Stellate Ganglion Block Improves the Proliferation and Function of Splenic CD4+ T Cells Through Inhibition of Posthemorrhagic Shock Mesenteric Lymph–Mediated Autophagy

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Abstract—Severe hemorrhagic shock leads to excessive inflammation and immune dysfunction, which results in high mortality related to mesenteric lymph return. A recent study showed that stellate ganglion block (SGB) increased the survival rate in rats suffering hemorrhagic shock. However, whether SGB ameliorates immune dysfunction induced by hemorrhagic shock remains unknown. The aim of the present study was to verify the favorable effects of SGB on the proliferation and function of splenic CD4+ T cells isolated from rats that underwent hemorrhagic shock and to investigate the mechanism related to the SGB interaction with autophagy and posthemorrhagic shock mesenteric lymph (PHSML).

Male rats underwent SGB or sham SGB and conscious acute hemorrhage followed by resuscitation and multiple treatments. After 3 h of resuscitation, splenic CD4+ T cells were isolated to measure proliferation and cytokine production following stimulation with ConA in vitro. CD4+ T cells isolated from normal rats were treated with PHSML drained from SGB-treated rats, and proliferation, cytokine production, and autophagy biomarkers were detected. Hemorrhagic shock reduced CD4+ T cell proliferation and production of interleukin (IL)-2, IL-4, and tumor necrosis factor-α–induced protein 8–like 2 (TIPE2). SGB or administration of the autophagy inhibitor 3-methyladenine (3-MA) normalized...
these indicators. In contrast, administration of rapamycin (RAPA) autophagy agonist or intravenous injection of PHSML inhibited the beneficial effects of SGB on CD4+ T cells from hemorrhagic shocked rats. Furthermore, PHSML incubation decreased proliferation and cytokine production, increased LC3 II/I and Beclin-1 expression, and reduced p-PI3K and p-Akt expression in normal CD4+ T cells. These adverse effects of PHSML were also abolished by 3-MA administration, as well as incubation with PHSML obtained from SGB-treated rats. SGB improves splenic CD4+ T cell function following hemorrhagic shock, which is related to the inhibition of PHSML-mediated autophagy.

**KEY WORDS:** Hemorrhagic shock; Stellate ganglion block; CD4+ T cells; Posthemorrhagic shock mesenteric lymph; Autophagy; Spleen.

**INTRODUCTION**

Hemorrhagic shock is an acute and critical pathological process that is more common in traumatic hemorrhage, postpartum hemorrhage, visceral rupture, and peptic ulcers [1]. The immune system plays an important role in maintaining homeostasis of the internal environment. Severe hemorrhagic shock induces hyperinflammation and immunosuppression, leading to sepsis and multiple-organ dysfunction [2, 3, 4, 5]. T cells have always been one of the important regulatory components in the cellular immune response [6, 7]. Studies have shown that the function of T cells is significantly inhibited after hemorrhagic shock. CD4+ T cell dysfunction plays a vital role in the pathogenesis of immunosuppression after hemorrhagic shock. Meanwhile, the role of various inflammatory mediators entering the systemic circulation through the mesenteric lymph pathway and causing organ damage has not been established. Blockage of posthemorrhagic shock mesenteric lymph (PHSML) return reduces splenic histological injury and normalizes cellular immune function [8, 9]. Therefore, PHSML return is a key causal factor that aggravates multiple-organ injury and immune dysfunction [10, 11].

