpha$  and neutrophils in the BAL fluid and the ratio of neutrophils/total cells in BAL were remarkably reduced in the anti- $TNF-\alpha$  inh group compared to the other three groups (Fig. 5c–e). The increase in neutrophil infiltration and thickness of the alveolar membrane was also attenuated in the anti- $TNF-\alpha$  inh group compared with the other three groups (Fig. 5f). These results suggested that pulmonary inflammation was inhibited by pretreatment with  $TNF-\alpha$  antibody inhalation but not with IgG inhalation or an intravenous  $TNF-\alpha$  antibody injection.

#### Discussion

We sought to identify the initiating factors involved in ECC-induced ALI. Proinflammatory  $TNF-\alpha$  seemed a reasonable candidate because in many pathological conditions it is highly presented in inflamed airways and acts



**Fig. 5** Pretreatment with TNF- $\alpha$  antibody by inhalation from airway, but not via intravenous injection, attenuated acute lung injury. **a** Arterial oxygen pressure. **b** TNF- $\alpha$  release in blood. **c, d** Neutrophil count and TNF- $\alpha$  release in bronchoalveolar lavage fluid. **e** Ratio of neutrophils/total cells in bronchoalveolar lavage fluid.

**f** Representative photomicrographs of hematoxylin & eosin–stained left lung tissue sections ( $\times 20$ ) harvested at the end of the study. Values are presented as mean  $\pm$  SD ( $n = 10$ ). \* $P < 0.01$ , \*\* $P < 0.001$  vs. baseline. NS nonsignificant

as a potential activator of neutrophil in multiple settings [15]. However, the role of TNF- $\alpha$  at the onset of ECC-induced ALI has not been previously examined. Our study at first demonstrated that the airway nociceptors were stimulated as early as 10 min accompanied by lung injury after ECC. Furthermore, we proved that the release of TNF- $\alpha$  in lung was also quickly increased in a time-related manner during ECC, which was similar to the activity of nociceptors. Finally, we found that pretreatment with TNF- $\alpha$  antibody inhalation, but not intravenous injection, could improve pulmonary function and inhibit inflammatory response. This fact further confirms that the ALI was initiated by pulmonary TNF- $\alpha$  and suggests that pulmonary, but not circulating, TNF- $\alpha$  may be one of promoters of lung inflammation.

In the present study, we employed a rabbit ECC model for recording nociceptor activity, mainly because the rat's vagus nerve is too small to separate easily and the electrodes for recording action potentials are too big for small animals such as rats. From the data of this study, we believe that TNF- $\alpha$  released in the initial phase of ECC-induced ALI contributed to activation of the pulmonary nociceptors. As reported previously, nociceptor activation must be multifactorial and cannot be explained by edema alone [3] because the activation occurs within several minutes, while pulmonary edema develops gradually. If the edema or water flux is the only stimulus, the increased activity should follow the time course of edema development. In the first several minutes of ALI/ARDS, the influence of lung extravascular fluid and interstitial edema

would be minimal. Thus, non-lung-water factors must be involved in stimulating these nociceptors. Some previous studies have indicated that TNF- $\alpha$  can activate or sensitize airway nociceptors [16, 17]. The present study provided additional evidence that the airway nociceptor was probably activated by TNF- $\alpha$  in lung because both the nociceptor activity and the pulmonary TNF- $\alpha$  level had increased within 10 min after ECC. However, our results only indirectly support that the increased activity is due to an increase in pulmonary TNF- $\alpha$ , and whether other inflammatory mediators like IL-1 $\beta$ , IL-6, and IL-8 that are involved in the early initiation of airway nociceptors still needed to be identified.

Despite continuous technical improvements, ALI after ECC is still a significant clinical problem [18, 19]. With the use of a rat ECC model, the present study was the first to demonstrate that pulmonary interstitial inflammation happened almost simultaneously at the ECC-induced ALI. In ALI, a high level of local inflammatory cytokines will promote F-actin recombination and Mac-1 assembly at the head of neutrophils, and thus increase the adhesive capacity and accumulation of neutrophils in alveolus and pulmonary interstitium, causing local inflammation in the lung tissue [20]. Therefore, diagnosis and treatment of the elevated inflammatory response at the initiation of ECC will be of great benefit to the patient. Pulmonary function was not influenced immediately after ECC, as evidenced by that significant PaO<sub>2</sub>/FiO<sub>2</sub> decrease that was observed 4 h after termination of ECC. However, the TNF- $\alpha$  was sensitive to the ECC-induced ALI, the level of which was increased from 10 min after onset of ECC. These data indicate that the elevated TNF- $\alpha$  level was more prospective in the diagnosis of ALI in the early stage.

