Intrapulmonary Delivery of Human Umbilical Cord Mesenchymal Stem Cells Attenuates Acute Lung Injury by Expanding CD4⁺CD25⁺ Forkhead Boxp3 (FOXP3)⁺ Regulatory T Cells and Balancing Anti- and Pro-inflammatory Factors

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Key Words

UC-MSC • CD4⁺CD25⁺ Foxp3⁺Treg • Acute lung injury • LPS

Abstract

Background: Systemic and local inflammatory processes play key, mainly detrimental roles in the pathophysiology of acute lung injury (ALI). The present study was designed to determine whether human umbilical cord mesenchymal stem cells (UCMSC) are able to act on CD4⁺CD25⁺ Foxp3⁺Treg cells and lead to an improvement in ALI. **Methods:** Mice were administered intratracheally endotoxin (lipopolysaccharide [LPS]) and received intrapulmonary 1×10⁶ UCMSC 4 hours after challenge. The CD4⁺CD25⁺ Foxp3⁺Treg, survival time, body weight, histology and lung injury scores were assessed after transplantation of UCMSC. In addition, anti-inflammatory factor IL10 and pro-inflammatory mediators production including tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-2(MIP-2) and interferon-γ (IFN-γ) were detected. **Results:** Transplantation of UCMSC resulted in significant increase in the level of CD4⁺CD25⁺ Foxp3⁺Treg in

ALI. Increased level of anti-inflammatory factor IL-10 and reduced levels of TNF-α, MIP-2 and IFN-γ were simultaneously observed in ALI in comparison with control mice. **Conclusion:** Our data demonstrate for the first time that transplantation of UCMSC ameliorates ALI by enhancing the diminished levels of alveolar CD4⁺CD25⁺ Foxp3⁺Treg and balancing anti- and pro-inflammatory factors in ALI mice.

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Introduction

Acute lung injury (ALI) is a syndrome of rapid-onset bilateral pulmonary infiltrates of non-cardiac origin, characterized by alveolar-capillary injury, inflammation with neutrophil accumulation, and release of pro-inflammatory cytokines, and systemic and local inflammatory processes play a key detrimental role in the pathophysiology of ALI [1, 2]. ALI can develop acute respiratory distress syndrome (ARDS), result in persistent respiratory failure and prolonged dependence

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on mechanical ventilation, increase susceptibility to multi-organ dysfunction, mortality and financial cost. ALI has a substantial impact on public health. In the United States ALI produces nearly 150,000 hospitalizations and 75,000 deaths yearly [3]. Recent data indicate that despite improvements in supportive care, ARDS from ALI is still high at 40% [4, 5]. Systemic inflammatory response syndrome (SIRS) is systemic inflammatory response to serious body injury. Beal et al found that excessive inflammatory response led to organ dysfunction, which develop ALI or ARDS [6]. It is known that systemic and local inflammatory processes play detrimental roles in the ALI. However, little is known about endogenous counter-regulatory immune mechanisms. D'Alessio et al. reported that increased levels of CD4⁺CD25⁺ Foxp3⁺Treg were associated with a reduced risk of death from ALI causes [7], which suggests CD4⁺CD25⁺ Foxp3⁺Treg may play a role in this paracrine-mediated protection of ALI.

Mesenchymal stem cells (MSC), representing a population of stem cells with great therapeutic potentials, can be obtained from a number of tissues including fetal and adult bone marrow, fetal lung, fat tissue and umbilical cord [8-11]. Human umbilical cord MSC (UCMSC) are currently being evaluated for potential clinical application due to their little ethical problem, easy isolation and great expansion ability. Like bone marrow MSC, UCMSC possess apparent immune properties of hypo-immunogenicity and immune suppression. UCMSC express human leukocyte antigen (HLA) major histocompatibility complex (MHC) class I, but not HLA MHC class II and co-stimulatory molecules such as CD40, CD80 and CD86. The hypo-immunogenic character of MSC allows their use in xenotransplantation studies without obvious immune response [12-16]. In another aspect, UCMSC are able to secrete factors to create an immunosuppressive milieu [9].

Previous studies have suggested a therapeutic potential of MSC in ALI. These cells could act through complex paracrine pathway and finally lead to functional recovery of ALI [17]. UCMSC are capable of modulating the activity of innate and adaptive immune cells including dendritic cells, T cells and B cells [18, 19]. One of the mechanisms underlying the action of MSC is to elicit CD4⁺CD25⁺T cells [20]. There are some differences in the levels of CD4⁺CD25⁺ Foxp3⁺Treg during the inflammatory responses to sepsis and ischemia [7, 20-26]. However, little is known concerning the dynamic changes of the pro-inflammatory and anti-inflammatory properties of CD4⁺CD25⁺Foxp3⁺Treg in ALI mice

after treatment with UCMSC.

