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Distribution and differentiation of myeloid-derived suppressor cells after fluid resuscitation in mice with hemorrhagic shock*

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Abstract: Objective: To investigate the distribution and differentiation of myeloid-derived suppressor cells (MDSCs) in hemorrhagic shock mice, which are resuscitated with normal saline (NS), hypertonic saline (HTS), and hydroxyethyl starch (HES). Methods: BALB/c mice were randomly divided into control, NS, HTS, and HES resuscitation groups. Three subgroups (n=8) in each resuscitation group were marked as 2, 24, and 72 h. Flow cytometry was used to detect the MDSCs, monocytic MDSCs (M-MDSCs), and granulocytic/neutrophilic MDSCs (G-MDSCs) in peripheral blood nucleated cells (PBNCs), spleen single-cell suspension, and bone marrow nucleated cells (BMNCs). Results: The MDSCs in BMNCs among three resuscitation groups were lower 2 h after shock, in PBNCs of the HTS group were higher, and in spleen of the NS group were lower (all P<0.05 vs. control). The M-MDSC/G-MDSC ratios in PBNCs of the HTS and HES groups were lower (both P<0.05 vs. control). At 24 h, the MDSCs in PBNCs of the NS and HTS groups were higher, while the spleen MDSCs in the HTS group were higher (all P<0.05 vs. control). The M-MDSC/G-MDSC ratios were all less in PBNCs, spleen, and BMNCs of the NS and HTS groups, and were lower in BMNCs of the HES group (all P<0.05 vs. control). At 72 h, the elevated MDSCs in PBNCs were presented in the HTS and HES groups, and in spleen the augment turned up in three resuscitation groups (all P<0.05 vs. control). The inclined ratios to M-MDSC were exhibited in spleen of the NS and HTS groups, and in PBNCs of the NS group; the inclination to G-MDSC in BMNCs was shown in the HES group (all P<0.05 vs. control). Conclusions: HTS induces the earlier elevation of MDSCs in peripheral blood and spleen, and influences its distribution and differentiation, while HES has a less effect on the distribution but a stronger impact on the differentiation of MDSCs, especially in bone marrow.

Key words: Hemorrhagic shock; Hydroxyethyl starch; Hypertonic saline; Myeloid-derived suppressor cells; Normal saline
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1 Introduction

Hemorrhagic shock is a condition of circulating blood volume loss simultaneous with inadequate microcirculation and tissue perfusion, which results in hypoxia, hypo-perfusion, and ischemia-reperfusion injury. These pathophysiological changes may induce multiple organ dysfunction syndrome (MODS) and immune dysfunction, which are the most common causes of mortality in hemorrhagic shock (Liu *et al.*, 2015; Arun *et al.*, 2016; Huber-Lang *et al.*, 2016). Thus, the fundamental purposes of any investigation for hemorrhagic shock resuscitation should be not only improving microcirculatory flow, but also preventing MODS and immune dysfunction. As well as the restoration of microcirculation, many studies also focus on the local and systemic inflammatory response after resuscitation with different fluids (Watters *et al.*, 2006; Wang *et al.*, 2009; Motaharinia *et al.*,

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2015; Öztürk *et al.*, 2015). The crystalloid or colloidal solutions, such as normal saline (NS), hypertonic saline (HTS), and hydroxyethyl starch (HES), are widely used fluids in clinical practice and research. Actually, HTS has been already proven to attenuate the increase of some cytokine production and the activity of immune cells in peripheral circulation (Lu *et al.*, 2008; 2010; 2013; Chen *et al.*, 2011; Motaharinia *et al.*, 2015). It was also found that HES ameliorated oxidative stress and inflammatory response in the rat model during hemorrhagic shock resuscitation (Watters *et al.*, 2006; Wang *et al.*, 2009; Chen *et al.*, 2013).

The immune dysfunction in hemorrhagic shock is determined by the activation of several key immune cells and cytokines in the circulation and immune organs, including spleen, lymphonodus, and bone marrow. Myeloid-derived suppressor cell (MDSC), one of such critical immune-regulated cells, is a heterogeneous cell population consisting of monocytic and granulocytic innate immune cells to suppress T cell responses (Youn *et al.*, 2008). There has been plenty of research on its role in cancer. Studies also found the variety of the amount, distribution, and function of MDSCs in many other pathological states like infection, trauma, hemorrhagic shock, and sepsis (Brudecki *et al.*, 2012; Janols *et al.*, 2014; Lai *et al.*, 2014; Ost *et al.*, 2016). However, the detailed mechanisms of its regulation and function in those states have not been fully understood. In our previous study, compared with NS or HES in the rat model of severe and controlled hemorrhagic shock, we found that HTS could induce an early dramatical increase of MDSCs in peripheral circulation (Lu *et al.*, 2013). The monocytic MDSC (M-MDSC) and the granulocytic/neutrophilic MDSC (G-MDSC) are the two major subsets, which had been recognized. They all have an immunosuppressive function, but also exhibit different effects in various microenvironmental and pathologic states (Heim *et al.*, 2014; O'Connor *et al.*, 2015; Ost *et al.*, 2016; Zhou *et al.*, 2016). Here, we established a mice model of trauma and controlled hemorrhagic shock, which was then resuscitated with NS, HTS, and HES solutions, respectively. We also evaluated the differences of the distribution and subphenotypes of MDSCs in peripheral blood, spleen, and bone marrow nucleated cells (BMNCs) in 2, 24, and 72 h, with the purpose of comparing the effects of three resuscitation fluids after hemorrhagic shock.

