Mesenchymal stem cells protect against sepsis-associated acute kidney injury by inducing Gal-9/Tim-3 to remodel immune homeostasis

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Mesenchymal stem cells protect against sepsis-associated acute kidney injury by inducing Gal-9/Tim-3 to remodel immune homeostasis

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ABSTRACT

Objective: The aim of the present study was to investigate the specific mechanism by which MSCs protect against SA-AKI.

Methods: Male C57BL/6 mice underwent cecal ligation and puncture operation to induce sepsis and then received either normal IgG or MSCs (1* 10^6 cells intravenously) plus Gal-9 or soluble-Tim-3 3h after surgery.

Results: After cecal ligation and puncture operation, injection with Gal-9 or MSCs plus Gal-9 had a higher survival rate than the IgG treatment group. Treatment with MSCs plus Gal-9 decreased serum creatinine and blood urea nitrogen levels and improved recovery of tubular function. Reduce the levels of IL-17, RORγt and induce the expression of IL-10 and FOXP3. Additionly**,** remodel the balance of TH17/Treg. However, when used soluble Tim-3 to block the Gal-9/Tim-3 pathway, the septic mice developed badly kidney injury and exhibited a higher mortality. Treatment with MSCs plus soluble Tim-3 blunted the therapeutic effect of MSCs, and inhibited the induction of Tregs and suppressed the inhibition of the differentiation to Th17 cells.

Conclusion: Treatment with MSCs significantly reversed the TH1/TH2 balance. And the important mechanism for MSCs protection against SA-AKI maybe through the Gal-9/Tim-3 pathway.

Key words: Mesenchymal stem cells, sepsis-associated acute kidney injury, Gal-9/Tim-3, immune. homeostasis

INTRODUCTION

Acute kidney injury (AKI) is a common critical illness involving multiple systems. It is a syndrome associated with rapid deterioration of renal function caused various etiologies. Sepsis is one of the most important causes of AKI, with an annual incidence rate of greater than 50%[1], greatly increasing the difficulty of sepsis treatment and medical costs. In recent years, despite the rapid development of critical care medicine and nephrology, the mortality rate for sepsis-associated AKI (SA-AKI) is still high. Therefore, the prevention and treatment of SA-AKI is a top priority for clinicians. Stem cell research is currently a hotspot with respect to the treatment of AKI. As a type of adult stem cell, mesenchymal stem cells (MSCs) not only have the proliferation and differentiation abilities but also have immune regulatory functions[2]. Although MSCs have been studied in relation to SA-AKI, their specific protective mechanisms in SA-AKI remain unclear.

Galectin-9 (Gal-9) belongs to the galectin family and is a natural ligand of Tim-3. Almost all cells express Gal-9[3]. However, in the inactivated immune system, Gal-9 is mainly expressed by naive CD4+ effector T cells, and regulatory T (Treg) cells. After activation, Gal-9 on the surface of effector T cells is downregulated, but Gal-9 on the surface of Treg cells is maintained[4]. Studies have confirmed that the Gal-9/Tim-3 pathway can negatively regulate the TH1 immune response, the binding of Gal-9 and Tim-3 can induce the apoptosis of TH1 and TH17 cells that express Tim-3, thereby inducing Treg cells and immune tolerance[5]. Studies have reported that the Gal-9/Tim-3 pathway is involved in the immune disorders of SA-AKI[6].

I In recent years, the protection of MSCs in sepsis and AKI have been confirmed. For example, MSCs can significantly improve the survival rate of septic mice and related organs damage[7]. The mechanism includes the secretion of prostaglandin E2 to induce the conversion of M1 macrophages to M2 macrophages[8]. MSC intervention significantly increased the percentage of peripheral blood Treg cells and improved the function of Treg cells, restoring immune homeostasis in septic rats[9].

MSCs can improve AKI caused by ischemia/reperfusion injury (IRI), crush syndrome, and sepsis. The mechanism were mainly focused on the following points: ① paracrine and endocrine mechanisms – MSCs secrete trophic growth factors or inflammatory regulatory factors, inhibit the apoptosis of vascular endothelial cells and tubular epithelial cells, promote local cell regeneration, and reduce local inflammatory cell infiltration in the kidneys[10]; ② immunomodulatory function – MSCs are involved in the induction of Tregs and the conversion of M1 macrophages to M2 macrophages[11]. Recent studies have also found that MSCs can remodel the balance of TH1/TH2, thereby reducing the production of interferon γ (IFN-γ) by CD4+ TH1 cells, reducing the release of interleukin-17 (IL-17) by TH17 cells, and increasing the release of IL-4 by TH2 cells[12]. More importantly, MSCs can promote the production of IL-10 by Tregs cells. In vitro, coculture of MSCs and naive T cells, MSCs can significantly increase the differentiation of naive T cells to Treg cells[13]. Our previous studies have confirmed that MSCs can improve SA-AKI. However, the mechanism by which MSCs improve SA-AKI remains unclear. In this study, the effect of MSCs on the immune homeostasis of SA-AKI was assessed by blocking or activating the Gal-

9/Tim-3 pathway to reveal the specific mechanism by which MSCs protect against SA-AKI.

Method

Animal protocols

Male C57BL/6 mice at 6-8 weeks were provided by the Experimental Animal Center of Qingdao University. Prior to the establishment of the model, the animals were housed for 1 week to adapt to the conditions. All animal experiments were approved by the Animal Ethics Committee of Qingdao University.

Mouse model of caecal ligation and puncture (CLP)

The sepsis model of cecal ligation and puncture (CLP) was generated as described previously[7]. Mice were anesthetized with 2% pentobarbital, the abdominal cavity was opened layer by layer to locate the cecum, and the distal end was carefully dissociated. Feces in the cecum was gently squeezed toward the distal end of the cecum. Seventy-five percent of the cecum was ligated with a 4-0 silk suture, and the cecum was punctured twice with a 21-gauge needle. A small amount of feces was squeezed on the surface of the cecum, and then the cecum was retracted into the abdominal cavity and sutured. In the sham operation group, only cecal separation was performed, without ligation and puncture.

Culture of MSCs

Bone marrow-derived MSCs of C57/BL6 mice were purchased from Cyagen Biosciences (Sunnyvale, Calif). The culture process was initiated according to the

manufacturer's instructions.Sixth to eight-generation MSCs were collected for use; After 3h of CLP, mice were injected with either of 10^6 MSCs, IgG(BD Biosciences, San Diego, CA), Gal-9(100 ug/mouse, R&D Systems, Minneapolis, MN), and soluble Tim-3 （100 ug/mouse, eBio-Science, San Diego, CA）by tail vein[14].

Survival analysis

Mice in each group (20 per group) were observed every 24 h for 7 days. The mice that survived were sacrificed after 7 days.

Enzyme-linked immunosorbent assay

The animals were anesthetized and killed at 24 h (n = 8 per group). Specific detection of IL-4, IL-6 and TNF-α was accomplished by sandwich enzyme-linked immunosorbent assay according to the manufacturer's instructions (R&D Systems, Minneapolis, Minn).