Another important finding of this study is that TNF- $\alpha$  antibody given via the airway, but not vein, potentially can prevent ECC-induced ALI. As we know, TNF- $\alpha$  plays a vitally important role in pulmonary inflammation [21]. TNF- $\alpha$  itself could potentially activate pulmonary inflammatory cells and promote them to secrete proinflammatory cytokines, including TNF- $\alpha$ . Thus, TNF- $\alpha$ -induced TNF- $\alpha$  release constitutes a positive feedback mechanism for enhanced inflammation during ECC [14]. However, it seems that neutralization of TNF- $\alpha$  in systemic circulation by its antibody was not effective in attenuating ALI; a double-blind randomized controlled trial did not find an improvement in survival with TNF- $\alpha$  monoclonal antibody therapy after septic shock [22]. Similarly, our study confirmed that venous injection of TNF- $\alpha$  antibody cannot reduce ALI after ECC. However, when changing the method of administration to inhalation, TNF- $\alpha$  antibody exhibited great protection by inhibiting pulmonary inflammation and ameliorating lung function. The exact mechanism of this phenomenon is not clear, but its

explanation may include the following reasons: First, TNF- $\alpha$  is produced mainly by macrophages and monocytes, which are abundant in lung tissue. During inflammation, both macrophages and monocytes are activated to recruit inflammatory cells. Therefore, delivery of antibody directly to the airway against TNF- $\alpha$ -mediated inflammation may be the best choice. Also, as we mentioned above, TNF- $\alpha$  quickly accumulated in great amounts in the lung after ECC, and neutralization of TNF- $\alpha$  in the alveolus and pulmonary interstitium may potentially inhibit the positive feedback of TNF- $\alpha$ -induced TNF- $\alpha$  release and reduce ALI/ARDS at the early stage.

## Conclusions

In conclusion, by using animal ECC models, we demonstrated that pulmonary nociceptor activity and TNF- $\alpha$  were quickly upregulated at the onset of ECC-induced ALI, and neutralization of TNF- $\alpha$  by inhalation of its antibody could improve pulmonary function and inhibit inflammation. These results indicate that TNF- $\alpha$  plays an important role in the pathogenesis of ALI and acts as an initiating cytokine at the early stage of ECC-induced ALI.

**Acknowledgments** The research was supported by National Natural Science Foundation of China (30772153 to Dr. Zhou, 81100180 to Dr. Li, and 30872455 to Dr. Du).

**Conflict of interest** The authors have no conflicts of interest or financial ties to disclose.

## References

1. Matthay MA, Zimmerman GA, Esmon C, Bhattacharya J, Collier B, Doerschuk CM, Floros J, Gimbrone MA Jr, Hoffman E, Hubmayr RD, Leppert M, Matalon S, Munford R, Parsons P, Slutsky AS, Tracey KJ, Ward P, Gail DB, Harabin AL (2003) Future research directions in acute lung injury: Summary of a national heart, lung, and blood institute working group. *Am J Respir Crit Care Med* 167:1027–1035
2. Spragg RG, Bernard GR, Checkley W, Curtis JR, Gajic O, Guyatt G, Hall J, Israel E, Jain M, Needham DM, Randolph AG, Rubenfeld GD, Schoenfeld D, Thompson BT, Ware LB, Young D, Harabin AL (2010) Beyond mortality: future clinical research in acute lung injury. *Am J Respir Crit Care Med* 181:1121–1127
3. Lin S, Walker J, Xu L, Gozal D, Yu J (2007) Behaviours of pulmonary sensory receptors during development of acute lung injury in the rabbit. *Exp Physiol* 92:749–755
4. Apostolakis E, Filos KS, Koletsis E, Dougenis D (2010) Lung dysfunction following cardiopulmonary bypass. *J Card Surg* 25:47–55
5. Abraham E, Carmody A, Shenkar R, Arcaroli J (2000) Neutrophils as early immunologic effectors in hemorrhage- or endotoxemia-induced acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 279:L1137–L1145