2 Materials and methods

2.1 Animals

Eighty male BALB/c mice, 10–15 weeks of age, weighing 20–26 g, were purchased from the Laboratory Animal Centre of Medical Institute of Zhejiang Province, China. They were fed with food and water ad libitum in specific pathogen-free (SPF) conditions at the Key Laboratory of Combined Multi-organ Transplantation, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China. All animal experiments were approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University, Hangzhou, China.

2.2 Experimental protocol

Mice were anesthetized with 40 mg/kg pentobarbital (intraperitoneally (IP)). In brief, along the abdominal medial line, a 2-cm surgical incision was made through the skin and abdominal wall, deep into the enterocoelia. The small intestine was pushed away carefully to expose the inferior vena cava. A 0.5-mm external diameter needle tip was used to draw blood through the inferior vena cava. The 50% of the total circulating blood volume was drawn (about 0.03 ml/g of each mouse body weight) in 30 min to establish the controlled hemorrhagic shock model. A small amount of heparin was reserved in the needle to avoid blood clotting. Then the abdominal wall was closed and covered with sterile gauze. After 30 min observation, a volumetric infusion pump was used to resuscitate mice. The resuscitation procedure is finished at a constant speed in 30 min, and then suturing of the abdominal wall and skin wounds in layers takes place after confirming no blood leakage. Mice were sacrificed 2, 24, and 72 h after closing the abdominal cavity. The spleen, bone marrow, and peripheral blood were taken for the subsequent analyses. All procedures were performed under sterile conditions.

Mice were randomly assigned into control (n=8), NS (n=24), HTS (n=24), and HES (n=24) groups. The control group received no operative intervention except anesthesia. According to the sacrifice time, the NS, HTS, and HES groups were divided into 2, 24, and 72 h subgroups, respectively. Mice in the NS group underwent all the operation procedures such as anesthesia, open surgery, inferior vena cava injection, and blood drawing. They also received 9 g/L NaCl as

the resuscitation fluid, and the dosage is three times the blood loss volume. The operative procedures in the HTS and HES groups are the same as those in the NS group, except the HTS group used 30 g/L NaCl solution and the HES group used HES (130/0.4) for resuscitation. The dosages of HTS and HES were both 1:1 volume to shed blood. The actual dose used in each group was readjusted according to the directions from previous studies (Lu *et al.*, 2008; 2013).

2.3 Collection and processing of samples

Heparin was used for anticoagulation in all samples. The spleen, BMNCs, and blood cell suspensions were prepared according to the protocol. Red blood cell lysis buffer was used to remove the red blood cells from the samples. The peripheral blood nucleated cells (PBNCs) refer to all the nucleated cells in peripheral blood after wiping off red blood cells. The BMNCs are designated as all the nucleated cells in bone marrow after cleaning off red blood cells.

2.4 Flow cytometry

The anti-mouse CD11b fluorescein isothiocyanate (FITC) antibodies (catalog number: 11-0112), anti-mouse CD16/32 purified antibodies (catalog number: 14-0161), anti-mouse Ly6C phosphatidylethanolamine (PE) antibodies (catalog number: 12-5932), anti-mouse Ly6G (Gr-1) PerCP-Cyanine5.5 antibodies (catalog number: 45-5931) and their isotype controls used for detecting the MDSCs were from eBioscience, Inc. (San Diego, USA). HES 130/0.4 was obtained from the Fresenius Kabi Deutschland GmbH (Bad Homburg, Germany). The machine (FACSCalibur, Beckman-Coulter, USA) was used for flow cytometric analysis. Cells (1×10^5) were collected for each flow cytometry analysis. The G-MDSC was $CD11b^+Ly6G^+Ly6C^{low/int}$ and the M-MDSC was $CD11b^+Ly6G^-Ly6C^{high}$. The MDSCs were labeled as $CD11b^+$, coupling with $Ly6G^+$ or $Ly6C^{high}$.

2.5 Statistical analysis

SPSS 16.0 software (SPSS Institute, Chicago, IL, USA) was used for statistical analysis. The data were presented as mean±standard deviation (SD). Normal distribution test used was the one-sample Kolmogorov-Smirnov test. Continuous data conforming to a normal distribution were analyzed using one-way analysis of variance (ANOVA) and the least significant difference *t* test (LSD-*t*); the nonparametric test Kruskal-Wallis *H* was used for continuous data without a normal distribution. A *P*-value of <0.05 was considered statistically significant for all tests.

3 Results

3.1 Distribution and differentiation of MDSCs in mice PBNCs after resuscitation

The representative illustrations of flow cytometry, MDSC count, and M-MDSC/G-MDSC ratio in mice PBNCs are shown in Fig. 1. The MDSCs in PBNCs of the HTS group presented a significant elevation at 2 h point after shock stress (P=0.001 vs. control); this elevation was sustained from the 24 h to the 72 h points (both P<0.001). In the NS group, the MDSCs demonstrated no obvious variation at 2 h, but a significant increase at 24 h (P=0.011), and then was back to the control level at 72 h. The noticeable rise of MDSCs only was presented in the HES group at 72 h (P<0.001).