Stellate ganglion block (SGB), a common method of blocking sympathetic nerves, reduces the release of inflammatory factors, enhances the activity of T cells, and increases the secretion of anti-inflammatory cytokines [12]. Previous studies have shown that SGB prolongs the survival time of rats with hemorrhagic shock and reduces damage to the intestinal barrier [13]. However, it is unclear whether SGB affects the function of CD4+ T cells after acute hemorrhage. The bidirectional effect of autophagy on cell structure and function has received attention, and excessive autophagy inhibits the differentiation and function of CD4+ T cells [14]. However, following hemorrhagic shock, whether autophagy occurs in CD4+ T cells or is related to PHSML return–induced CD4+ T cell dysfunction is unknown. Furthermore, the relationship between CD4+ T cell autophagy and SGB also remains unknown. Therefore, we hypothesized that SGB improves splenic CD4+ T cell function through inhibition of PHSML return–induced autophagy. To test this hypothesis, this study observed the effect of autophagy activators or inhibitors and intravenous injection of PHSML on the proliferation and cytokine production of CD4+ T cells from rats that underwent SGB and hemorrhagic shock. To confirm the beneficial effect of SGB, we further investigated the effect of PHSML from SGB-treated rats (PHSML-SGB) on proliferation, cytokine production, and autophagy markers in CD4+ T cells isolated from normal rat spleens.

**METHODS**

**Animals**

Male Wistar rats (280–320 g) were purchased from Sibeifu Biotechnology Co., Ltd (Beijing, China), and maintained in a clean-grade animal house. All surgeries were performed under anesthesia according to the Use of Laboratory Animals Guidelines from the National Institutes of Health. The Animal Ethics Committee of Hebei North University approved the experimental procedures.

**Experimental Procedures**

First, twenty rats underwent pretreatment with SGB and sham SGB (n = 10), and then the model of hemorrhagic shock under anesthesia was established for PHSML drainage. The PHSML and PHSML-SGB were stored at −80 °C for subsequent experimental research. Second, forty-two rats were divided into seven groups (n = 6 each group) as follows: Sham group, Shock group, Sham + SGB group, Shock + SGB group, Shock + 3-methyladenine (3-MA) group, Shock + SGB + Rapamycin (RAPA) group,
and Shock + SGB + PHSM group. SGB pretreatment and a hemorrhagic shock model in conscious rats were performed for the isolation and proliferation of splenic CD4+ T cells and for observation of their cytokine production. Third, fifteen rats were used for the isolation of splenic CD4+ T cells. Then, CD4+ T cells were treated with various mesenteric lymph extracts, including PHSM (4%), PHSM-SGB (4%), PHSM (4%) plus 3-MA (5 mM), and phosphate-buffered saline (PBS) or dimethyl sulfoxide (DMSO, 1 %), as a control. The final concentration of mesenteric lymph used for in vitro experiments was determined according to our previous studies [15, 16]. The proliferation, cytokine production, and autophagy of splenic CD4+ T cells were detected. Finally, eighteen rats were used to collect normal mesenteric lymph (NML), PHSM, and PHSM-SGB for cytokine determination.

**SGB Pretreatment**

SGB pretreatment was performed in accordance with the method in our laboratory [13]. Briefly, inhalation anesthesia was induced in each rat with isoflurane, and 0.2 mL ropivacaine hydrochloride (AstraZeneca AB, Sweden) at a 2.5% concentration was injected into the body surface landmarks of the right stellate ganglion. After the rats awakened naturally, positive Horner syndrome (droopy eyelid on the right side) was observed for confirmation of SGB. If there was no obvious droopy eyelid, SGB treatment was considered a failure, and the rat was relinquished. Sham SGB treatment was performed with the injection of an equal amount of 0.9% saline.