The present study was designed to determine if intrapulmonary transplantation of UCMSC decreases the risk of death from ALI mice. The impact of UCMSC on CD4⁺CD25⁺ Foxp3⁺Treg was also investigated in order to reveal the mechanism of action through which UCMSC exhibit therapeutic effect on ALI mice.

Materials and Methods

Animals

Healthy male strain Balb/C mice were provided by the Institute of Experimental Animal, Beijing, China. The mice were 8 to 10-week-old, 18 to 25g each, fed a standard diet and acclimated in a quiet quarantine room for 1 week before the experiments. All experiments were conducted under protocols approved by Animal Care and Use Committee of Peking Union Medical College.

Isolation and Identification of UCMSC

UCMSC were obtained from full-term caesarian section deliveries with informed consent of the mothers. Tissue obtaining for study was approved by the institutional review board of the Chinese Academy of Medical Science and Peking Union Medical College. UCMSC were isolated and expanded from the umbilical cord tissue as described previously [9]. Briefly, the cord was cut with scissor into small pieces (1-2 cm²) and digested with digested with 0.075% collagenase II (Sigma, St Louis, MO) for 30 min and with 0.125% trypsin (Gibco, Grand Island, NY) for second 30 min with gentle agitating at 37° C. The digested mixture was passed through a 100-μm filter to collect cell suspensions after terminated the trypsin activity with serum. Cells were washed with phosphate- buffered saline (PBS) for three times and placed in non-coated plastic flasks in the presence of Dulbecco's minimum essential medium/F12(1:1) (Gibco) supplemented with 10% fetal calf serum (Invitrogen, Sydney, Australia) and antibiotics (penicillin, 100μg/ml plus streptomycin, 10μg/ml) (Sigma). Three days later nonadherent cells were removed from culture and the adherent cells were cultured to confluence. UCMSC were cultured at 37° C in a humidified atmosphere maintaining 5% CO₂ and passed about 70% confluence. UC-MSC of passage five to six were used in the research.

Flow cytometry

For surface phenotype analysis, primary staining was performed using FITC-, PE-or PerCPC -conjugated monoclonal antibodies, cells surface markers included (CD4, CD25, CD45, CD34, CD29, CD44, CD73, human leukocyte antigen /HLA-II). Primary antibodies were purchased from Becton Dickinson (BD, San Diego, CA) and FOXP3 from Biolegend. For intracellular staining of Foxp3, cells were fixed and permeabilized with Foxp3 staining buffer (Biolegend), then stained with PE-conjugated anti-mouse Foxp3 mAbs (0.2 μg per 10⁶ cells; Biolegend). Cells were analyzed by

Fig. 1. Resolution of lung injury is markedly improved in the mice treated with UCMSC. Mice were challenged with i.t. LPS. (A and B) Survival (n=20 animals per group) (A) and body weight relative to baseline (B) were plotted after injury. (C and D) lung injury scores (C) and BAL total protein (D) were determined in LPS mice after treatment with PBS or with UCMSC (n=3 animals per group per time point). Histopathologic mean lung injury scores from 20 lung sections. Log-rank test (mortality curves) and unpaired Student's t test (other injury parameters), $P < 0.05$.

flow cytometry in a FACSCalibur, using the CellQuest software (Becton Dickinson).

Multipotent differentiation

To evaluate osteogenic and adipogenic differentiation of UCMSC, cells of passage 5-6 were seeded at 1000 cells/cm² and incubated more than 24 h before changing to the induction media as described previously [15].

Experimental groups

Mice were anesthetized with inhaling ethyl ether before exposure of the trachea, Escherichia coli LPS (O55:B5 Sigma-Aldrich L2880) at the dose of 4mg/kg for mice or sterile water as control was instilled intratracheally (i.t.) via a 20-gauge catheter. 1×10^6 UCMSC or PBS as control was administered intratracheally 4 hours after challenge. At 1-3 and 7 days after instillation, 10 mice per group were anesthetized and killed by exsanguinations from their inferior vena cava. Lobes of the lung tissue were fixed in 10% formalin for paraffin embedding. Mice were recorded for the survival and weight ever day after challenge.

