Available online at www.blood-genomics.com

Blood and Genomics

2023, 7(1): 67−70

B & G

*Brief Report*

# The inflammation mediated by mesenchymal stem cells enhances blood group antigen-antibody responses *via* IL-1β

Qiwang Liu[△], Xiaoshuang Wu[△], Yaozhen Chen, Zhixin Liu, Wenting Wang, Qunxing An[*], Xingbin Hu[*]

*Department of Blood Transfusion, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710032, China.*

## ABSTRACT

While it is known that the inflammatory state of the recipient may regulate the production of blood group antibodies, the cellular and molecular mechanisms are unclear. Mesenchymal stromal cells (MSCs) can produce inflammatory factors in response to inflammation and regulate inflammatory immunity. To explore how inflammatory MSCs exert influence on blood group antigen-antibody responses, the inflammatory MSC culture solution was added to the blood group antigen-antibody response system. Flow cytometry was used to detect the effect of different concentrations of inflammatory MSC supernatant on the production of blood group antibodies. Western blotting was used to determine whether the IL-1β receptor was expressed on the surface of red blood cells (RBCs). The intensity of antigen-antibody response was detected by serological methods. The results demonstrated that IL-1β produced by inflammation-induced MSCs could directly bind to the surface of erythrocytes and interfere with blood group antigen-antibody responses. This study suggests that inflammatory MSC-derived IL-1β might enhance the strength of blood antigen-antibody responses.

**Keywords:** inflammation, mesenchymal stromal cell, IL-1β, transfusion reaction

## INTRODUCTION

Red blood cells (RBCs) supply oxygen to tissues throughout the body. Most trauma patients have low-functioning or an insufficient number of RBCs, and thus require blood transfusion. However, even if the ABO and Rh blood group systems are completely compatible, patients may still be stimulated by other blood group system antigens, such as the Kell, Duffy, and Kidd systems. About 3%–10% of RBC transfusion recipients produce harmful antibodies against blood group antigens[1].

Transfusion reactions that cause hemolysis are closely related to inflammation, but the mechanism is unclear[2]. Under inflammatory conditions, immune-cell-mediated responses to external blood group antibodies are major factors in hemolysis[3]. In recent years, many studies have reported that liver cells, intestinal epithelium, skin cells, and mesenchymal stromal cells (MSCs) can regulate the body's inflammatory response[4]. After transfusion, exogenous erythrocytes have extensive contact with vascular endothelial cells, hepatocytes, and MSCs, which may induce hemolysis and tissue damage caused by inflammation.

In a previous study, NLRP3 inflammasomes in

[*]Corresponding to: Qunxing An and Xingbin Hu, Department of Blood Transfusion, Xijing Hospital, Fourth Military Medical University, 127 Changle West Road, Xincheng District, Xi'an, Shaanxi 710032, China. E-mails: bestar01@163.com and hxbyqh@163.com.

[△]These authors contributed equally to this work. [△]These authors contributed equally to this work.

Conflict of interests: The authors declared no conflict of interests.

https://doi.org/10.46701/BG.2023012022024

MSCs were shown to be activated by lipopolysaccharides (LPSs) or nigericin[5]. Moreover, our previous research also demonstrated that MSCs can affect the physicochemical parameters of RBCs[6]. Therefore, we hypothesize whether inflammatory MSCs are involved in the response of blood group antigens and antibodies. The study aims to explore the potential relationship between inflammatory MSCs, blood group antigens, and antibody response, as well as its mechanism.

## MATERIALS AND METHODS

### Isolation of MSCs

MSCs were isolated and obtained as per our previously reported strategy[7]. Briefly, femurs from sacrificed C57BL/6 mice were crushed. After obtaining the bone chips, type-I collagenase was digested to release MSCs. $CD45^+$ and $Ter119^+$ cells were sorted using a mouse compact bone mesenchymal progenitor cell enrichment kit (#19771, STEMCELL Technologies, Inc., Vancouver, Canada). All animal procedures were approved by the Experimental Animal Committee of the Fourth Military Medical University.