6. Strieter RM, Kunkel SL, Keane MP, Standiford TJ (1999) Chemokines in lung injury: Thomas A. Neff Lecture. *Chest* 116(1 Suppl):103S–110S
7. Takala A, Jousela I, Takkinen O, Kautiainen H, Jansson SE, Orpana A, Karonen SL, Repo H (2002) A prospective study of inflammation markers in patients at risk of indirect acute lung injury. *Shock* 17:252–257
8. Matthay MA, Zimmerman GA (2005) Acute lung injury and the acute respiratory distress syndrome: four decades of inquiry into pathogenesis and rational management. *Am J Respir Cell Mol Biol* 33:319–327
9. Imai Y, Kawano T, Iwamoto S, Nakagawa S, Takata M, Miyasaka K (1999) Intratracheal anti-tumor necrosis factor- $\alpha$  antibody attenuates ventilator-induced lung injury in rabbits. *J Appl Physiol* 87:510–515
10. Dos Santos CC, Slutsky AS (2000) Mechanisms of ventilator-induced lung injury: a perspective. *J Appl Physiol* 89:1645–1655
11. Li T, Luo N, Du L, Liu J, Gong L, Zhou J (2012) Early and marked up-regulation of TNF- $\alpha$  in acute respiratory distress syndrome after cardiopulmonary bypass. *Front Med* 6:296–301
12. Lin S, Li H, Xu L, Moldoveanu B, Guardiola J, Yu J (2011) Arachidonic acid products in airway nociceptor activation during acute lung injury. *Exp Physiol* 96:966–976
13. Yu J, Lin S, Zhang J, Otmishi P, Guardiola JJ (2007) Airway nociceptors activated by pro-inflammatory cytokines. *Respir Physiol Neurobiol* 156:116–119
14. Du L, Zhou J, Zhang J, Yan M, Gong L, Liu X, Chen M, Tao K, Luo N, Liu J (2012) Actin filament re-organization is a key step in lung inflammation induced by systemic inflammatory response syndrome. *Am J Physiol Lung Cell Mol Physiol* 47(5):597–603
15. Baluk P, Yao LC, Feng J, Romano T, Jung SS, Schreiter JL, Yan L, Shealy DJ, McDonald DM (2009) TNF- $\alpha$  drives remodeling of blood vessels and lymphatics in sustained airway inflammation in mice. *J Clin Invest* 119:2954–2964
16. Hu Y, Gu Q, Lin RL, Kryscio R, Lee LY (2010) Calcium transient evoked by TRPV1 activators is enhanced by tumor necrosis factor- $\alpha$  in rat pulmonary sensory neurons. *Am J Physiol Lung Cell Mol Physiol* 299:L483–L492
17. Li HF, Yu J (2009) Airway chemosensitive receptors in vagus nerve perform neuro-immune interaction for lung-brain communication. *Adv Exp Med Biol* 648:421–426
18. Hall RI, Smith MS, Rocker G (1997) The systemic inflammatory response to cardiopulmonary bypass: pathophysiological, therapeutic, and pharmacological considerations. *Anesth Analg* 85:766–782
19. Voss B, Krane M, Jung C, Brockmann G, Braun S, Günther T, Lange R, Bauernschmitt R (2010) Cardiopulmonary bypass with physiological flow and pressure curves: pulse is unnecessary! *Eur J Cardiothorac Surg* 37:223–232
20. Zarbock A, Ley K (2008) Mechanisms and consequences of neutrophil interaction with the endothelium. *Am J Pathol* 172:1–7
21. Mukhopadhyay S, Hoidal J, Mukherjee T (2006) Role of TNF $\alpha$  in pulmonary pathophysiology. *Respir Res* 7:1–9
22. Abraham E, Anzueto A, Gutierrez G, Tessler S, Pedro GS, Wunderink R, Nogare AD, Nasraway S, Berman S, Cooney R, Levy H, Baughman R, Rumbak M, Light RB, Poole L, Allred R, Constant J, Pennington J, Porter S (1998) Double-blind randomised controlled trial of monoclonal antibody to human tumour necrosis factor in treatment of septic shock. *Lancet* 351:929–933