The effect of erythrocyte transfusion on macrophage pyroptosis and inflammation in a sepsis model

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Abstract

Background. Sepsis is one of most common causes of death in the intensive care unit (ICU) due to infection and inflammation. The Duffy antigen receptor for chemokines (DARC) regulates pro-inflammatory cytokines, thus playing an important role in inflammation.

Objectives. This study aimed to elucidate the correlation among erythrocyte transfusion, macrophage pyroptosis and inflammation in the progression of sepsis.

Materials and methods. Alanine aminotransferase (ALT/GPT) activity was measured with the ALT/GPT activity measurement kit (Jiancheng Bio, Nanjing, China) according to the kit manual. The ET-1 concentration was measured with enzyme-linked immunosorbent assay (ELISA) using the endothelin-1 (ET-1) measurement kit (Jiancheng Bio) according to the kit manual. Apoptosis was evaluated using flow cytometry-based Annexin V staining assay. The cells were collected using centrifugation and resuspended in binding buffer. Ultrastructural analysis of pyroptotic body, the levels of interleukin (IL)-1β, IL-18, IL-33, MIP-2, CXCL8, reactive oxygen species (ROS), and LTB4 were measured with ELISA.

Results. Our results showed that septic rats had impaired hepatic function and ET-1 levels. Erythrocyte transfusion upregulated DARC expression in the sepsis model. Erythrocyte transfusion also affected pyroptosis in macrophages, reduced the production of inflammatory cytokines, such as IL-1β, IL-18 and IL-33, and alleviated cytotoxicity in the sepsis model.

Conclusions. Erythrocyte transfusion may function as a therapeutic tool against sepsis by regulating pyroptosis, inflammation and cytotoxicity.

Key words: transfusion, erythrocyte, pyroptosis, sepsis, DARC

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Background

The Duffy antigen receptor for chemokines (DARC) is an atypical receptor that regulates pro-inflammatory cytokines.[1] It is expressed in multiple tissues, including kidney and brain.[2,3] It has also been reported that DARC plays a role in erythrocyte function. For example, DARC found on the surface of erythrocytes facilitates chemokine reception.[4] *Staphylococcus aureus* has been shown to target DARC to lyse erythrocytes within the mammalian host.[5] The DARC is also engaged in *Plasmodium* spp.-induced red blood cell invasion.[6] Furthermore, DARC regulates inflammatory responses in adipose tissues,[7] asthma[1] and fractures.[8] Thus, DARC may act as a critical regulator in inflammation-related pathological processes.

Sepsis is one of the most common causes of death among hospitalized patients in the intensive care unit (ICU).[9] It has multiple effects on erythrocytes.[10] Sepsis alters erythrocyte morphology and blood flow in capillary networks.[11] The mechanism underlying the effect of sepsis on erythrocytes remains unclear; however, several factors such as inflammation and oxidative stress are involved in this process.[12] Previous studies have explored the effect of erythrocyte transfusion in patients with sepsis.[13] However, the mechanism is still controversial. More and more studies demonstrate that oxygen delivery remains sufficient during sepsis while erythrocyte transfusion increases the oxygen-carrying capacity of the blood flow.[14]

Pyroptosis is a programmed and inflammatory form of cell death, which is different from the long-standing form of cell death. It is one of the mechanisms of programmed cell death (PCD). Pyroptotic cells undergo nuclear condensation and chromatin DNA fragmentation,[15] releasing pro-inflammatory mediators.[16] Pyroptosis contributes to the pathogenesis of several diseases, including cancer,[17] atherosclerosis,[18] liver disease,[19] etc. In sepsis, pyroptosis is a hallmark that marks progress and recovery.[20,21] In this study, we found that erythrocyte transfusion affected pyroptosis in macrophages and attenuated inflammation in a sepsis model. This work elucidated the correlation among erythrocyte transfusion, macrophage pyroptosis and inflammation in the progression of sepsis, thereby presenting a potential therapeutic method for treating sepsis.

Materials and methods

Establishment of a rat sepsis model

Two-month-old male Sprague Dawley rats (250 g) were purchased from Shanghai Model Organisms (Shanghai, China). Rats were housed under pathogen-free conditions with a 12-hour light/dark cycle. Rats received a single intraperitoneal injection of 10 mg/kg of lipopolysaccharide (LPS; Sigma-Aldrich, St. Louis, USA). Control animals received an equal volume of intraperitoneal saline.