### Treatment of MSCs

After stimulating $10^6$ MSCs with 5 nmol/L nigericin for 1.5 hours, the NLRP3 inflammasome in the MSC was activated, and the supernatant of the MSC culture medium was collected to treat RBCs in equal proportions.

### Flow cytometry analysis

Healthy humans donated blood for this study after volunteering and providing the informed consent. RBCs were isolated from their blood, and then treated with MSCs activated by nigericin, and single-cell suspensions were prepared. Human anti-B blood group antigens (S10980059, Shanghai Raas Blood Products Co., Ltd., Shanghai, China) were added before flow cytometry. IL-1β receptor flow antibody (ab246519, Abcam, USA) was used to detect the IL-1β receptor in human RBCs. Flow cytometric data were acquired using FACSCalibur (BD Immunocytometry Systems, San Jose, CA, USA) and analyzed using FlowJo software.

### Blood group antigen-antibody titration

After erythrocytes were treated with murine MSCs stimulated with nigericin or IL-1β (#12426, Cell Signaling Technology, Danvers, MA, USA), the suspended cells were collected. The samples were then centrifuged and washed once with saline, and the blood group antigens were titrated with human RBC antibodies (IgM).

### Western blotting analysis

Healthy human RBCs were lysed using ice-cold RIPA lysis buffer. Protein concentrations were determined using a Pierce BCA Protein Assay Kit (#23225, Thermo Fisher Scientific, Inc., Waltham, MA, USA). Equal amounts of protein were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and blocked with the following primary antibodies: IL-1β receptor (ab246519, Abcam, USA) and CD235 (PA5-115 298, eBioscience, San Diego, USA). Signals were detected using an enhanced chemiluminescence kit (Amersham Biosciences, Little Chalfont, UK). The Western blotting was analyzed using Image Lab software (Bio-Rad Laboratories, Hercules, USA).

### Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism 6 software (La Jolla, San Diego, USA). Student's *t*-test or one-way ANOVA with Tukey's post-hoc or Dunnett's correction were used where appropriate. Statistical significance was set at $P<0.05$.

## RESULTS

### MSCs enhance the blood group antigen-antibody response

To determine whether the blood group antigen-antibody response was interfered with MSCs during inflammation, the supernatant from the MSC culture medium was added to the reaction system. The results showed that, upon addition of cultured MSCs to stimulate an inflammatory response, the binding strength of anti-B antibody to human B-type RBCs increased when compared with the control lacking MSCs (***Fig. 1A–B***). In addition, the group with added MSCs culture supernatant and equally diluted anti-B antibodies showed a stronger antigen-antibody response than the controls (***Fig. 1C***). It was also observed that nigericin-treated MSCs directly increased the intensity of the antigen-antibody response (***Table 1***).

### IL-1β enhances the intensity of the blood group antigen-antibody response

The IL-1β receptors on RBC membranes were checked using flow cytometry analysis. As shown in

***Fig. 2A***, the IL-1β receptor was displayed on the surface of RBCs. Western blotting analysis also demonstrated that the IL-1β receptor was present on the RBC membrane (***Fig. 2B***). It was found that IL-1β could directly enhance the intensity of the blood group antigen-antibody response (***Fig. 2C***, ***Table 2***).