At the 2 h point, the differentiation of MDSCs in PBNCs inclined to G-MDSCs in the NS, HTS, and HES groups with a significant difference (all P=0.001). Later, the M-MDSC/G-MDSC ratio in the HES group went back to the control level at 24 h; the inclination to G-MDSC continued to be upheld in the HTS group (P<0.001). In the NS group, the ratio of M-MDSC/G-MDSC also deviated to G-MDSC compared with the control at 24 h (P=0.049). However, this ratio reversed the inclination to M-MDSC at 72 h (P=0.014). No significant differences of M-MDSC/G-MDSC ratio in PBNCs were found in the HTS or HES groups compared with the control group.

3.2 Distribution and differentiation of MDSCs in mice spleen after resuscitation

The representative illustrations of flow cytometry, MDSC count, and M-MDSC/G-MDSC ratio in mice spleen are shown in Fig. 2. The MDSCs in the spleen of the NS group were below the control level at 2 h (P=0.008), returned back at 24 h, and then displayed a significant increase at 72 h (P=0.014 vs. control). In the HTS and HES groups, the MDSCs both remained at the control level at 2 h. The MDSCs in the HTS group increased obviously at 24 h

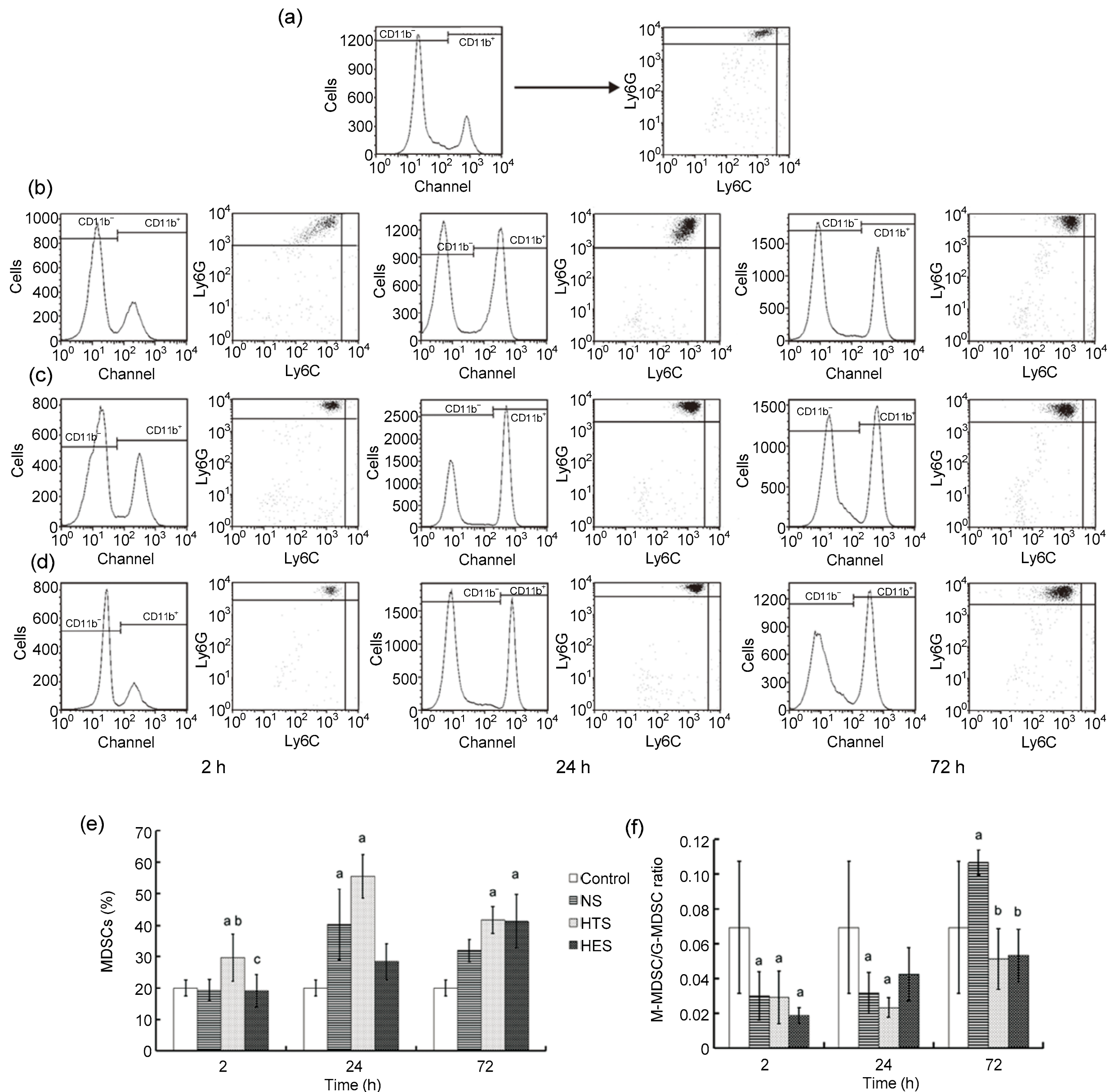

Fig. 1 Variations of MDSCs and M-MDSC/G-MDSC ratio in mice PBNCs at different time points