Assessment of kidney function

The mice were anesthetized and killed at 24h after surgery. Blood (500 μL/mouse) was collected from the tail vein and then centrifuged at 3000 rpm for 15 min ; the upper layer (serum) was retained for the determination of serum creatinine (Cr) and blood urea nitrogen (BUN).

Histopathological analysis

Kidneys were harvested at 24 h after CLP. The kidneys were collected and placed in 10% formaldehyde. After fixation at 4°C for 48 h, the tissue was dehydrated and embedded in paraffin for pathological staining. Pathological scoring was performed using a double-blind method. Tubular injuries were scored by 3 independent observers. Semiquantitative scoring was performed based on the previously reported [7]: 0 = no necrosis, 1 = <10%, 2 ≤ 11-25%, 3 = 26-45% and 4 = 46-75%. Six mice in each group were observed, and at least 20 high-magnification fields (magnification: 400×) were visualized for each section.

Real-time PCR

Total RNA was extracted from the renal sample homogenates using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription into complementary DNA (cDNA) was performed using a TaqMan reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed using SYBR Green Master Mix (Toyobo) and ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA). GAPDH levels were used for normalization. The sequences of real-time PCR primers are shown in Table 1.

Western blot

The protein levels of RORγt , IL-17, Foxp3, and IL-10 were measured by Western blotting. Total proteins were extracted from the kidney tissue using complete radioimmunoprecipitation lysis buffer (RIPA), and protein levels were measured using a bicinchoninic acid assay kit (Thermo Scientific, Bremen, Germany). A total of 100 μg of protein was separated and transferred onto polyvinylidene difluoride (PVDF)

membranes. Then, anti- RORγt, IL-17, Foxp3, IL-10 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and anti-actin (Cell Signaling Technology) antibodies were used as primary antibodies at 4°C overnight, and horseradish peroxidase-conjugated anti-rabbit/mouse IgG (Santa Cruz Biotechnology) was used as the secondary antibody. Protein levels were semiquantitatively determined based on the optical density using ImageJ software and were expressed as the protein/actin ratio. All samples were measured in duplicate.

Flow cytometry

Antibodies used for multicolor flow cytometric analysis were as follows: CD4-PE-CY7 (FITC, GK1.5, eBiosciences) , CD25-APC (Bio-Legend), Foxp3- PC5(FJK-16S; eBio-sciences) ,IL-17-PE (eBioscience). For staining of intracellular markers, cells were incubated for 20 min at 4°C in Cytofix/Cytoperm (Biolegend) to permeabilize cell membranes. Intracellular markers were stained according to standard laboratory procedures. All data were acquired using a FACSCalibur cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star, Ashland, OR, USA).

Statistical methods

The survival rate for mice in each group was evaluated by Kaplan–Meier analysis. Statistical analysis was performed using SPSS statistical software (SPSS version 10.1). The results were presented as mean ± SD. Normal and abnormal distributed variables were compared using Student t-test and Mann- Whitney U test, respectively. The value

of α was corrected using [2α/n (n−1)] to ensure α = 0.05. The mRNA level was calculated using the $2^{-\Delta\Delta CT}$ method. $P < 0.05$ was considered statistically significant.

Result

1.Effect of exogenous Gal-9 activation of Gal-9/Tim-3 on the 7-day survival in septic mice treated with MSCs

In the absence of antibiotics, we established a sepsis model by cecal ligation and perforation. High mortality rates were observed at 24 hours after CLP surgery in all groups, indicating that the sepsis model was successfully established. On day 7, the survival rate for CLP + IgG mice was only 25%, while the survival rate for mice in the CLP + Gal-9 + MSCs group was 45%. Compared with CLP + IgG, the 7-day survival rates of CLP + IgG + MSCs, CLP + Gal-9, and CLP + Gal-9 + MSCs groups were significantly lower; however, there were no statistically significant differences between the CLP + Gal-9 group and CLP + Gal-9 + MSCs group (Fig1).

2.Effect of exogenous Gal-9-activation of Gal-9/Tim-3 on the renal functions in septic mice treated with MSCs

I The levels of Cr and BUN in CLP + IgG, CLP + IgG + MSCs, CLP + Gal-9, and CLP + Gal-9 + MSCs groups were all significantly increased, suggesting that the model was successfully established. Compared with CLP + IgG, the levels of Cr and BUN in CLP + IgG + MSCs and CLP + Gal-9 groups were significantly decreased. Although the Cr and BUN levels in CLP+Gal-9+MSC group were decreased compared with CLP+Gal-9 group, there was no significant difference(Fig 2).

3.Effect of exogenous Gal-9-activation of Gal-9/Tim-3 on pathological of kidney in septic mice treated with MSCs

As described previously, the pathological changes in the kidneys in all septic groups were manifested as brush border loss, tubular degeneration, and vacuolization in the

proximal tubules. compared with CLP + IgG group, the tubular injury scores in CLP + IgG + MSCs and CLP + Gal-9 groups were significantly lower. Although the tubular injury scores in CLP + Gal-9 + MSC group were lower than in CLP + Gal-9 group, there was no significant difference (Fig 3)

4. Effect of exogenous Gal-9 activation of Gal-9/Tim-3 on Treg and TH17 cells in kidney of septic mice treated with MSCs

The percentage of TH17 cells in CLP + IgG group was significantly higher, and the mRNA(Fig 4A)and protein expression(Fig 4B) levels of RORγt and IL-17 were higher, while the mRNA and protein expression levels of FoxP3 and IL-10 were lower. These findings are consistent with previous studies, indicating that Tregs and TH17 cells play key roles in the pathogenesis of sepsis. The percentage of TH17 cells was significantly lower in the CLP + IgG+MSCs intervention group than in CLP+IgG group(Fig 4C), while the percentage Treg cells was significantly higher(Fig 4D); This finding suggests that in SA-AKI, treatment with MSCs can induce the TH17/Treg ratio. We speculate that MSCs may through remodel the balance of TH17/Treg via Gal-9/Tim-3 to protect SA-AKI. To verify this hypothesis, we used exogenous Gal-9 to activate the Gal-9/Tim-3 pathway, we found that the percentage of TH17 cells in kidney significantly decreased (Fig 4C)and the mRNA and protein expression levels of RORγt and IL-17 were significantly reduced(Fig 4A,4B), whereas the mRNA and protein expression levels of FoxP3 and IL-10 were increased(Fig 4A,4B). However, there were no statistically significant differences between the CLP + Gal-9 group and CLP + Gal-9 + MSCs group.

This finding demonstrated the pathway of Gal-9/Tim-3 in regulation of TH17/Treg ratio about MSCs is very important.

5. Effect of blocking Gal-9/Tim-3 on the 7-day survival of septic mice treated with MSCs

We used soluble Tim-3 to block the Gal-9/Tim-3 pathway in vivo. On day 7, there was no significant difference between the CLP + soluble-Tim-3 group and the CLP + soluble-Tim-3 + MSCs group(Fig5A). Next, we tested the bacterial load at 24 hours after CLP. Mice in all group were showed severely bacterial load (Figure 5B). but, there were no statistically significant differences between the CLP + soluble-Tim-3 group and CLP + soluble-Tim-3 + MSCs group(Fig5).