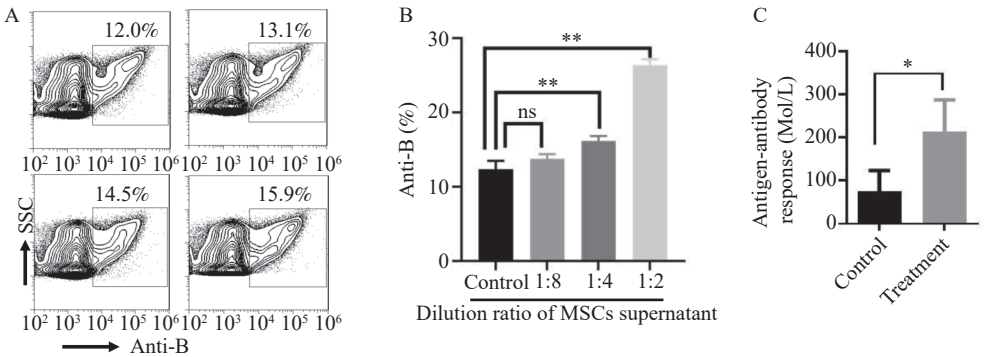


***Fig. 1* Detection of the effect of mesenchymal stromal cells (MSCs) on red blood cell (RBC) antigens and reactivity under the inflammatory condition.** A: Flow cytometry analysis of human erythrocyte blood group antigens. The upper left is the untreated control group. The upper right is the supernatant and the total culture system with a ratio of 1:8. The lower left has a volume ratio of 1:4, and the lower right has a volume ratio of 1:2. B: Quantitative analysis of the effect of MSCs supernatant on the fluorescence intensity of B antigen. C: The difference between the control group (B antigen-antibody reaction) and the treatment group (B antigen-antibody reaction with MSC culture supernatant). **: $P<0.01$; *: $P<0.05$; ns: no statistical significance ($n=3$).

***Table 1* Human blood type antibody titration**

| Anti-B dilution | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |
|---|---|---|---|---|---|---|---|---|
| Control | $3+^{w}$ | $2+^{w}$ | 1+ | 1+ | $1+^{w}$ | ± | 0 | 0 |
| Control | 3+ | $2+^{w}$ | 1+ | 1+ | $1+^{w}$ | ± | 0 | 0 |
| Control | $3+^{w}$ | 2+ | 1+ | $1+^{w}$ | ± | 0 | 0 | 0 |
| Treatment | $4+^{w}$ | 3+ | $3+^{w}$ | 2+ | 1+ | 1+ | $1+^{w}$ | ± |
| Treatment | 4+ | $4+^{w}$ | $3+^{w}$ | $2+^{w}$ | 1+ | 1+ | $1+^{w}$ | ± |
| Treatment | $4+^{w}$ | 3+ | $3+^{w}$ | 2+ | $2+^{w}$ | 1+ | $1+^{w}$ | ± |

After doubling dilution of the anti-B antibody with a titer of 256, the reactivity of different titers of antibodies in the treatment group (supernatant: total volume 1:2) and the control group was detected. $^{w}$: a weak reaction; ±: agglutination can be observed under microscope; 1+: large granule surrounded by free red blood cells (RBCs); 2+: RBCs are dispersed into small clots with free RBCs; 3+: erythrocytes agglutinate into small pieces with clear serum; 4+: erythrocytes agglutinate into a mass with clear serum.

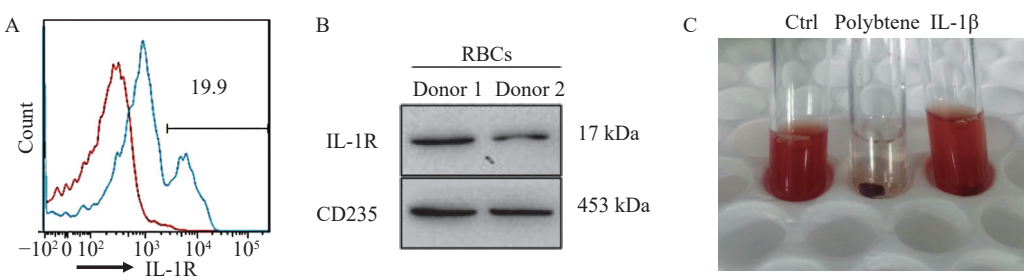


***Fig. 2* Verification of the presence of the IL-1β receptor on human red blood cells (RBCs), and detection of the effect of IL-1β on human erythrocyte antigen-antibody responses.** A: Flow cytometry analysis of the IL-1β receptor on the surface of RBCs. B: Western blotting analysis of the IL-1β receptor on RBCs. C: IL-1β was directly added to the human erythrocyte antigen-antibody reaction system. Ctrl: negative control; Polybrene: positive control.