Measurement of ALT/GPT activity

Alanine aminotransferase (ALT/GPT) activity was measured with the ALT/GPT activity measurement kit (Jianchen Bio, Nanjing, China) according to the kit manual.

Measurement of endothelin-1 concentration

The endothelin-1 (ET-1) concentration was measured using enzyme-linked immunosorbent assay (ELISA) using the ET-1 measurement kit (Jiancheng Bio) according to the kit manual.

Erythrocytes isolation and transfusion

Whole blood was centrifuged at 500 × g for 10 min at 4°C. The supernatant containing plasma was aspirated and erythrocyte pellets were rinsed with wash buffer. Then, erythrocytes were again centrifuged at 500 × g for 10 min at 4°C and rinsed with wash buffer. Freshly collected erythrocytes were transfused into the sepsis model via tail vein injection.

Flow cytometry

Erythrocytes membrane protein DARC was determined using anti-DARC-FITC antibody (Abcam, Cambridge, UK). Followed by the fixation with 2% paraformaldehyde for 15 min, citrated blood was incubated with HEPES for 2 min. The antibody was added to the sample and incubated for 45 min in the dark. Cells were washed and suspended in Dulbecco's phosphate-buffered saline (DPBS). Flow cytometry was performed using a BD Accuri C6 (Becton Dickinson Biosciences, Franklin Lakes, USA). Data were analyzed using CFlow Plus software v. 1.0.227.04 (Becton Dickinson). Macrophage population was differentiated using flow cytometry (FCM) as previously described.[22]

Annexin V staining assay

Apoptosis was evaluated using FCM-based Annexin V staining assay. Briefly, cells were collected using centrifugation and resuspended in binding buffer. Then, 5 µL of Annexin V-FITC and 5 µL of propidium iodide (PI) were added to the cells and incubated at room temperature for 5 min in the dark. Finally, Annexin V-FITC binding was analyzed (Ex = 488 nm; Em = 350 nm) using FITC signal detector and PI staining using a phycoerythrin emission signal detector.

Ultrastructural analysis of pyroptotic body

Ultrastructural alteration in the pyroptotic body was detected with a scanning electron microscope (SEM). Samples were prepared as previously reported.[23] Images were captured under the JEOL NeoScope JCM-7000 Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan).

ELISA

The levels of interleukin (IL)-1β, IL-18, IL-33, macrophage inflammatory protein 2 (MIP-2), Invitrogen anti-IL-8 (CXCL8) (Invitrogen, Carlsbad, USA), reactive oxygen species (ROS), and leukotriene B4 (LTB4) were measured with ELISA. The ELISA kit (Jiancheng Bio) was used to detect the changes in the concentration of the abovementioned cytokines. The measurement was performed according to the kit manual.

Cytotoxicity assay

The concentration of LDH was measured with the LDH detection kit (Jiancheng Bio) according to the kit manual.

Statistical analyses

Data were expressed as mean ± standard error of the mean. Statistical significance was determined using unpaired Student's t-test or one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test using GraphPad Prism software v. 5.0 (GraphPad Software, San Diego, USA). A p-value <0.05 was considered statistically significant.

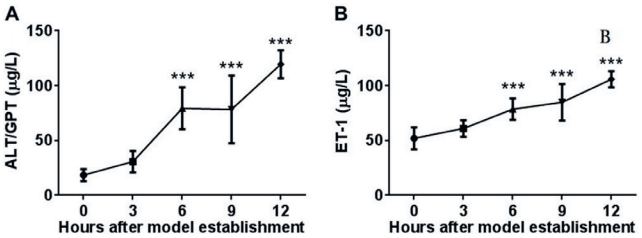

Fig. 1. The alteration in the levels of ALT/GPT (A) and ET-1 (B) in septic rats. The statistical difference was determined using a one-way ANOVA followed by the Bonferroni post hoc test; ***p < 0.001 compared to 0 h after model establishment

Results

Septic rats showed impaired hepatic function and altered endothelin-1 levels

Firstly, we examined the hepatic function and ET-1 levels in the sepsis model. The concentration of ALT/GPT was significantly higher in septic rats at 6 h, 9 h and 12 h after model establishment (Fig. 1A, p < 0.001, n = 3) compared with those measured at the 0 h. Meanwhile, ET-1 concentration at 6 h, 9 h and 12 h after model establishment (Fig. 1B, p < 0.001, n = 3) was also significantly higher than at the 0 h. Thus, the sepsis model was successfully established.