6. Effect of blocking Gal-9/Tim-3 on renal function of septic mice treated with MSCs

Compared with CLP + IgG group, the Cr and BUN levels in CLP + IgG + MSCs group were significantly lower. However, the Cr and BUN levels in CLP + soluble-Tim-3 group not only did not decrease but also tended to increase. Although the Cr and BUN levels o in CLP + soluble-Tim-3 + MSCs group were lower than in CLP + soluble-Tim-3 group, the difference between the group have no statistically significance(Fig6).

7. Effect of blocking Gal-9/Tim-3 on the pathological damage in kidney of septic mice treated with MSCs

The renal tubular injury score in CLP + IgG + MSCs group was significantly lower than in CLP + IgG group. However, the renal t tubular injury score in CLP + soluble Tim-3 group did not decrease significantly, indicating that soluble Tim-3 did not protect against SA-AKI. Compared with CLP + soluble-Tim-3 group, the renal tubular injury score in CLP + soluble-Tim-3 + MSCs group also did not significantly decrease (Fig7).

These results indicated that blocking Gal-9/Tim-3 disrupted the protective effect of MSCs in SA-AKI.

8. Effect of blocking Gal-9/Tim-3 on Treg and TH17 cells in kidney of septic mice treated with MSCs

We blocked the Gal-9/Tim-3 pathway with soluble Tim-3. Compared with CLP + IgG group, the percentage of TH17 cells in the kidney tissue in CLP + IgG + MSCs group did not significantly decrease, and the mRNA（Fig8a） and protein（Fig8b） expression of the RORγt and IL-17 did not significantly decrease else; In addition, the mRNA（Fig8a） and protein（Fig8a） levels of FoxP3 and IL-10 were not significantly different from those in the CLP + IgG group. By blocking the Gal-9/Tim-3 pathway with soluble Tim-3, the regulation of the TH17/Treg（Fig8c-1, Fig8c-2, Fig8d-1, Fig8d-2） ratio by MSCs was no longer statistically significant, indicating that Gal-9/Tim-3 pathway regulation of the TH17/Treg ratio plays an important role in the protection against SA-AKI by MSCs.

Discussion

Sepsis is a life-threatening condition caused by microbial infection that trigger an excessive inflammatory response. Consistent with the opinion, the results from the present study also showed more badly mortality after CLP operation, indicating the development of sepsis. In addition to the direct damage of pathogens and their toxins, the immune function also plays a crucial role in the process of sepsis. Treg cells are classic immune regulatory cells. Previous studies have shown that Treg cells can mediate the TH1/TH2 balance during the pathophysiological process of sepsis, thereby affecting the inflammatory responses. Kinsey et al[15] used antibodies (PC61) to

eliminate Treg cells in mice 5 days before ischemia-reperfusion injury-induced AKI model. As a result, the infiltration of inflammatory cells in kidney were significantly increased, and the secretion of IL-6, TNF-α and TGF- β (but not IL-10) increased, thereby aggravating renal injury. To further verify the relationship between Treg cells and AKI, Kinsey injected Treg cells from wild-type mice and Foxp3-deficient mice into RAG-1 mice (lacking T and B cells) before AKI modeling. The former alleviated AKI while the latter increased the infiltration of local neutrophils and macrophages in the kidneys, thereby aggravating AKI. These findings demonstrate that, in IRI-AKI, Tregs can directly inhibit innate immune responses.

Gandolfo et al[16] used PC61 to eliminate Treg cells in mice 24 h after AKI injury and found that it could still lead to increased Cr, while the transfer of Treg cells from wild-type mice 24 h after injury significantly reduced tubular necrosis scores and promoted tubular epithelial cell regeneration, suggesting that Treg cells are involved in AKI repair. Our study also confirmed that although the local Treg cells in septic kidney tissues were slightly increased, however, because the imbalances of TH1/TH2 to TH1, it eventually resulted in kidney injury.

TH17 cells are a newly identified T cell subpopulation that secrete IL-17 and has clearly been the focus of recent research. TH17 cells as the main source of IL-17 can recruits neutrophils and promotes inflammatory mediator release[17]. Studies have shown that in the peripheral blood of septic mice, IL-17 increased in a time-dependent manner. The injection of antibodies to neutralize IL-17 before CLP injury significantly improved the survival and reduced the expression of inflammatory factors and

chemokines. Even the injection of anti-IL-17 is delayed 12h after CLP injury, it still result in the improvement of sepsis[17].In addition to septic animal model, Brunialti et al [19] also detected significantly increased IL-17 and the percentage of IL-17-producing TH17 cells in the peripheral blood of patients with sepsis. In recent years, the role of IL-17 and TH17 cells in kidney diseases has been established. Elevated IL-17 expression was detected in kidney tissues from patients with focal segmental glomurular sclerosis glomerulonephritis, membranoproliferative glomerulonephritis, and minimal change disease [20].

Li et al[21] found that IL-17 was significantly increased in IRI-AKI mice model. He showed that whether use IL-17a$^{-/-}$ mice or after IL-17A neutralization were all had significantly less neutrophil infiltration and tissue injury 24 hours after kidney IRI, indicating that IL-17 participated in the innate immune inflammation associated with kidney IRI. Our study found a significant increase of TH17 cells in the kidney of septic mice. Although the percentage of Treg cells also increased slightly, it was much less than the increase in TH17 cells, suggesting a role of the TH17-mediated TH1 inflammatory response in the pathogenesis of SA-AKI.

Recent studies have found that in addition to the regulation of TH1 cells, the Gal-9/Tim-3 signaling pathway is also involved in the regulation of TH17 and Treg cells [22, 23]. When downregulation and/or blockade of Tim-3 pathway by an antagonist mAb against Tim-3 and/or soluble Tim-3 protein in sepsis, the levels of TH1- and TH17-related cytokines (IFN-γ, IL-17, IL-2, and IL-6) increased, and the

immunosuppressive function of Treg cells significantly decreased; furthermore, the expression of cytokine IL-10 also significantly decreased, suggesting that Tim-3 plays important roles in maintaining the homeostasis of sepsis in both humans and a mouse model [24]. In addition to evidence from animal studies, in rheumatoid arthritis patients, higher Gal-9 and Tim-3 expression was associated with better clinical outcomes and/or reduced disease severity. which provides additional evidence that galectin-9 is anti-inflammatory[25].

In our study, we also showed that the expression of IL-4, IL-6 and TNF-α were increased at 24h after the injury of CLP. Instead, activating the Gal-9/Tim-3 pathway by Gal-9 reduces the expression of IL-4, IL-6 and TNF-α. These results suggests that gal-9 /Tim-3 plays an important role in regulating inflammation in sepsis.

Tim-3 expression is dysregulated during the pathological process of sepsis, and is a negative immune regulator, correlated with severity of sepsis, The expression of Tim-3 in patients with severe sepsis is significantly lower than that in patients with mild sepsis[26]. Consistent with this, in a mouse model of sepsis, Tim-3 expression was significantly reduced in the acute/severe phase, and blockade of the Tim-3 pathway by Tim-3 antibodies and Tim-3 fusion proteins reduced the survival rate, increased proinflammatory factor release, and exacerbated septic injury[27].