We took $CD11b^+$ immunofluorescence single positive cells from the total clusters of cells according to the isotype controls. Then we adjusted the window to Ly6C (*x*-coordinate) and Ly6G (*y*-coordinate), and dropped the Ly6C and Ly6G gates according to isotype controls and data itself. Finally, we counted the MDSC number ($CD11b^+Ly6G^-Ly6C^{high}$+$CD11b^+Ly6G^+Ly6C^{low/int}$+$CD11b^+Ly6G^+Ly6C^{high}$) and M-MDSC/G-MDSC ratio ($CD11b^+Ly6G^-Ly6C^{high}$/$CD11b^+Ly6G^+Ly6C^{low/int}$). (a) Representative illustration of flow cytometry for the control group. (b) Representative illustration of flow cytometry for the NS group at different time points. (c) Representative illustration of flow cytometry for the HTS group at different time points. (d) Representative illustration of flow cytometry for the HES group at different time points. (e) The variation of MDSCs in PBNCs at different time points. (f) The variation of M-MDSC/G-MDSC ratio in PBNCs at different time points. Column charts with average and standard deviation are demonstrated. [a] $P<0.05$ vs. the control group; [b] $P<0.05$ vs. the NS group; [c] $P<0.05$ vs. the HTS group. HES: hydroxyethyl starch; HTS: hypertonic saline; NS: normal saline; MDSC: myeloid-derived suppressor cell; M-MDSC: monocytic MDSC; G-MDSC: granulocytic/neutrophilic MDSC; PBNCs: peripheral blood nucleated cells

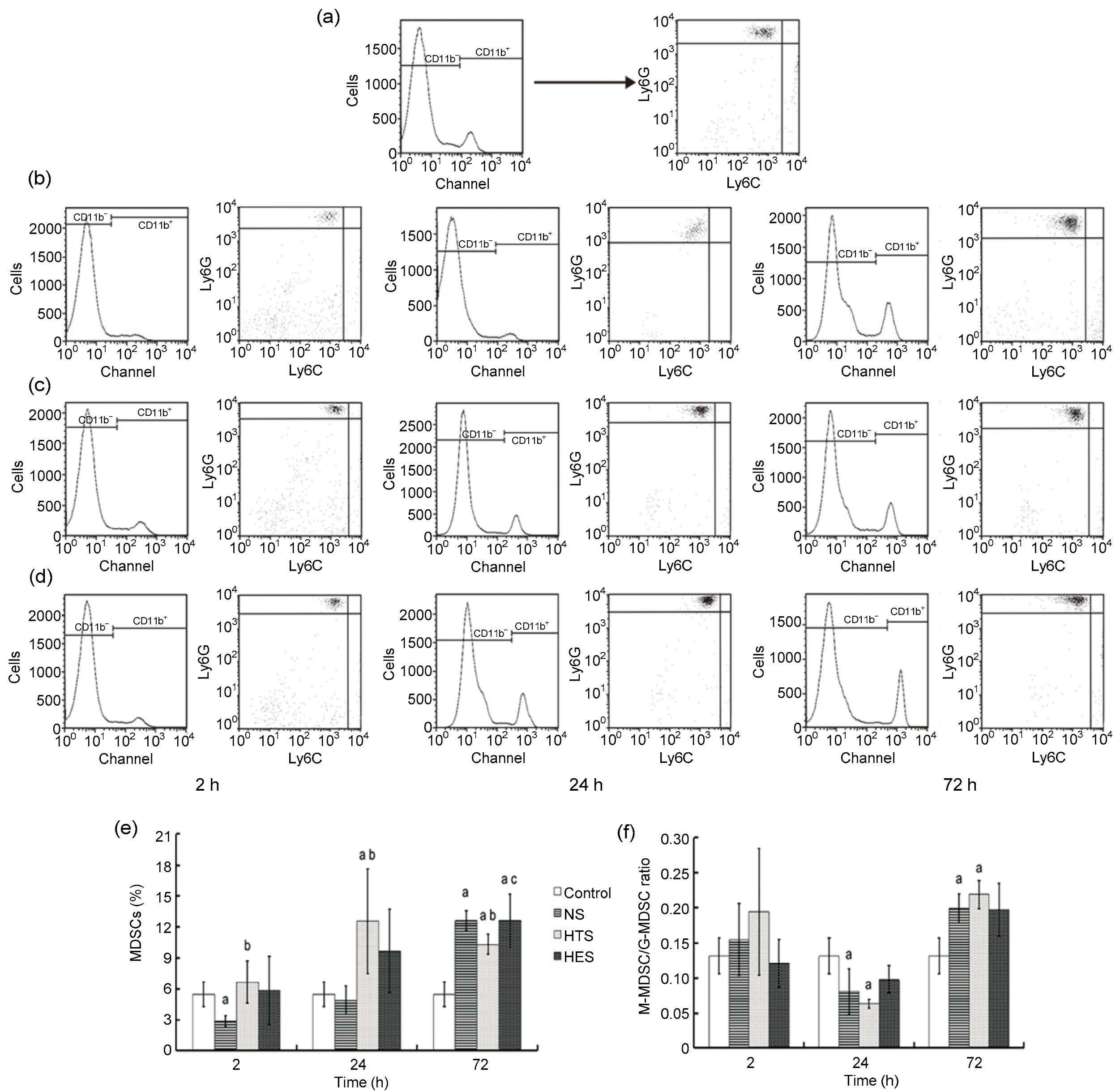

Fig. 2 Variations of MDSCs and M-MDSC/G-MDSC ratio in mice spleen at different time points