Erythrocyte transfusion affected macrophages in septic rats

Secondly, we injected rats with erythrocytes to evaluate the potential benefit of erythrocyte transfusion. Flow cytometry results showed that the expression of DARC in septic rats injected with erythrocytes (Fig. 2A) was upregulated compared to the control animals (Fig. 2B). Flow cytometry

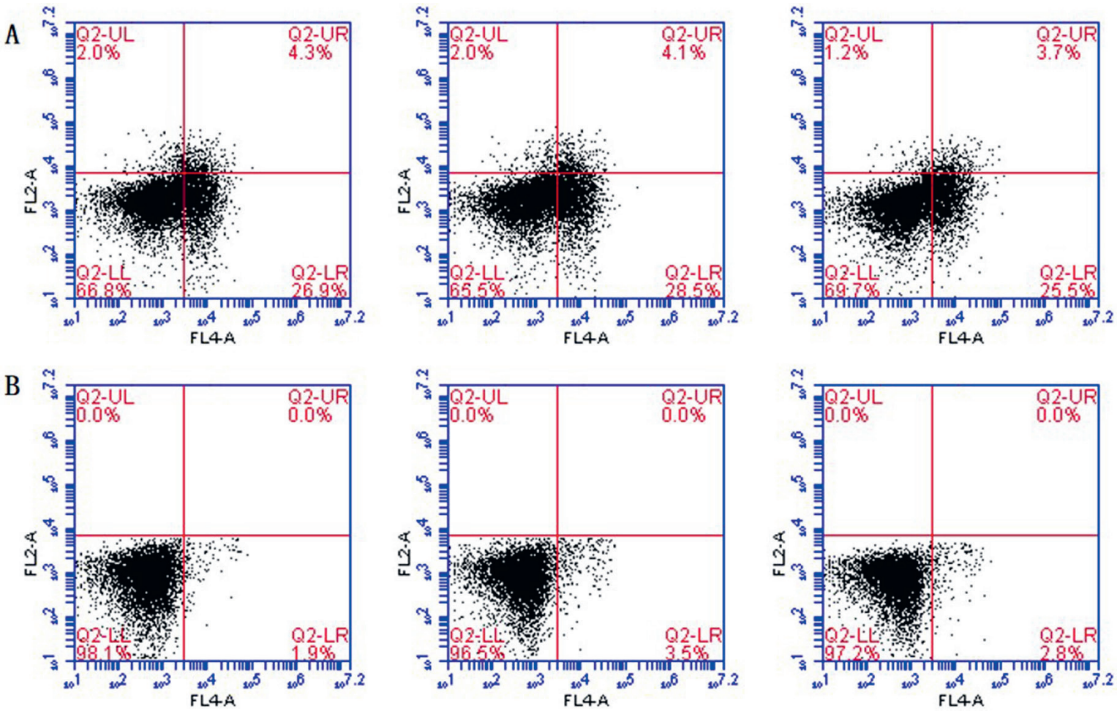

Fig. 2. Flow cytometry showed that (A) erythrocyte injection enhanced DARC level in septic rats in comparison to those injected with saline (B)

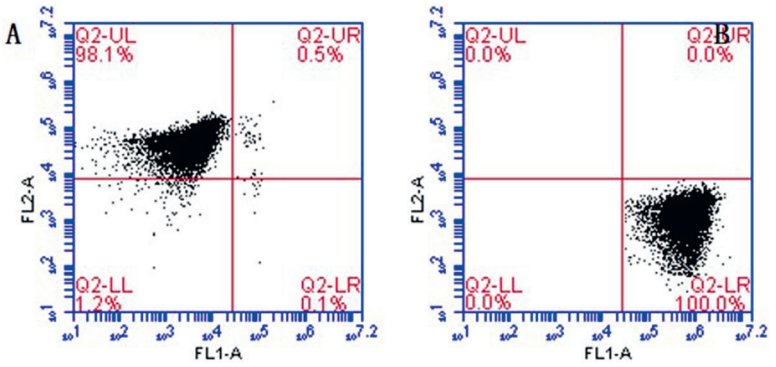

Fig. 3. Flow cytometry indicated that the ratio of macrophages was significantly different between sepsis model rats injected with erythrocytes (A) or saline (B)