In an in vitro study of sepsis, lipopolysaccharide (LPS) caused the upregulation of the expression of inflammatory factors such as IFN-γ, TNF-α and IL-17, indicative of excessive proliferation and activation of TH1 and TH17 cells[28], while Gal-9 inhibited

the upregulation of these factors and promoted the production of the TH2 and Treg cell-related cytokines IL-4 and IL-10[29].

These results indicate that the Gal-9/Tim-3 pathway can regulate the of TH17/Treg cells and related secretion factors in sepsis and kidney diseases. Our previous study found that IL-17 knockout improved SA-AKI and increased IL-10 while reducing IL-6 levels[30]. This finding indicates that IL-17 is involved in the pathogenesis of SA-AKI. In addition, in an IRI-AKI model, increasing the percentage of Treg cells and the secretion of IL-10 significantly improved AKI, while clearing Treg cells accelerated the deterioration of AKI and reduced IL-10 levels [31]. In our study, we further confirmed that the number of IL-10-secreting Treg cells in SA-AKI was significantly reduced, while the number of IL-17-secreting TH17 cells was significantly increased, and after exogenous Gal-9 activation of the Gal-9/Tim-3 pathway, the number of IL-17-secreting TH17 cells also decreased, while the number of IL-10-secreting Treg cells increased. In addition, blockade of the Gal-9/Tim-3 pathway with Tim-3 further aggravated the local TH17/Treg imbalance in the kidneys of septic mice, resulting in an increase in TH17 cells and a decrease in the number of IL-10-secreting Treg cells. In summary, TH17/Treg cells and IL-17/IL-10 secreted by TH17/Treg cells are important pathogenic factors involved in SA-AKI. Inducing TH17/Treg cells to establish a new balance may become a new target for SA-AKI treatment. The Gal-9/Tim-3 pathway may be an important mechanism affecting the TH17/Treg ratio. Further regulation of the TH17/Treg balance by manipulating the Gal-9/Tim-3 pathway may become a breakthrough strategy.

Previous studies have found that MSCs can induce IL-10 secretion by Treg cells in IRI-AKI model[31]. Our previous studies demonstrated that MSCs inhibited the secretion of IL-17 by TH17 and increased IL-10 expression in SA-AKI mice.

Recent studies have found that MSCs can inhibit T cell function through the secretion of Gal-1 and Gal-3. Silencing the expression of Gal-1 and/or Gal-3 in MSCs with small interfering RNA can partially or even completely eliminate the proliferative effect of MSCs on T cells[32].

The present study found that the administration of MSCs in SA-AKI mice significantly reversed the TH1/TH2 balance, resulting in a significant decrease in the percentages of IL-17-secreting TH17 cells and a significant increase in IL-10-secreting Treg cells. In addition, the activation of the Gal-9/Tim-3 pathway by exogenous Gal-9 reduced the number of IL-17-secreting TH17 cells and increased the number of IL-10-secreting Treg cells. Blocking the Gal-9/Tim-3 pathway with Tim-3 further aggravated the local TH17/Treg imbalance in the kidneys of septic mice, resulting in an increase in TH17 cells and a decrease in Il-10-secreting Treg cells. Therefore, we speculate that reestablishing the TH17/Treg balance by MSCs through the Gal-9/Tim-3 pathway might be an important mechanism for protection against SA-AKI.

Our experiment has some limitations. For example, although our previous study verified that injection of MSCs not home to kidney[7], we did not further verify how MSCs affect the Gal-9/Tim-3 pathway in kidney. So, in the future, using in vitro experiments, we will further verify whether MSCs affect the Gal-9/Tim-3 pathway

through the secretion of Gal-9 or through other mechanisms, such as exosomes derived from MSCs or other paracrine mechanisms.

Conclusions

In summary, Our findings suggest that MSCs can ameliorate SA-AKI by reversing the balance of TH1/TH2, and the important mechanism for MSCs protection against SA-AKI maybe through the Gal-9/Tim-3 pathway. Our study also offers additional evidence that administered MSCs protect against SA-AKI by remodel immune homeostasis and sheds new light on the mechanisms of the beneficial effects of MSCs on AKI.

Ethics approval and consent to participate

All the experimental procedures were performed with the approval of the Experimental Research Institute of the QingDao University and followed the guidelines of the Institutional Animal Care and Use Committee.

Acknowledgements

All authors read and approved the final manuscript.

All authors consent for publication.

Authors' contributions

Congjuan Luo and Yan Xu generated the hypothesis; Congjuan Luo, Feng Luo, Xiaofei Man, Quandong Bu, Haiyan Zhou and Long Zhao participated in study design and coordination; Congjuan Luo, Hui zhang, and Lin Che performed the experiments; Congjuan Luo subjected the mice to behavioral tasks during the experimental period; Feng Luo and Congjuan Luo performed the statistical analysis; Congjuan Luo and Feng Luo wrote the manuscript; and Feng Luo and Yan Xu made critical revisions to the manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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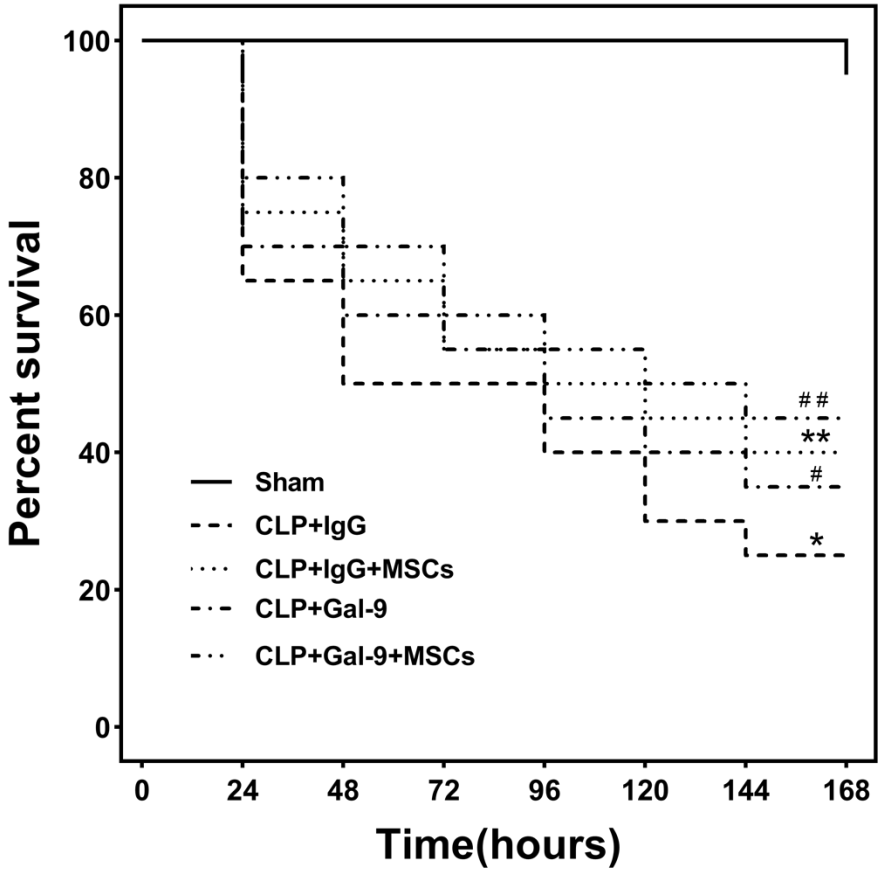