***Table 2* Human erythrocyte blood group antigen-antibody reaction**

| | Control | Polybrene | IL-1β |
|---|---|---|---|
| Group 1 | 0 | 3+ | 1+ |
| Group 2 | 0 | 4+ | 1+ |
| Group 3 | 0 | 3+ | $2+^{w}$ |

The strength of the antigen-antibody reaction under the stimulation of IL-1β was verified by serological methods.

## DISCUSSION

The number of patients who produce abnormal blood group antibodies after RBC transfusion is large, which raises questions about the safety of RBC transfusion[1]. In the present study, IL-1β signal existed after the addition of inflammatory MSCs, suggesting that it may play a role in the blood group antigen-antibody response[5]. Based on these findings, more attention should be paid to patients with inflammation who require blood transfusion.

The results illustrated that IL-1β produced by inflammation-induced MSCs can directly bind to the surface of erythrocytes and interfere with blood group antigen-antibody responses *in vitro* and provided a directly evidence that IL-1β may act on the surface of RBCs. This study suggests that inflammatory MSC-derived IL-1β may enhance the strength of the blood antigen-antibody response. Therefore, targeting IL-1β produced by MSCs can be useful in attenuating the intensity of the blood group antigen-antibody response, which may weaken transfusion reactions, especially in hemolytic transfusion reactions. Hopefully, attenuating IL-1β from MSCs can relieve the blood group antigen-antibody response. Although some studies have reported that MSCs can be used to treat various diseases[8], our study suggests that inflammatory MSCs decrease transfusion success. Consequently, further investigation is required, both in the fields of transfusion and disease treatment, to gain a better view of the use of MSCs in clinical applications.

## Acknowledgments

This study was supported by the grant from the National Natural Science Foundation of China (No. 81800740).

## References

[1] Gibb DR, Calabro S, Liu D, et al. The Nlrp3 inflammasome does not regulate alloimmunization to transfused red blood cells in mice[J]. *EBioMedicine*, 2016, 9: 77−86.

[2] Elayeb R, Tamagne M, Bierling P, et al. Red blood cell alloimmunization is influenced by the delay between Toll-like receptor agonist injection and transfusion[J]. *Haematologica*, 2016, 101(2): 209−218.

[3] Yi T, Li J, Chen H, et al. Splenic dendritic cells survey red blood cells for missing self-CD47 to trigger adaptive immune responses[J]. *Immunity*, 2015, 43(4): 764−775.

[4] Bernardo ME, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation[J]. *Cell Stem Cell*, 2013, 13: 392−402.

[5] Chen Y, Qin X, An Q, et al. Mesenchymal stromal cells directly promote inflammation by canonical NLRP3 and non-canonical caspase-11 inflammasomes[J]. *EBioMedicine*, 2018, 32: 31−42.

[6] Chen Y, Zhang J, Gu S, et al. Mesenchymal stromal cells can be applied to red blood cells storage as a kind of cellular additive[J]. *Biosci Rep*, 2017, 37(5): BSR20170676.

[7] Hu X, Garcia M, Weng L, et al. Identification of a common mesenchymal stromal progenitor for the adult haematopoietic niche[J]. *Nat Commun*, 2016, 7: 13095.

[8] Zhang K, Jiang Y, Wang B, et al. Mesenchymal stem cell therapy: a potential treatment targeting pathological manifestations of traumatic brain injury[J]. *Oxid Med Cell Longev*, 2022, 2022: 4645021.

**Received 3 October 2022, Revised 7 April 2023, Accepted 10 April 2023**