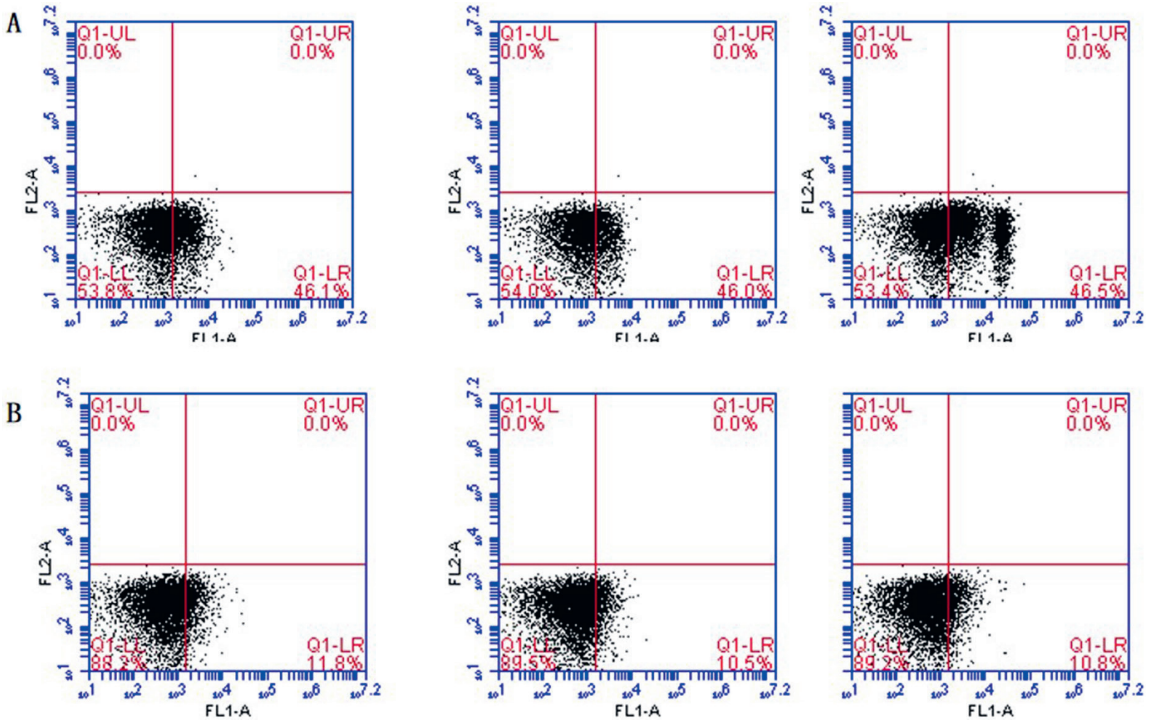

Fig. 4. Flow cytometry showed the alteration in the ratio of apoptotic cells in the blood collected from erythrocyte-injected (A) and saline-injected septic rats (B)

analysis also indicated that the ratio of macrophages in the blood collected from rats treated with erythrocyte transfusion was different from that of the control group (Fig. 3A,B). Furthermore, macrophages of erythrocyte-injected rats underwent more severe apoptosis compared to those administered with saline (Fig. 4A,B). Ultrastructural alteration in the pyroptotic body was detected using SEM. Compared to the saline controls, erythrocyte-injected rats had fewer pyroptotic bodies in macrophages (Fig. 5A,B). Thus, erythrocyte transfusion could attenuate the pyroptosis of macrophages in septic rats.

Erythrocyte transfusion attenuated inflammation in septic rats

Thirdly, we explored whether erythrocyte transfusion could affect inflammatory responses in the sepsis model. The ELISA results showed that the concentration of inflammatory factors (i.e., IL-1β, IL-18, IL-33) and other inflammation-related proteins (i.e., MIP-2, CXCL8, ROS, LTB4) was significantly lower in septic rats injected with erythrocytes as compared with the control animals (Fig. 6). Thus, erythrocyte transfusion suppressed inflammation in the sepsis model.