Figure 1. Seven-day survival rate for mice in each group: Each group consisted of 20 animals. Kaplan-Meier curves represent the survival rate in each group. compared with

CLP + IgG group, #P<0.05， **P<0.05; compared with the CLP + Gal-9 group, ##P> 0.05

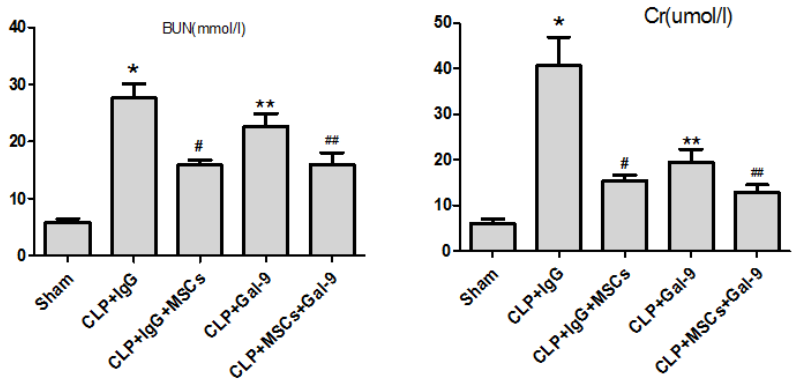


Figure 2. The levels of serum Cr and BUN 24 h after CLP injury in each group: values are mean ± SD; compared with CLP + IgG group, #P<0.05， **P<0.05; compared with the CLP + Gal-9 group, ##P> 0.05

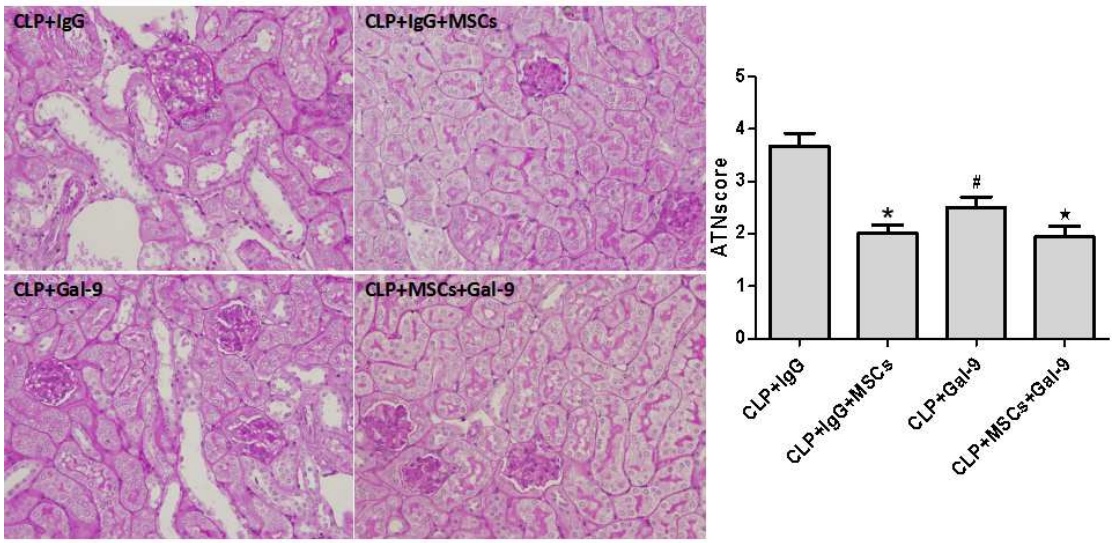


Figure 3. The acute tubular necrosis (ATN) scores 24 h after CLP in each group: values are mean ± SD; compared with CLP + IgG group, *P<0.05,#P<0.05; compared with the CLP + Gal-9 group, ★P >0.05

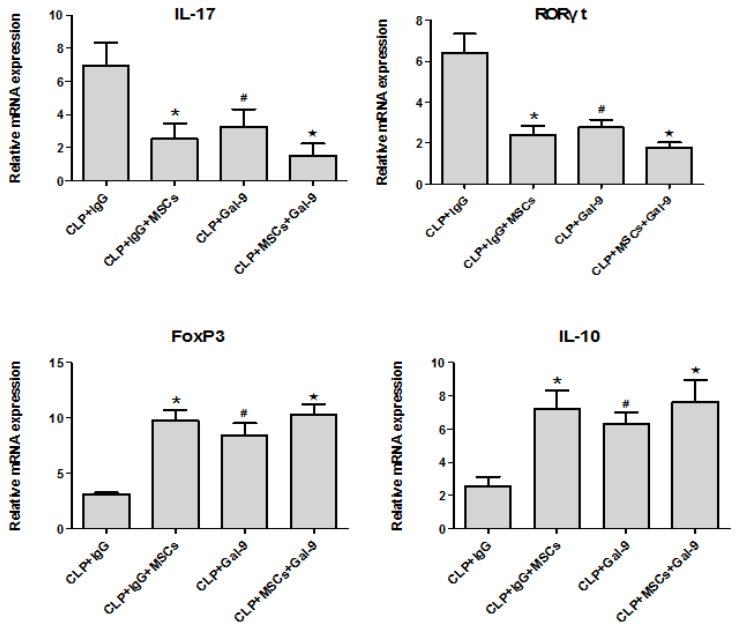


Figure 4A: The mRNA expression of IL-17、RORγt、FoxP3 and IL-10 in kidney tissues 24 h after CLP operation.. compared with CLP + IgG group, *P<0.05,#P<0.05; compared with the CLP + Gal-9 group, ★P >0.05;

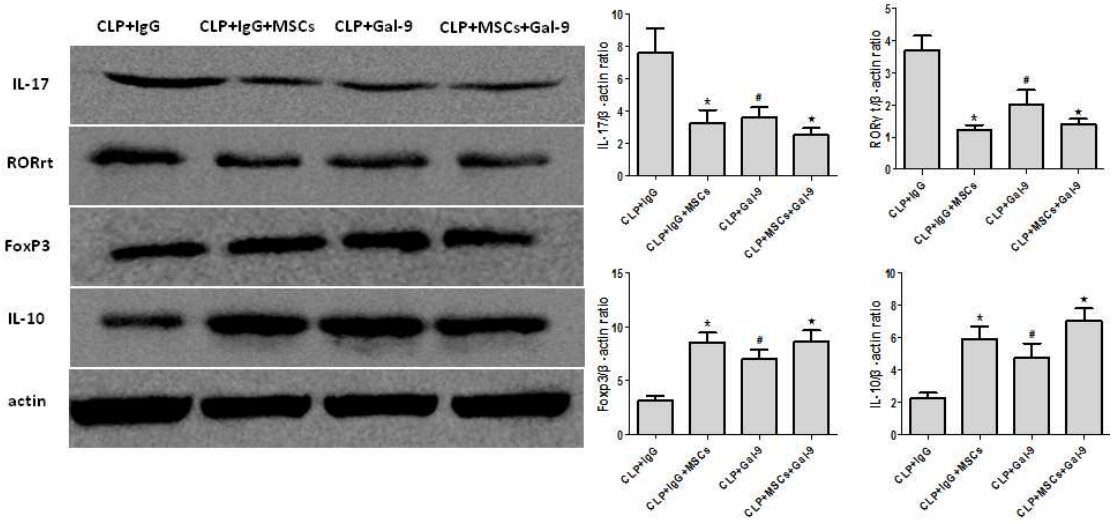


Figure 4B: The Western blotting expression and quantitative analysis of IL-17、RORγt、FoxP3 and IL-10 in kidney tissues. The protein levels are presented as mean ± SD. compared with CLP + IgG group, $^{*}P<0.05$,$^{\#}P<0.05$; compared with the CLP + Gal-9 group, $^{\star}P>0.05$