**Collection of PHSM**

Rats treated with SGB or sham SGB were subjected to hemorrhagic shock under anesthesia. After inhalation anesthesia with isoflurane, the rats were anesthetized with 1% pentobarbital sodium (5 mL/kg), and polyethylene tubing was aseptically cannulated to both bilateral femoral arteries and the right femoral vein. The tubing was pulled from the middle of the scapula through a subcutaneous tunnel on the back and was closed with heparin sodium after fixation. The right femoral artery was connected to the PowerLab biological signal acquisition system to monitor MAP, and the contralateral femoral artery was connected to a withdrawal-infusion pump for blood withdrawal. After the surgery and a 20-min stabilization period, acute bleeding was performed rapidly through the left femoral artery at a rate of 0.6 mL/min, and the MAP reached 40 mmHg within 10 min and was maintained at 40 ± 2 mmHg for 1 h. Subsequently, fluid resuscitation was performed with shed whole blood and an equal amount of Ringer’s solution within 30 min. Meanwhile, 3-MA (30 mg/kg, M9281, Sigma-Aldrich, USA), RAPA (10 mg/kg, A8167, APExBIO, TX, USA), and PHSM were administered. The concentrations of 3-MA and RAPA were determined according to the literature [17, 18]. The sham rats underwent the same operation but did not undergo hemorrhage or fluid resuscitation.

**Splenic Tissue Management**

Three hours after resuscitation of the shocked rats and the corresponding time point in the sham rats, splenic tissues were aseptically removed from the rats. Three splenic samples from each group were collected for CD4+ T cell isolation for observation of cell proliferation and cytokine production after stimulation with ConA in vitro. The other splenic samples were further used for other experiments (not shown in this study).
CD4+ T Cell Isolation

The collected spleen was placed into a 5-mL EP tube filled with cold phosphate-buffered saline (PBS). Then, the spleen was minced and ground with a syringe piston in a 70-μm sieve to produce a single cell suspension. After centrifugation at 1500 rpm for 5 min, the isolated splenocytes were resuspended in PBS. The splenocyte suspension was carefully added to the upper layer of an equal amount of lymphocyte separation solution with a pipette, and the mixed solution was centrifuged at 3000 rpm for 20 min. Then, the cloudy ring-shaped lymphocytes in the middle layer were carefully pipetted into a fresh 15-mL centrifuge tube and washed three times with PBS. After cell counting, 1 × 10^7 cells resuspended in 80 μL of PBS were mixed with 20 μL of CD4 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) for incubation for 15 min at 4 °C. CD4 microbead-labeled CD4+ T cells were obtained by a separation column (Miltenyi Biotec) in the magnetic field of a MACS separator. After washing and centrifugation, the final cell concentration was determined to be 2 × 10^6 cells/mL, and the cells were resuspended in RPMI 1640 containing 10% heat-inactivated fetal bovine serum and 1% antibiotic at 37 °C.

Purity Identification of Splenic CD4+ T Cells

The isolated cells (5 × 10^5) were resuspended in 100 μL of PBS, and then 2 μL of CD3-PE antibody, 2 μL of CD4-FITC antibody, and two other antibodies were added simultaneously. However, there was no negative control antibody. After incubation at 4 °C for 15 min, the purity of CD4+ T cells was identified using flow cytometry (C6, BD Biosciences Inc., San Jose, CA). The results from flow cytometry analysis showed that more than 90% of the isolated cells were CD4+ T cells (Supplementary Fig. 1), which demonstrated that the isolated cells could be used for subsequent experiments.

Cell Proliferation Analysis

CD4+ T cells isolated from rats subjected to acute hemorrhage or the corresponding treatments were resuspended in RPMI-1640 culture medium, and the final concentration was adjusted to 2 × 10^6 cells/mL. CD4+ T cells (100 μL) were further plated into 96-well plates and stimulated with ConA (5 μg/mL, Sigma) at 37 °C and 5% CO2 for 48 h according to previous studies [19, 20], and then the cells were incubated with 10 μL of CCK-8 for 4 h. CD4+ T cells (2 × 10^6 cells/mL) harvested from normal rats were first stimulated with ConA for 48 h and further treated with PHSMIL, PHSMIL-SGB, or PHSMIL plus 3-MA (5 mM) for 6 h and then incubated with CCK for 4 h. The absorbency was measured using a SpectraMax M3 microplate reader (Molecular Devices, San Jose, CA) at 450 nm, which indicated the level of CD4+ T cell proliferation.