We took $CD11b^+$ immunofluorescence single positive cells from the total clusters of cells according to the isotype controls. Then we adjusted the window to Ly6C (*x*-coordinate) and Ly6G (*y*-coordinate), and dropped the Ly6C and Ly6G gates according to isotype controls and data itself. Finally, we counted the MDSC number ($CD11b^+Ly6G^-Ly6C^{high}$+$CD11b^+Ly6G^+Ly6C^{low/int}$+$CD11b^+Ly6G^+Ly6C^{high}$) and M-MDSC/G-MDSC ratio ($CD11b^+Ly6G^-Ly6C^{high}$/$CD11b^+Ly6G^+Ly6C^{low/int}$). (a) Representative illustration of flow cytometry for the control group. (b) Representative illustration of flow cytometry for the NS group at different time points. (c) Representative illustration of flow cytometry for the HTS group at different time points. (d) Representative illustration of flow cytometry for the HES group at different time points. (e) The variation of MDSCs in mice spleen at different time points. (f) The variation of M-MDSC/G-MDSC ratio in mice spleen at different time points. Column charts with average and standard deviation are demonstrated. [a] $P<0.05$ vs. the control group; [b] $P<0.05$ vs. the NS group; [c] $P<0.05$ vs. the HTS group. HES: hydroxyethyl starch; HTS: hypertonic saline; NS: normal saline; MDSC: myeloid-derived suppressor cell; M-MDSC: monocytic MDSC; G-MDSC: granulocytic/neutrophilic MDSC

(P=0.039), whereas those in the HES and control groups showed no statistical significance. The MDSCs in the HTS and HES groups were higher than those in control at 72 h (both P<0.001). However, this MDSC count in the HTS was much lower than that in the NS and HES groups with a statistical significance at 72 h (P=0.014 and P=0.013, respectively).

The trends of M-MDSC/G-MDSC ratio in the spleen of the NS and HTS groups were similar, which showed no significant difference at 2 h, but distinctly inclined to G-MDSC at 24 h (P=0.02 and P<0.001, respectively), then reversed to M-MDSC clearly at 72 h (P=0.017 and P=0.001, respectively). In the HES and control groups, the variations of M-MDSC/G-MDSC ratio had no statistical significance at all the time points.

3.3 Distribution and differentiation of MDSCs in mice BMNCs after resuscitation

The representative illustrations of flow cytometry, MDSC count, and M-MDSC/G-MDSC ratio in mice BMNCs are shown in Fig. 3. The MDSCs in BMNCs among three resuscitation groups were all below the control level at 2 h (all P<0.001). Simultaneously, the MDSCs in the HES group obviously descended compared with the NS and HTS groups (P=0.003 and P<0.001, respectively). After that, the differences of MDSCs across the four main groups presented no statistical significance at both 24 and 72 h (P=0.051 and P=0.061, respectively).

The variations of M-MDSC/G-MDSC ratio in BMNCs among the four groups had no statistical significance at 2 h (P=0.087). At 24 h, the M-MDSC/G-MDSC ratios in the NS, HTS, and HES groups all inclined to G-MDSC (P<0.001, P=0.005, and P<0.001, respectively), and returned to the control level at 72 h excluding the HES group, in which the ratio still inclined to G-MDSC (P=0.003).

4 Discussion

Our results showed that at the 2 h point after shock stress, which we considered as the immediate immune response time, resuscitation with NS, HTS, or HES could decrease the MDSC number in bone marrow, but the largest drop happened in the resuscitation with HES. At the same time, the MDSCs in NS resuscitation were significantly less than those in the other three groups in the spleen, which implied that HTS and HES had less influence on the MDSC count than NS in the spleen microenvironment. The M-MDSC/G-MDSC ratio in the spleen displayed no difference among these four groups at this immediate time. It revealed that the spleen has a strong buffering capacity to withstand the effects of these three resuscitation fluids on the differentiation of MDSCs to M-MDSCs and G-MDSCs.

The M-MDSC/G-MDSC ratios had no significant difference between the NS and control groups in bone marrow at the immediate time after shock. However, in the HTS group, the MDSC count reduced in bone marrow with increasing and inclining differentiation into G-MDSC in peripheral blood. More interestingly, the decline of MDSCs in bone marrow in the HES group was larger than that in the HTS group, but it did not couple with the increasing count in peripheral blood. Only the same incline of the differentiation into G-MDSC displayed in the HES group. These results imply that HTS might influence the distribution and differentiation regulation of MDSCs, while HES had a less effect on the distribution but a stronger impact on the differentiation of MDSCs.