Histology and lung injury scores

Lungs from both UCMSC and PBS groups were excised 3 days and 7days after endotoxin-induced lung injury and fixed with 10% formalin i.t. under 20 cm of H₂O pressure. After fixed for 24 hours, lungs were embedded in paraffin, cut into 5 μ m sections, and stained with H&E. The degree of lung injury scores were scored in six sections from the lower lobes using the following criteria: no injury, 0; injury to 25% of the field, 1; injury to 50% of the field, 2; injury to 75% of the field, 3; diffuse injury, 4 [27].

Broncho-alveolar lavage (BAL) and plasma cytokine and protein measurements

BAL and plasma samples were obtained from mice at 48h and 72h after endotoxin-induced lung injury in the UCMSC and PBS-treated groups. BAL was done after euthanizing the mice and placing a 20-gauge catheter into the trachea through which 1ml of cold PBS was flushed back and forth five times. IFN- γ , TNF- α , IL-10 and MIP-2 were measured in both the BAL and plasma samples with Enzyme-linked immunosorbent assay (ELISA) kits (Westang Biotech Co, Ltd, PR China) according to the manufacturer's instructions. BAL protein, a marker of endothelial and epithelial permeability, was measured in both UCMSC and PBS-treated groups by the protein assay kit (JianChen Biotech Co, Ltd, PR China). All samples and standards were measured in triplicate.

Statistical analysis

Analysis of treatment effects between the two groups was performed using the GraphPad Prism software, log-rank test (mortality curves), a one-way analysis of variance analysis and Student's unpaired t-test used. $P < 0.05$ was considered statistically significant.

Results

Transplantation of UCMSC improves LPS-induced ALI

To examine if the intrapulmonary transplantation of UCMSC in ALI mice could increase the percentage of CD4⁺CD25⁺ Foxp3⁺Treg and improve survival or

Fig. 2. UCMSC inhibit lung inflammation: Histology of lung obtained from a Balb/C mouse at 3 days following LPS lung injury and treatment with PBS sham (A) or UCMSC (B). Magnification (all sections).

accelerate recovery in response to the lung injury induced by LPS, we first investigated the therapeutic action of UCMSC in an experimental model of ALI induced by (i.t.) infusion of LPS, which displays human ALI-like clinical, histopathologic, and immunologic features [7]. We adoptively transplanted UCMSC into ALI mice receiving a standard dose of LPS (4mg/kg). Macroscopic examination of lung showed significant pneumonitis, inflammatory exudates, and distortion of the normal architecture of the lung in the control mice. There are severe infiltrates involving 60-75% of the lung at 3 days post-LPS injury. These changes are drastically attenuated 3 days after UCMSC intrapulmonary transplantation (Fig. 2A and B). Consistently, PBS-shamed mice developed a severe illness characterized by decreased activity, lack of grooming and sustained weight loss resulting in high mortality. By contrast, UCMSC treated mice showed an improved activity, better weight gain and higher survival rate. Of the 20 control mice, 9 died after LPS administration, while only 2 of 20 mice receiving UCMSC died (Fig. 1A). In addition, lung histology and lung injury score (Fig. 1C) also revealed accelerated recovery of injury in UCMSC infused mice compared with the control. The surviving UCMSC-treated mice were clinically improved (e.g. grooming, activity, and weight gain) in comparison with the controls.

These data indicate that intrapulmonary transplantation of UCMSC reduces the inflammation of mice in response to LPS and improves the survival rate and lung injury recovery in ALI mice.

Reduction of inflammatory mediators by transplanting UCMSC into ALI mice

Secondly we studied the effect of UCMSC intrapulmonary administration on the production of inflammatory mediators that are linked to LPS-induced ALI. BAL protein, a marker of endothelial and epithelial