Erythrocyte transfusion inhibited LDH activity in the sepsis model

To further analyze the effect of erythrocyte transfusion, lactate dehydrogenase (LDH) activity in the sepsis model was measured. The LDH activity of septic rats injected with erythrocytes was significantly lower compared with the control group (Fig. 7), suggesting that erythrocyte transfusion alleviated cytotoxicity in the sepsis model.

Discussion

The DARC is a transmembrane protein expressed on erythrocytes.[24] Since erythrocytes function as a primary reservoir of macrophage migration inhibitory factor in the whole blood, DARC might be correlated with

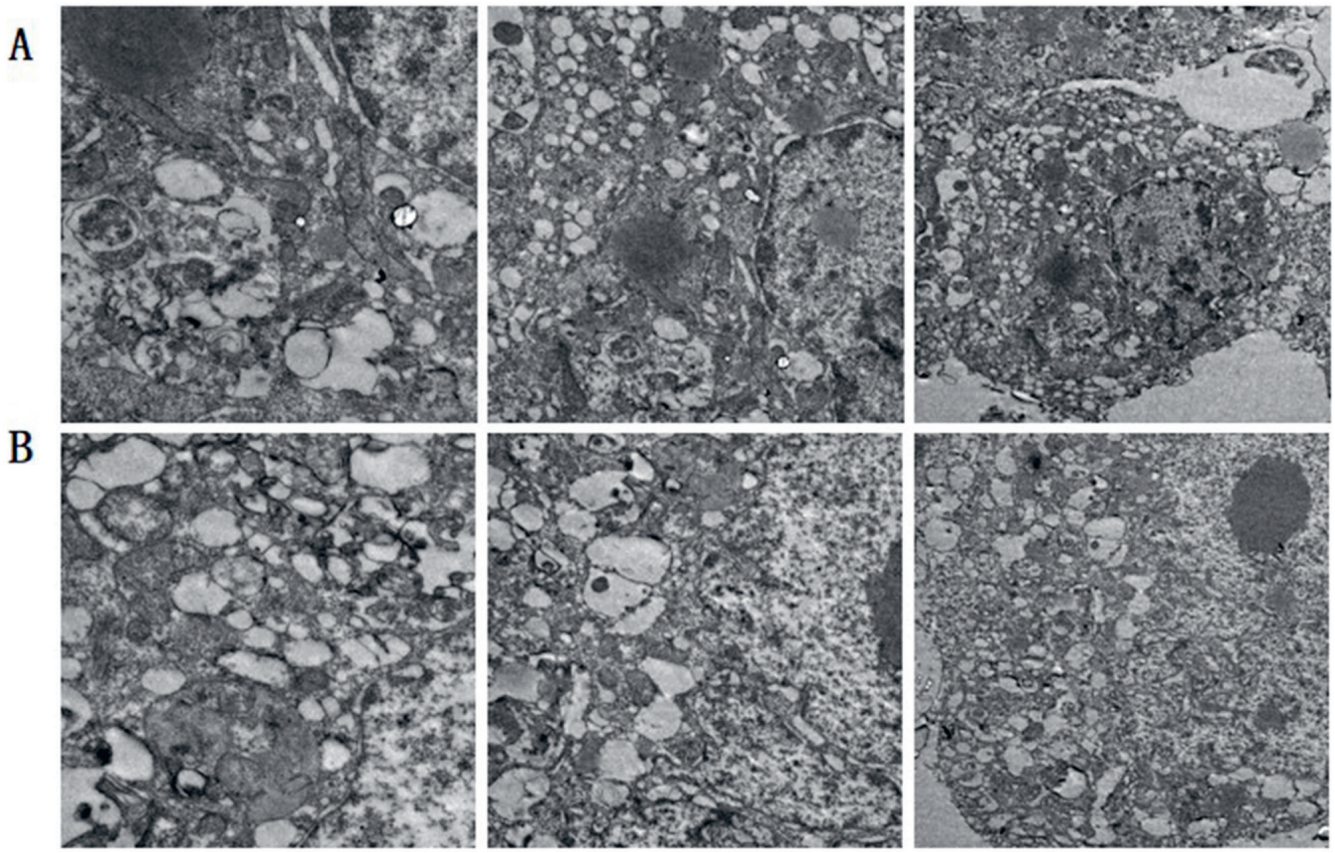

Fig. 5. Ultrastructural alteration of the pyroptotic body of macrophages collected from erythrocyte-injected (A) and saline-treated (B) septic rats was detected with SEM