At the 24 h point after shock, which we considered as the early immune response time, the MDSCs in bone marrow among the four groups displayed no statistically significant difference. In bone marrow, the MDSCs were more inclined to differentiate into G-MDSC in the HES group than in the control and HTS groups. It implies a stronger influence of HES on the bone marrow microenvironment and its induction of the MDSC differentiation into G-MDSC. The M-MDSC/G-MDSC ratios between the NS and HTS groups in peripheral blood and spleen had the same incline to G-MDSC as in bone marrow at this early time. This revealed that the influences of NS and HTS on the MDSC differentiation were working toward to a same level 24 h after resuscitation. In addition, the elevation of MDSC in peripheral blood across both NS and HTS groups implies that the influences of NS and HTS on the distribution and differentiation of peripheral blood MDSCs may last for 24 h after shock.

The direct effect of resuscitation fluid on MDSCs might finally abate at the 72 h point after shock, which we considered as the late immune response

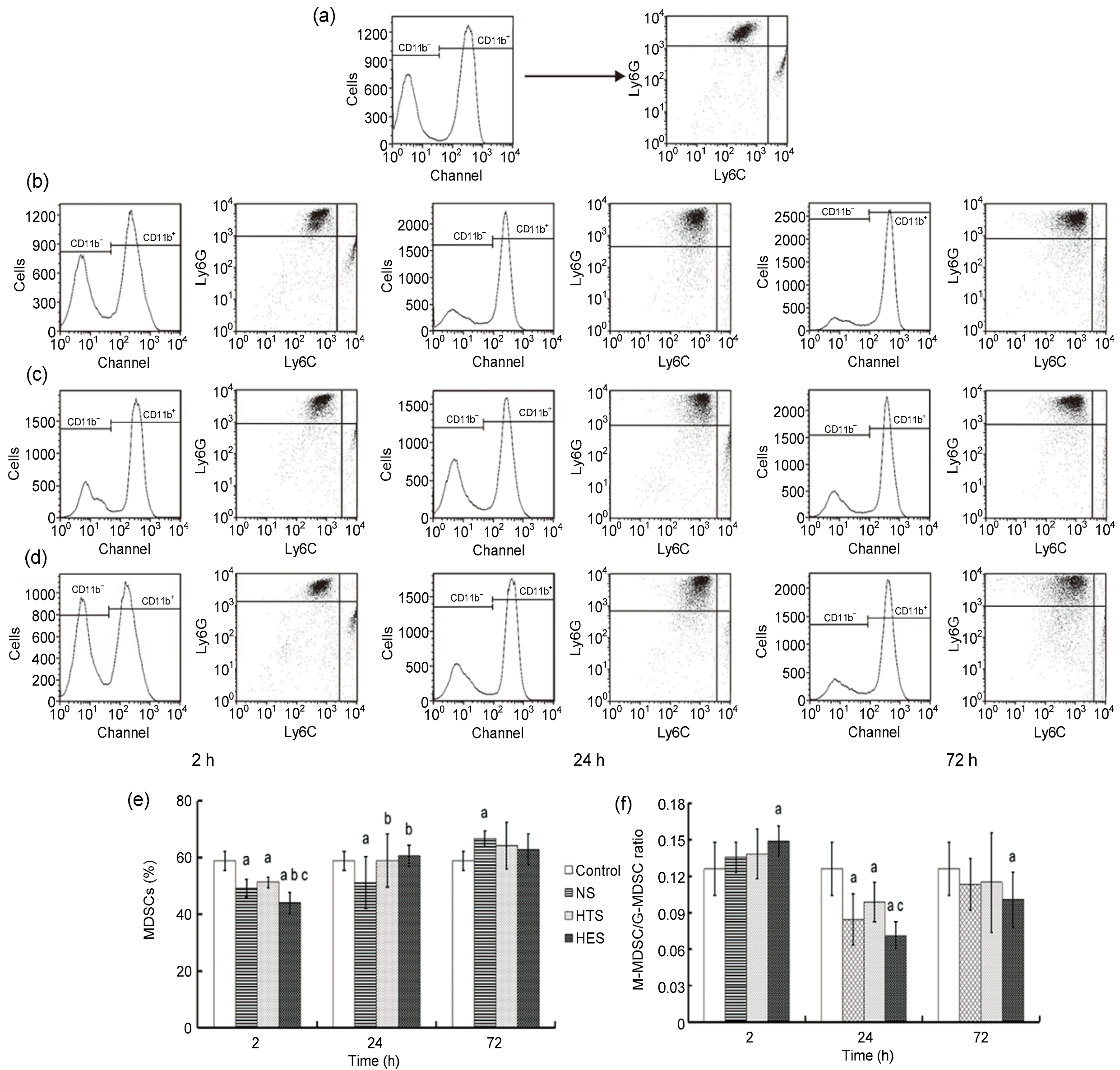

Fig. 3 Variations of MDSCs and M-MDSC/G-MDSC ratio in mice BMNCs at different time points