permeability, which is increased in inflammatory lung tissue and thought to be able to exacerbate lung injury by eliciting alveolar hyaline membrane formation, was significantly reduced in the UCMSC treated group ($2.02 \pm 0.302 \text{ mg/mL}$) as compared to control mice ($3.22 \pm 0.262 \text{ mg/mL}$) (Fig. 1D). BAL of UCMSC-treated mice contained reduced levels of inflammatory cytokines including IFN- γ , TNF- α and MIP-2 in comparison with PBS-shamed ALI mice (Fig. 3, DFH). Moreover, UCMSC increased the levels of the anti-inflammatory/regulatory cytokine IL-10 (Fig. 3B). The broad anti-inflammatory activity of UCMSC in the lung was accompanied by down-regulation of the systemic inflammatory response. We repeatedly measured blood cytokine concentrations of ALI mice (Fig. 3, ACEG). The serum concentrations of the pro-inflammatory cytokines IFN- γ (Fig. 3G), TNF- α (Fig. 3C) and MIP-2 (Fig. 3E) were significantly higher in control mice than those in the UCMSC-treated mice at specific time points after ALI ($p < 0.05$). In contrast, post lung damage serum levels of IL10 were highly elevated after intrapulmonary transplantation of UCMSC as compared to controls (Fig. 3A). The decrease of the inflammatory mediators was also a consequence of the diminished inflammatory permeability in the broncho-alveolar endothelial and epithelial tissues of the UCMSC-treated mice, as shown by the low production of BAL protein from UCMSC-treated mice compared to the control (Fig. 1D). These findings indicate that UCMSC suppress increased expression of local and systemic cytokines.

Resolution of lung injury was achieved even when we transferred apoptotic UCMSC (nonviable UCMSC were instilled i.t. 4 h after the instillation of endotoxin as additional controls. UCMSC ($5 \times 10^7/\text{ml}$) were made nonviable by incubating with an apoptosis induction kit containing Mitomycin C ($50 \mu\text{g/ml}$) for 20 min in culture 37°C . UCMSC were then stained with

Fig. 3. UCMSC treatment reduces local and systemic inflammatory responses in ALI. Levels of the proinflammatory cytokines (IFN- γ , TNF- α , MIP-2) were reduced and IL-10 was increased in mice receiving UC-MSC after endotoxin-induced ALI. (B,D,F,H) were showed significant changes in the BAL of mice treated with UC-MSC. (A,C,E,G) were showed significant changes in the plasma of mice treated with UCMSC. (* p <0.05, ** p <0.01). Data are expressed as mean $\pm$ SD.

Fig. 4. UCMSC deactivate effector Th1 cells and induce CD4⁺CD25⁺Foxp3⁺Treg cells *in vitro*. SMCs isolated from LPS-treated mice were cocultured with UC-MSC (treated) or sham (controlled) stimulated by PMA+conA. (B,D) cytokine production were assayed after 48 hours, (A1, C1, A,C) Intracellular cytokine expression was analyzed by flow cytometry between UCMSC group and the control group. * p <0.05, ** p <0.01.

0.4% trypan blue to ascertain viability. The apoptotic UCMSC were transplanted intrapulmonary into mice as late as 4 hours after i.t. LPS and the lung histology, BAL protein and inflammatory cytokines were analyzed. There was no significant difference was observed between apoptotic UCMSC and PBS (data not shown), suggesting that UCMSC (viable) likely act through a paracrine mechanism by secreting soluble factors to promote the recovery of ALI.

UCMSC down-regulate the Th1 cytokines and upregulate CD4⁺CD25⁺Foxp3⁺ regulatory T cells in ALI

Systemic and local inflammatory processes play a key detrimental role in the ALI, and the bias toward Th1 cytokines is crucial in the establishment of inflammation in ALI [7, 27, 28]. UCMSC could ameliorate LPS-induced ALI by reducing auto-reactive T-cell responses and migration to the lung. UCMSC

Fig. 5. UCMSC induce CD4⁺CD25⁺ Foxp3⁺Treg response *in vivo*. (A) The flow cytometric panels represent UCMSC treated group and the control group, intracellular cytokine expression was analyzed by flow cytometry(PerCP) in gated CD4 cells. (B) Percents of CD4⁺CD25⁺ Foxp3⁺Treg cells was determined in the broncho-alveolar isolated at 3 days in UCMSC-treated and PBS-shamed ALI mice. **p*<0.05.

were able to directly deactivate auto-reactive Th1 cells, because the addition of either UCMSC to spleen mononuclear cells (SMCs) cultures from ALI mice suppressed IFN- γ production while increasing the levels of IL-10 (Fig. 4). The effects of UCMSC were partially dependent on cell-cell contact. Determination of intracellular cytokine contents on the CD4 cells of the co-cultures showed that UCMSC decreased the percents of IFN-r-producing Th1 cells and increased the percents of Treg cells (Fig. 4). These findings suggest that intrapulmonary administration of UCMSC to ALI mice significantly suppress the differentiation of autoreactive/inflammatory Th1 cells and expands Treg cells.