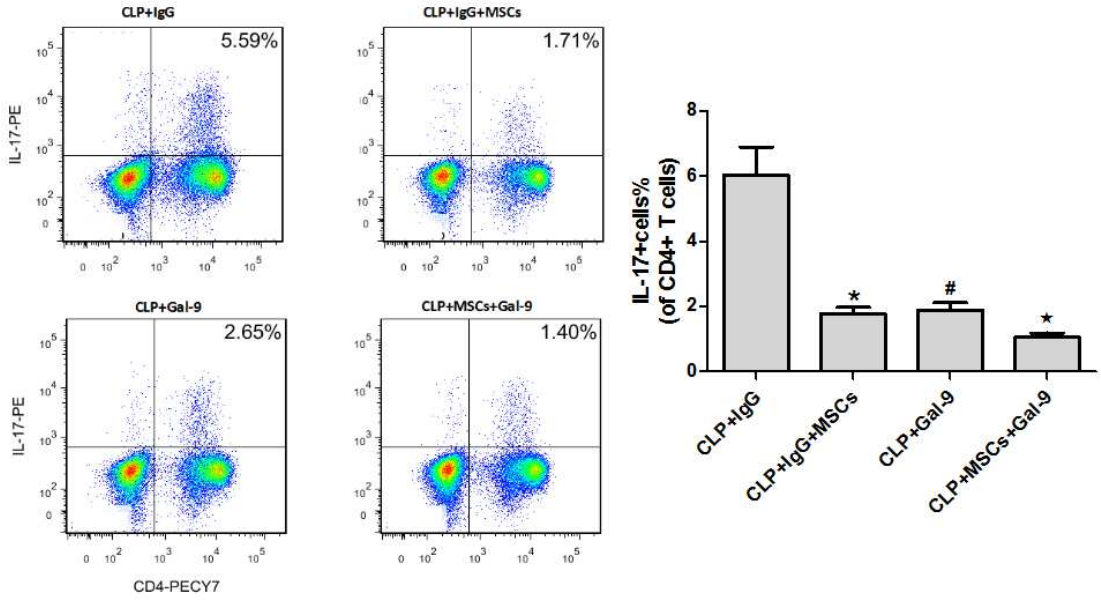


Figure 4C: The percentage of Th17 cells relative to CD4+ T cells were measured by flow cytometry after the preparation of single-cell suspensions. the values are mean ± SD. Comparisons between groups were performed using Student t-test. compared with CLP + IgG group, $^{*}P<0.05$,$^{\#}P<0.05$; compared with the CLP + Gal-9 group, $^{\star}P>0.05$

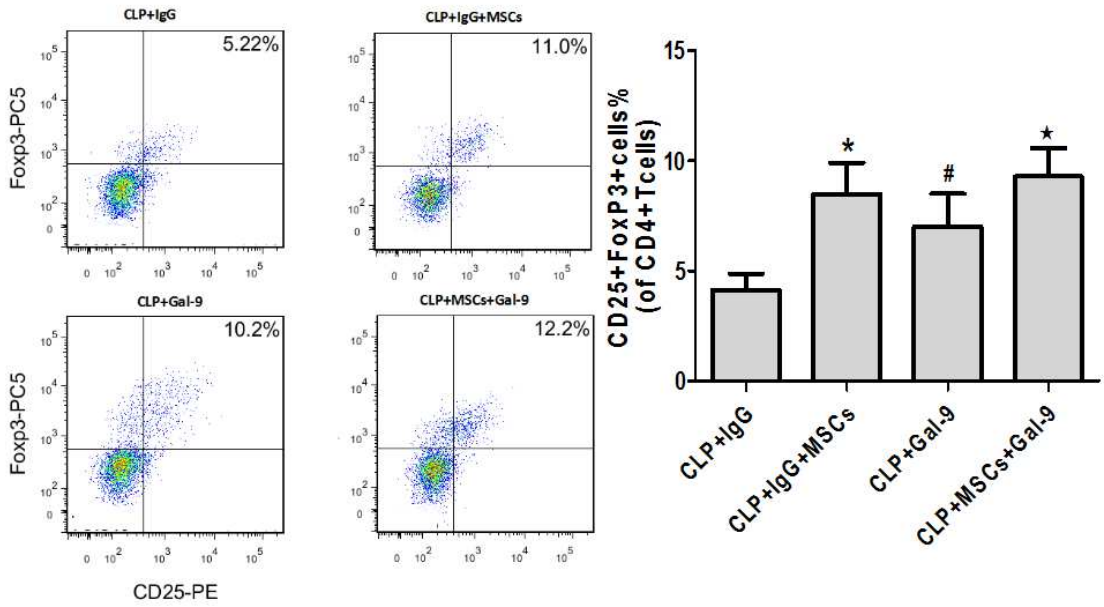


Figure 4D: The percentage of Treg cells relative to CD4+ T cells were measured by flow cytometry after the preparation of single-cell suspensions. the values are mean ± SD. Comparisons between groups were performed using Student t-test. compared with CLP + IgG group, $^{*}P<0.05$,$^{\#}P<0.05$; compared with the CLP + Gal-9 group, $^{\star}P>0.05$