We took CD11b$^+$ immunofluorescence single positive cells from the total clusters of cells according to the isotype controls. Then we adjusted the window to Ly6C (*x*-coordinate) and Ly6G (*y*-coordinate), and dropped the Ly6C and Ly6G gates according to isotype controls and data itself. Finally, we counted the MDSC number (CD11b$^+$Ly6G$^-$Ly6C^{high}+CD11b$^+$Ly6G$^+$Ly6C$^{low/int}$+CD11b$^+$Ly6G$^+$Ly6C^{high}) and M-MDSC/G-MDSC ratio (CD11b$^+$Ly6G$^-$Ly6C^{high}/CD11b$^+$Ly6G$^+$Ly6C$^{low/int}$). (a) Representative illustration of flow cytometry for the control group. (b) Representative illustration of flow cytometry for the NS group at different time points. (c) Representative illustration of flow cytometry for the HTS group at different time points. (d) Representative illustration of flow cytometry for the HES group at different time points. (e) The variation of MDSCs in mice BMNCs at different time points. (f) The variation of M-MDSC/G-MDSC ratio in mice BMNCs at different time points. Column charts with average and standard deviation are demonstrated. ^{a}*P*<0.05 vs. the control group; ^{b}*P*<0.05 vs. the NS group; ^{c}*P*<0.05 vs. the HTS group. HES: hydroxyethyl starch; HTS: hypertonic saline; NS: normal saline; MDSC: myeloid-derived suppressor cell; M-MDSC: monocytic MDSC; G-MDSC: granulocytic/neutrophilic MDSC; BMNCs: bone marrow nucleated cells

time. The results from the indirect effects of NS, HTS, and HES on the total immune system should be considered. It is worth noting that the bone marrow M-MDC/G-MDSC ratio in the HES group still inclined to G-MDSC compared to the control; the M-MDC/G-MDSC in peripheral blood of the NS group inclined to M-MDSC compared to the HTS and HES groups. The former observation implies the induction of HES on the MDSC differentiation into G-MDSC, and the latter confirmed the different effects of HTS and HES on the immune system compared to NS.

Significant inflammatory processes are often stimulated in response to trauma and hemorrhagic shock; they might not be attenuated even with the improvement of microcirculation after fluid resuscitation (Watters *et al.*, 2006; Zhang *et al.*, 2012; Liu *et al.*, 2015; Naumann *et al.*, 2016). Our previous studies have found that an acute decrease of regulatory T cells (Tregs) and the shift in the balance of Th1/Th2 and Tc1/Tc2 were presented at the early stage after hemorrhagic shock (Zhang *et al.*, 2012). Resuscitation with HTS could induce dramatic early migration and redistribution of MDSCs from bone marrow to peripheral circulation, compared to resuscitation with NS or HES (Lu *et al.*, 2013). The two major subpopulations G-MDSC and M-MDSC have different immunosuppressive mechanisms (Lai *et al.*, 2014; O'Connor *et al.*, 2015; Ost *et al.*, 2016). Normally, G-MDSCs could differentiate into neutrophile granulocyte, while M-MDSC could differentiate into monocyte. G-MDSCs have been found as the predominant subset in cancers, and are involved in sepsis which is infected by Gram-positive bacteria. Meanwhile, M-MDSCs are often discovered in acute or chronic infections regardless of the Gram staining (Youn *et al.*, 2008; 2013; Zoglmeier *et al.*, 2011; Nagaraj *et al.*, 2013; Janols *et al.*, 2014). Research has shown that under stimulation of docetaxel (DTX), M-MDSCs may lose their immunosuppressive function and differentiate into mature M1 macrophages, whereas G-MDSCs still have strong immunosuppressive activity (Dufait *et al.*, 2015; Zhou *et al.*, 2016).

HTS could temporarily enhance plasma sodium and osmolality, and these had been thought to be associated with the immunomodulatory effect. Although the increase of serum sodium and osmolality would begin to decline 0.5 or 1 h later, the impact on the immune system could last for up to 24 h after HTS administration (Bulger *et al.*, 2008; 2011; Junger *et al.*, 2012; Motaharinia *et al.*, 2015). Considering the anti-inflammatory and immunosuppressive effects of MDSCs, the rise of the MDSC count induced by HTS in the early stage should be helpful in relieving inflammation damage.

The MDSCs are generally believed to originate from the bone marrow. Our results show that all three resuscitation fluids can cause a significant decrease of the number of MDSCs in bone marrow at the immediate time after trauma and shock, of which HES had the largest impact. Moreover, there was a noticeable rise of MDSCs in PBNCs in the HTS group at the same time. It hints that HTS might induce the migration and redistribution of MDSCs from bone marrow to peripheral circulation, which was consistent with our previous research (Zhang *et al.*, 2012; Lu *et al.*, 2013). A distinct differentiation to G-MDSC was found in PBNCs at the immediate time in the HTS group. It also implies a faster and more drastical effect of HTS on the MDSCs in peripheral blood. However, NS and HTS seemed to have similar impacts on the MDSCs in the spleen and bone marrow at the immediate time. No elevation of MDSCs in peripheral blood or spleen was found at the immediate time in the HES groups, which implies that HES may induce the MDSCs to differentiate into mature cells, and this influence might depend on the hematopoietic microenvironment. The mature cells lost immunosuppressive activity, and performed diverse biological functions according to their subtypes. This may be conducive to clearing the infection, and aggravate inflammation in a sensitive condition.