The observed UCMSC down-regulation of the inflammatory Th1 response, together with the elevated levels of regulatory cytokines, prompted us to investigate the involvement of Treg in the *in vivo* immunosuppressive action of UCMSC. We found that UCMSC-treated ALI mice had significant higher percents of CD4⁺CD25⁺ Foxp3⁺Treg cells in the broncho-alveolar than control PBS mice (Fig. 5, AB). The increased percentage of CD4⁺CD25⁺ Foxp3⁺Treg cells is consistent with the less mortality, lung histology damage, lung injury score, BAL protein exudates and accelerated resolution of injury in UCMSC treated ALI animals as compared to the control. These results suggest that UCMSC intrapulmonary transplantation during development of ALI significantly induces the generation and/or activation of CD4⁺CD25⁺

Foxp3⁺Treg cells that efficiently improve survival and recovery of lung injury in ALI mice.

Immunophenotypic analysis and Multilineage differentiation of UCMSC

UCMSC were successfully isolated and *in vitro* expanded from human umbilical cord.

Flow cytometric results illustrated indicated that UCMSC were positive for CD29, CD44, CD73 and negative for CD45, CD34, HLA-II (Fig. 6). UCMSC were able to differentiate into osteogenic and adipogenic cells *in vitro* (Fig. 7). Together, our data clearly demonstrate that the cells which were obtained from the umbilical cord tissue fit the recommended definition of MSC.

Discussion

Acute lung injury (ALI) is characterized by rapid alveolar injury, inflammation, cytokine induction, and neutrophil accumulation. No effective treatment for acute lung injury currently exists. The predominant works in this area focus on interruption of early events in disease pathogenesis. Recently, mesenchymal stem cells (MSC) have emerged as a therapeutic modality in various inflammatory disease states. A number of ongoing randomized Phase I/II clinical trials are evaluating the

Fig. 6. For surface phenotype analysis, primary staining was performed using FITC-conjugated monoclonal antibodies, cells surface markers included (CD45, CD34, CD29, CD44, CD73, HLA-II).

Fig. 7. Multilineage differentiation of UCMSC. (A,C) Osteoblastic potential of UCMSC as shown by Alizarin red staining and Von kossa staining. (B) Differentiation of UCMSC into adipocytes as evaluated by Oil Red O staining.

effects of MSC infusion in patients with multiple sclerosis, graft-versus-host disease, Crohn's disease, Type I diabetes and severe chronic myocardial ischemia [12, 17, 28-35]. MSC transplantation is also being considered as a potential therapy in patients with inflammatory lung diseases. Several studies have demonstrated compelling benefits from the administration of MSC derived from bone marrow, umbilical cord or cord blood in animal models of lung injury [12]. These studies are leading to growing interest in the therapeutic use of MSC in ALI.

MSC have unique features relating with immune system. MSC are low immunogenicity, which express human leukocyte antigen major histocompatibility complex I(MHC-I), but do not express human leukocyte antigen major histocompatibility complex II(HLA MHC-II) and the costimulatory molecules CD40, CD80 and CD86 [16]. In another aspect, MSC are able to interact and regulate a variety of immune cells. Our previous studies found that UC-MSC were capable of inhibiting the proliferation of human peripheral blood mononuclear cells after mitogen

or allogeneic stimulation [18]. UC-MSC also significantly inhibited the activation of T lymphocytes by antigen-independent manner [18]. In parallel to this, UC-MSC can inhibit the mitogen-activated rat spleen cell proliferation (xenograft model) and the allogeneic mixed lymphocyte reaction of human peripheral blood mononuclear cells [16]. Based on their activities in immune system, MSC have been shown to be able to repair tissue damage by immunomodulatory effect. Ortiz et al. found that MSC injection could attenuate the lung injury in which IL-1RA plays a crucial role for the anti-inflammatory effect [36]. By differentiation to low immune cell-like cells UC-MSC were able to repair the carbon tetrachloride-induced liver injury in mice [14]. Intracerebral transplantation of UC-MSC can decrease the inflammatory response and accelerate brain function recovery after hemorrhagic stroke in rats [15, 37].

In the present study we have demonstrated a therapeutic use of UCMSC in a mouse model of ALI induced by LPS. Transplantation of UCMSC into ALI mice resulted in a significant increase in the survival rate of ALI mice. Of the 20 control mice, 9 died after LPS administration. By contrast, only 2 of 20 mice receiving UCMSC died. Consistent with the effect of UCMSC on the survival rate, the lung histology and lung injury scores revealed a significant improvement in UCMSC-treated mice in comparison with control mice. The results obtained here clearly indicate a therapeutic use of UCMSC in ALI, a disease with substantial impact on public health.