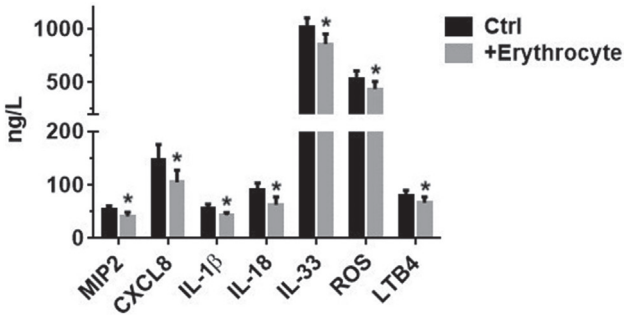

Fig. 6. ELISA showed that the levels of MIP-2, CXCL8, IL-1β, IL-18, IL-33, ROS, and LTB4 were different between erythrocyte-injected (A) and saline-treated (B) septic rats; * $p < 0.05$

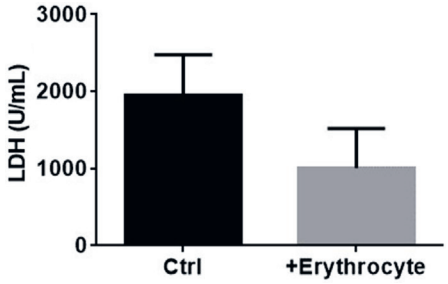

Fig. 7. LDH activity was affected in septic rats injected with erythrocytes

inflammation.[25] We found that the expression of DARC was upregulated in septic rats injected with erythrocytes when compared to the control model animals (Fig. 2). These findings provide direct evidence that DARC is involved in the therapeutic effect of erythrocyte transfusion in sepsis. Furthermore, we showed that erythrocyte transfusion decreased the level of pro-inflammatory factors (Fig. 6), indicating that transfused erythrocytes attenuate inflammation in sepsis. This is the first demonstration of its kind that shows the therapeutic effects of erythrocytes in sepsis treatment. Considering that inflammation is one of the biomarkers in sepsis,[26] a decrease in the level of pro-inflammatory factors may be a promising strategy in treating sepsis. Further investigations of the levels of lactate and C-reactive protein (CRP), and procalcitonin, neutrophil and monocyte activation are needed to comprehensively evaluate the effect of erythrocyte transfusion on sepsis.

Erythrocyte transfusion alleviated pyroptosis in the sepsis model (Fig. 5). Unlike apoptosis and necrosis, pyroptosis is accompanied by the release of pro-inflammatory mediators.[27] Attenuation in pyroptosis inhibited the release of pro-inflammatory factors, which is consistent with our findings that erythrocyte transfusion inhibited the level of pro-inflammatory factors in septic rats (Fig. 6). However, the underlying mechanism of sepsis-induced inflammation is still unclear. Several factors may contribute to potential mechanisms, including cAMP metabolism,[28] NLRP3 activation[29] and inflammasome activation.[30] All of these require further investigation.

Limitations

The results in this paper showed that septic rats had impaired hepatic function and altered ET-1 levels. Erythrocyte transfusion upregulated DARC expression in the sepsis model. Erythrocyte transfusion also affected pyroptosis in macrophages, reduced the production of inflammatory cytokines, such as IL-1β, IL-18 and IL-33, and alleviated cytotoxicity in the sepsis model.

Macrophages polarize to the classical M1 macrophage activation pathway and promote inflammatory cytokine response. Studies on the interaction between stored RBCs infusion and macrophages mainly focus on macrophages in peripheral blood. However, due to time constraints and small sample size, sepsis is affected by a variety of factors. Considering that the purpose of the study is to group and compare according to a single index, and other factors among the groups have not been corrected, a more rigorous, multi-center, large sample and further analysis are still needed to improve the accuracy and universality of the research results.

Conclusions

Erythrocyte transfusion may function as a therapeutic tool against sepsis by regulating pyroptosis, inflammation and cytotoxicity.

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