Except for the hemodynamic properties, studies showed that HTS and HES have different influences in microcirculation and immune system (Huang *et al.*, 2016). HTS can reduce endothelial and tissue edemas, improve microcirculation, inhibit the expression of the adhesion molecule in endothelial cells, suppress the activation and alter the inflammatory cytokine production of neutrophils, and also attenuate oxidative stress. HES exhibits an anti-inflammatory effect and ameliorates oxidative stress by different mechanisms (Chen *et al.*, 2013; Huang *et al.*, 2014; Wright *et al.*, 2014; Duan *et al.*, 2015; Liu *et al.*, 2015; Motaharinia *et al.*, 2015; Öztürk *et al.*, 2015; Anna

et al., 2016; Gamboni *et al.*, 2016). All of these factors cause the various distributions and differentiations of MDSCs.

The factors and signal pathways involved in MDSC function activation include STAT3, nuclear factor-κB (NF-κB), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), myeloid differentiation factor 88 (MyD88), and toll-like receptor signaling (Delano *et al.*, 2007). Studies based on different animal models suggest that HTS and HES could either directly or indirectly alter the expression of cytokines and activation of signaling pathways, including those above (Coimbra *et al.*, 1996; Loomis *et al.*, 2001; Ke *et al.*, 2006; Wang *et al.*, 2009; Chen *et al.*, 2013; Esnault *et al.*, 2013; Liu *et al.*, 2013; Choi *et al.*, 2014; Dong *et al.*, 2014; Huang *et al.*, 2014; Igarashi *et al.*, 2014; Wright *et al.*, 2014; Öztürk *et al.*, 2015; Chang *et al.*, 2016). Thus, the MDSC-related cytokines and signaling pathways in hemorrhagic shock resuscitation with different kinds of fluids need further research.

Studies showed the paradoxical functions of MDSCs in sepsis and trauma (Cuenca *et al.*, 2011). Research based on cancers had definitively proved their immunosuppressive function in a variety of neoplasms (Youn *et al.*, 2008). Other studies had also verified the inhibitory function of MDSCs in several chronic infections and autoimmune diseases (Makarenkova *et al.*, 2006). Nevertheless, recent studies even suggested that the expansion of MDSCs in some acute inflammatory processes, such as burns and sepsis, could enhance immune surveillance and innate immune responses, which might be helpful in infection control (Brudecki *et al.*, 2012; Lai *et al.*, 2014; Ost *et al.*, 2016). Additionally, it also implied that MDSCs have divergent gene expression profiles and functions in early and late sepsis. MDSCs could express nitric oxide synthase and proinflammatory cytokines which had proinflammatory function at early time, latterly expressing arginase, IL-10 and transforming growth factor-β (TGF-β) which were responsible for the immunosuppressive function (Brudecki *et al.*, 2012).

The functions of MDSC and its subtypes M-MDSC and G-MDSC in hemorrhagic shock were still unclear; it is necessary to prolong the observation time, add cellular phenotype study, use specific molecular markers, design function experiments in in vitro and in vivo cells, with cell tracking in signaling pathway in future research to help counter the main limitation of the present study.

Among the three resuscitation fluid groups, HTS could induce an earlier elevation of MDSCs in peripheral blood. It may restrain the excessive activation of the inflammation reaction, alleviate inflammatory damage, and probably weaken the ability to eradicate the infection. Or, conversely, it may increase the activation of inflammation reaction and the ability to eliminate the infection. HTS could influence the distribution and differentiation regulation of MDSCs, while HES may have a less effect on the distribution regulation but a stronger impact on the differentiation regulation of MDSCs, and induce the MDSCs inclining to G-MDSCs, especially in bone marrow. Therefore, the sequential use of HTS and HES in the different resuscitation stages may be a beneficial strategy, but this needs further research.

Compliance with ethics guidelines

Jiu-kun JIANG, Wen FANG, Liang-jie HONG, and Yuan-qiang LU declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：失血性休克小鼠液体复苏后髓源性抑制细胞的分布和分化

目　的：在失血性休克小鼠模型中使用不同的液体复苏，包括等渗盐水（NS）、高渗盐水（HTS）和羟乙基淀粉（HES），比较在不同时间点髓源性抑制细胞（MDSCs）在外周血、脾脏和骨髓组织中分布和分化的情况。

创新点：（1）创建失血性休克小鼠模型；（2）将 MDSCs 引入失血性休克液体复苏后免疫变化的研究中；（3）对骨髓、脾脏和外周血细胞中的 MDSCs 分布进行研究，并探讨了在失血性休克不同液体复苏后 MDSCs 的分化趋势，为临床上形成规范的救治方案提供了科学的实践资料。

方　法：将 BALB/c 雄性小鼠随机分成四组，除对照组外，其余三组在建立失血性休克小鼠模型后采用不同的液体复苏：NS 组、HTS 组和 HES 组。在模型建立后的 2、24 和 72 h 分批次处死小鼠，取外周血、脾脏和骨髓细胞组织，通过三色荧光标记流式细胞术进一步分析 MDSC 细胞含量，以及其两亚组单核髓源性抑制细胞（M-MDSC）和中性粒髓源性抑制细胞（G-MDSC）的比值。

结　论：HTS 可诱导 MDSCs 在外周血和脾脏中的早期积累，并影响 MDSCs 分化和分布；而 HES 对 MDSCs 的分布影响较小，但对 MDSCs 在骨髓中的分化影响较大。

关键词：失血性休克；羟乙基淀粉；高渗盐水；髓源性抑制细胞；等渗盐水