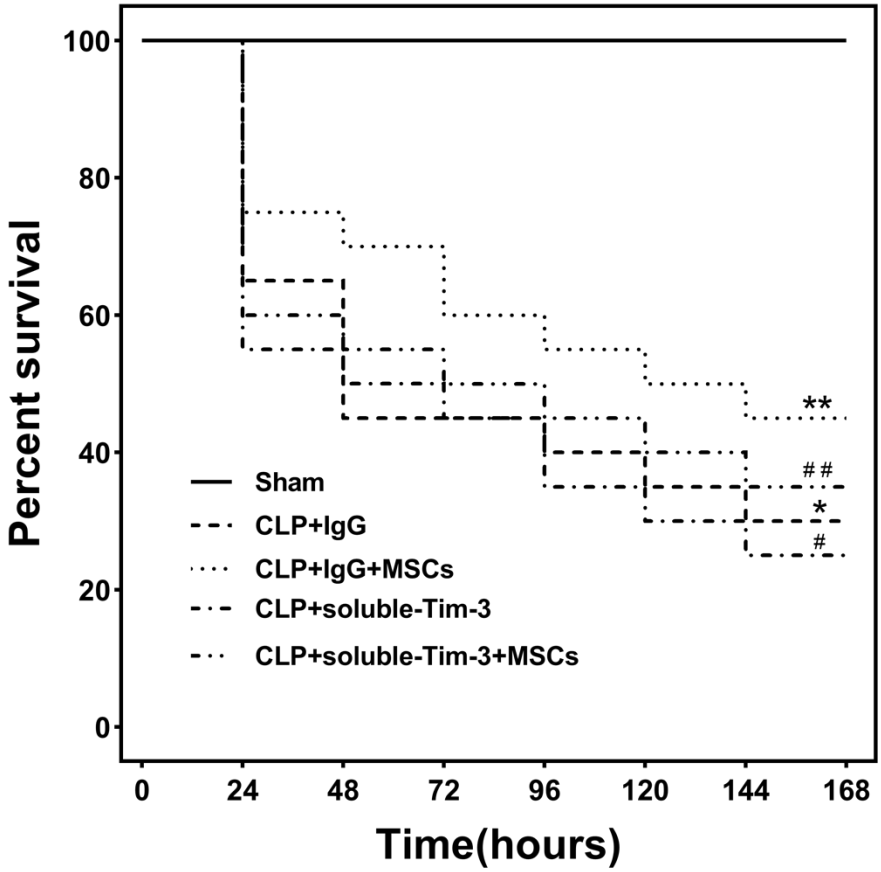


Figure 5. Seven-day survival rate for mice in each group: Each group consisted of 20 animals. Kaplan-Meier curves represent the survival rate in each group. compared with the CLP + IgG group, $^{\#}P<0.05$，$^{**}P>0.05$; compared with CLP+ soluble-Tim-3 group,

##P>0.05;

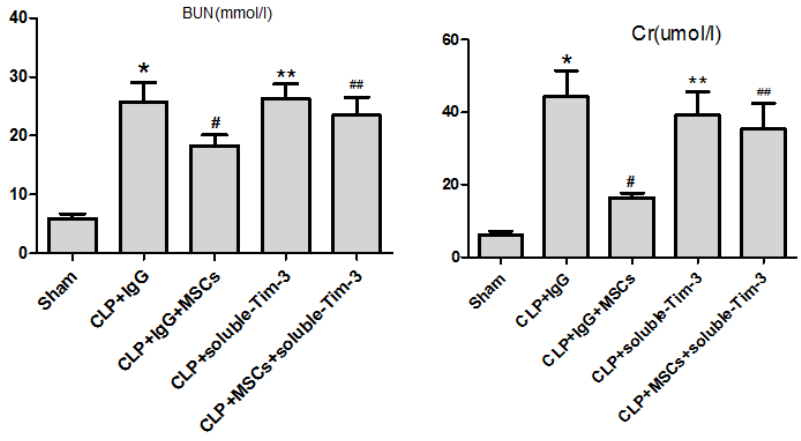


Figure 6. The levels of serum Cr and BUN 24 h after CLP injury in each group: values are mean ± SD; n = 8 in each group. compared with the CLP + IgG group, #P<0.05， **P>0.05; compared with CLP+ soluble-Tim-3 group, ##P>0.05

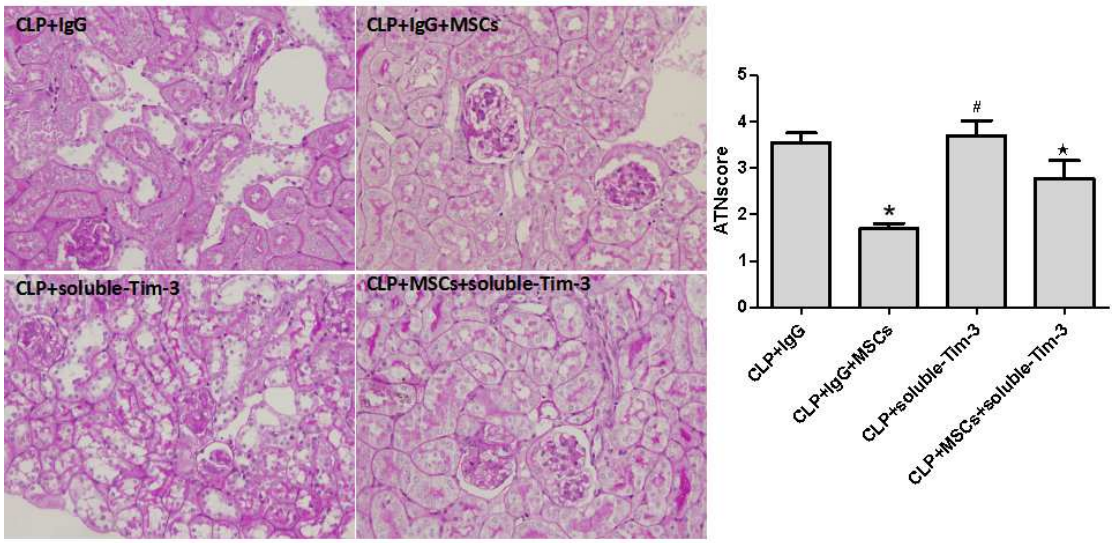
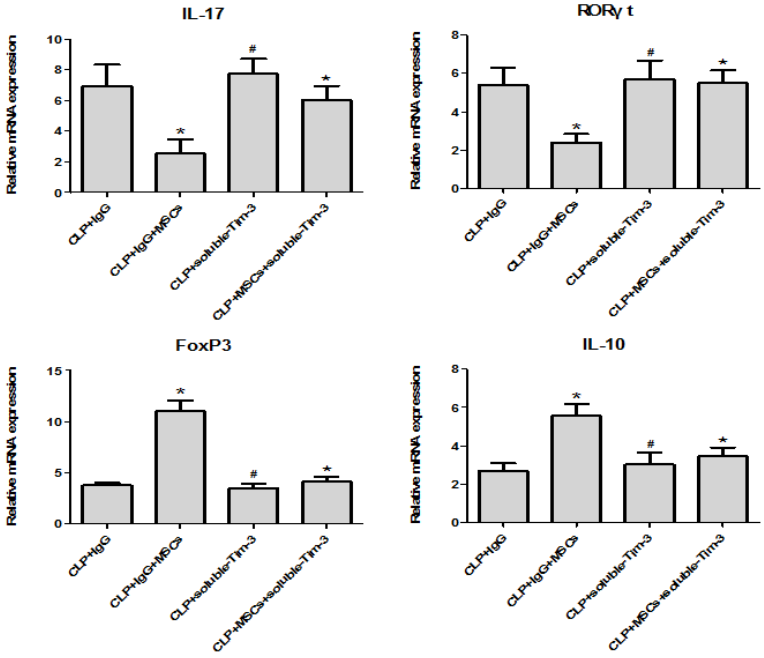


Figure 7. The acute tubular necrosis (ATN) scores 24 h after CLP in each group: values are mean ± SD; n = 8 in each group. compared with the CLP + IgG group, *P<0.05，#P>0.05; compared with CLP+ soluble-Tim-3 group, ★P >0.05