N. Gupta et al. reported that MSC administration led to higher levels of IL-13, IL-10 and IL-1ra [27], and shifted the balance from a proinflammatory to an anti-inflammatory cytokines in response to endotoxin in part by leading to the production of soluble factors such as IL-1ra and by inducing a Th2 type response. This is consistent with other reports that MSC can decrease T cell responses by shifting from a Th1 to Th2 type response [38–40]. Th17 cells produced a pro-inflammatory cytokines IL-17 which can induce activation of T cells and stimulation of fibroblasts, macrophages produce proinflammatory IL-1, IL-6, TNF- α and induced inflammation [41].

Although the present study and previously published data have shown an ameliorating effect of MSC in models of acute lung injury caused by LPS and in chronic lung injury induced by bleomycin and asbestos [12, 28], it remains unclear by which mechanism these cells ameliorate lung injury, under what conditions these cells must be applied to provide an advantage and when using these cells might cause exacerbation of the lung

injury. Pathogenesis of ALI is explained by injury to both the vascular endothelium and alveolar epithelium. Regulation of pulmonary inflammation involves an intricate balance of both pro- and anti-inflammatory mediators.

We have therefore been interested in understanding the mechanisms regulating the inflammatory process underlying ALI. It was interesting to note in this study that the intrapulmonary transplantation of UCMSC resulted in a significant increase in the percentage of CD4⁺CD25⁺ Foxp3⁺Treg cells, following by functional and survival benefits in ALI mice when the cells were given 4 h after ALI. CD4⁺CD25⁺ Treg cells have been shown to play a central role in the prevention of autoimmunity and in the control of immune responses by down-regulating the function of effector CD4⁺ or CD8⁺ T cells [7, 21, 22, 24]. Moreover, D'Allesio et al. have demonstrated that CD4⁺CD25⁺ Foxp3⁺Tregs resolved experimental lung injury in mice. The infusion of CD4⁺CD25⁺ Foxp3⁺Tregs as late as 24 hours after administration of LPS normalized resolution in Rag-1^{-/-} mice. Similarly, Treg depletion in WT mice delayed recovery [7]. Our results together with these previous data suggest that UCMSC may resolve ALI by upregulating CD4⁺CD25⁺ Foxp3⁺Treg cells.

We then determined whether the UCMSC directly produce or induce immune cells to secrete immunoregulatory cytokines *in vivo* in ALI, or both. The levels of anti-inflammatory factor IL-10 and pro-inflammatory cytokines IFN- γ , MIP-2 and TNF- α in both the plasma and BAL were examined after transplantation of UCMSC. We noted that transplantation of MSC significantly reduced systemic and pulmonary levels of IFN- γ , MIP-2 and TNF- α , while increased IL-10 level. IL-10 is a potent, endogenous anti-inflammatory molecule that has been shown to decrease lung inflammation [27]. The results obtained here bring new insights into the endogenous immune response after ALI mice receiving UCMSC. Specifically, we have described a previously unknown role of CD4⁺CD25⁺ Foxp3⁺Treg cells as lung-protective immunomodulators in ALI mice receiving UCMSC, a function that affects diverse cytokine-dependent and cellular inflammatory targets via IL-10. It has been reported that natural regulatory (CD4⁺CD25⁺FOXP⁺) T cells control the production of pro-inflammatory cytokines during infection [21] and CD4⁺CD25⁺ Foxp3⁺Treg cells do increase the production of IL-10 [42]. Taken together, both the increase of percentage of CD4⁺CD25⁺ Foxp3⁺Treg cells and the balance of the levels of pro-inflammatory IFN- γ , MIP-2 and TNF- α and anti-inflammatory IL-10 contribute to

the therapeutic effects of UCMSC in ALI mice in response to endotoxin damage.

In comparison with bone marrow or other tissues derived MSC, UCMSC may have several clinical advantages including easy collection and isolation, rapid expansion in culture to generate a clinically effective dosage, and less ethical problem [9, 10, 43]. The results obtained demonstrate that intrapulmonary transplantation of UCMSC in experimental endotoxin-mediated ALI provides a significant survival advantage that is explained by an expansion of CD4⁺CD25⁺ Foxp3⁺Treg cells and a reduction in lung vascular permeability and the quantity of lung edema through the balance of pro- and anti-

inflammatory cytokines., therefore raising the possibility to use UCMSC-based therapy for ALI in patients.

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