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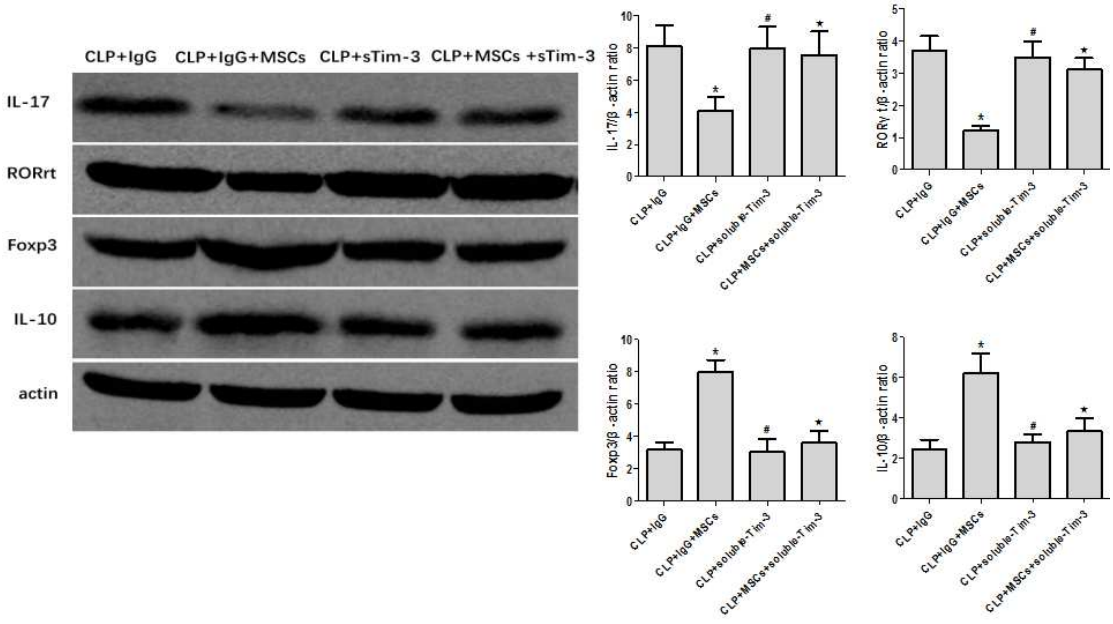

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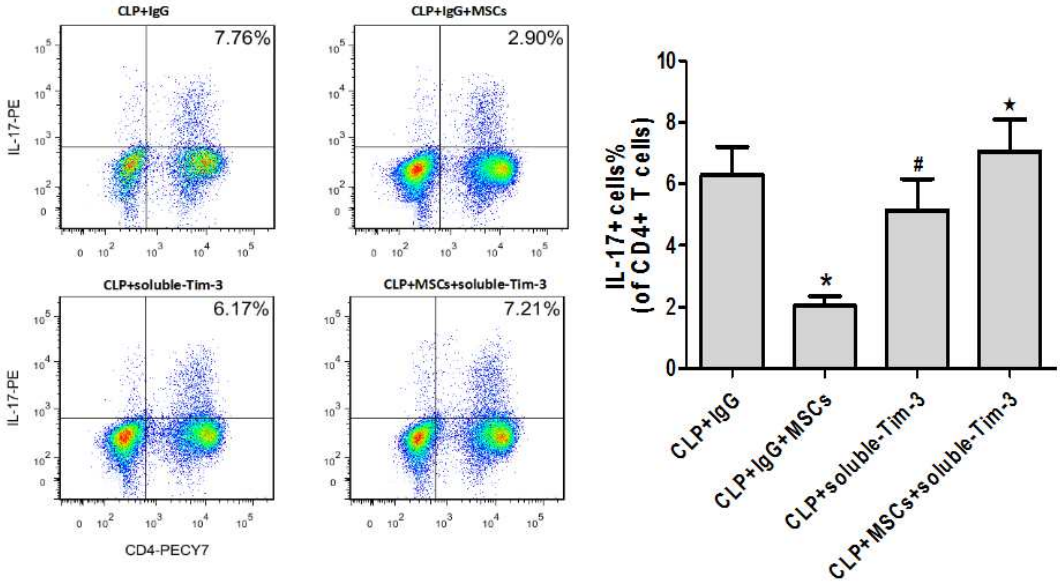

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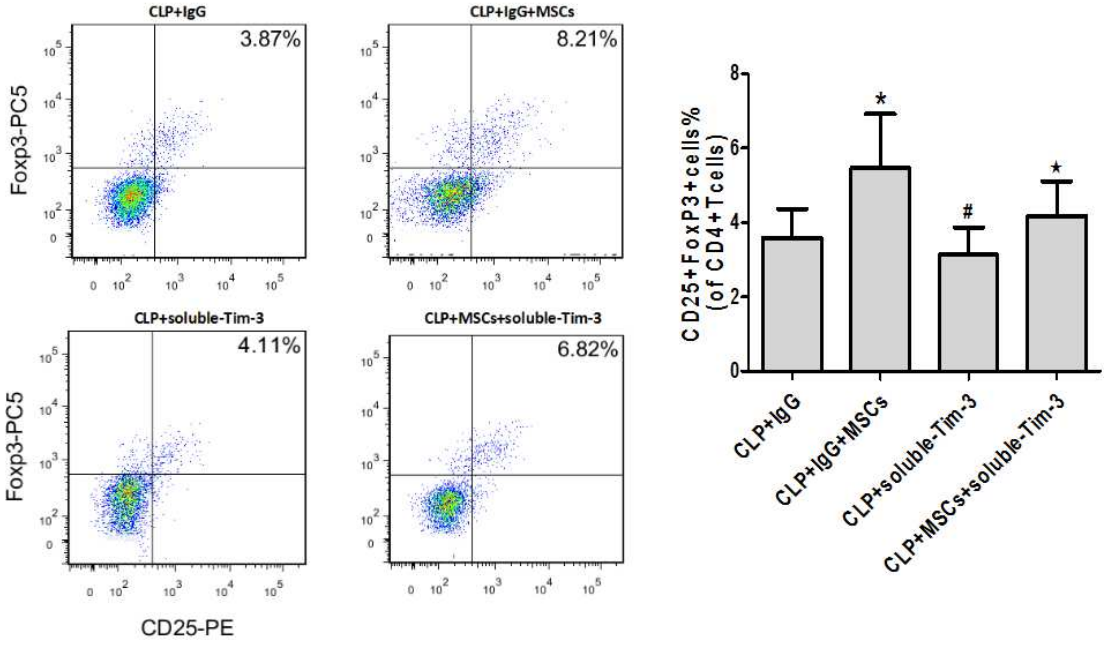

Figure 8D: The percentage of Treg cells relative to CD4+ T cells were measured by flow cytometry after the preparation of single-cell suspensions. the values are mean ± SD. Comparisons between groups were performed using Student t-test. compared with the CLP + IgG group, *P<0.05，#P>0.05; compared with CLP+ soluble-Tim-3 group, ★ P >0.05.