Cytokine Determination

CD4+ T cells in 200 μL of culture media (2 × 10^6/mL) were treated with ConA (5 μg/mL) at 37 °C and 5% CO2 for 48 h, and then the culture supernatant was collected. The levels of interleukin (IL)-2, IL-4, and tumor necrosis factor-α-induced protein 8–like 2 (TIPE2) in the culture supernatant were detected using rat-specific ELISA kits according to the manufacturer’s instructions (Wuhan Pure Biology Co., Ltd. Hubei, China). Meanwhile, the detection of IL-2, IL-4, and TIPE2 in mesenteric lymph was performed using ELISA kits.

Western Blot Analysis

Splenic CD4+ T cells derived from normal rats were treated with DMSO, PHSMIL, PHSMIL-SGB, or PHSMIL + 3-MA for 6 h. Proteins were extracted with RIPA lysis buffer and centrifuged at 12,000 rpm for 10 min at 4 °C. After quantification with a BCA kit purchased from Beijing Puli Gene Technology Co., Ltd. (Beijing, China), proteins were separated on 10–15% SDS-PAGE gels and then transferred to polyvinylidene fluoride (PVDF) membranes using a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad, USA). The membranes were blocked in 5% nonfat milk in Tris–HCl buffered solution-Tween (TBS-T) for 1 h and then incubated with primary antibodies at 4 °C overnight. All antibodies were purchased from Abcam (Cambridge, UK) as follows: LC3 (ab192890, 1:2000), Beclin-1 (ab192890, 1:2000), AKT (ab179463, 1:10,000), phosphorylated AKT (ab81283, 1:10,000), PI3K (ab191606, 1:1000), and phosphorylated PI3K (ab182651, 1:1000). The membranes were washed three times with TBS-T and incubated with secondary antibodies conjugated to horseradish peroxidase (HRP) for 1 h. Finally, protein signals were detected using ImageQuant™ LAS 4000 (General Electric Company, Boston, MA), and the grayscale values of the target proteins were quantified using Quantum One v4.6.2 software.
Stellate Ganglion Block Improves the Proliferation and Function of Splenic CD4 + T Cells Through...

Statistical Analysis

Data are presented as the mean ± standard error of the mean (SE). The significant differences between groups were analyzed with one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls (SNK) test. The level of statistical significance was considered at \( P < 0.05 \).

RESULTS

Effects of SGB on the Proliferation and Cytokine Production of Splenic CD4 + T Cells in Hemorrhagic Shock Rats

The CCK-8 assay (Fig. 1A) and ELISA results (Fig. 1B–D) revealed that there were no significant differences between the Sham and Sham + SGB groups (\( P > 0.05 \)). The proliferation and IL-2, IL-4, and TIPE2 levels in cell culture supernatants of the Shock group were significantly lower than those in the Sham group (\( P < 0.05 \)). When the rats were treated with SGB or 3-MA, hemorrhagic shock-induced decreases in these indices were significantly enhanced (\( P < 0.05 \)). In addition, RAPA administration or PHSML injection obviously inhibited the effect of SGB on these indices in rats that underwent hemorrhagic shock (\( P < 0.05 \)).

Effects of PHSML-SGB on the Proliferation and Cytokine Production of Splenic CD4 + T Cells in Normal Rats

In the analysis of splenic CD4 + T cells isolated from normal rats (Fig. 2), PHSM treatment reduced the proliferation and cytokine production of IL-2, IL-4, and TIPE2 compared to the control and DMSO groups (\( P < 0.05 \)), which were significantly increased following the administration of PHSML-SGB or PHSML + 3-MA (\( P < 0.05 \)).

Effects of SGB on the Cytokine Levels of Mesenteric Lymph Collected From Hemorrhagic Shock Rats

ELISA results (Fig. 3) revealed that there were no significant differences in IL-4 and TIPE2 levels among the NML, PHSM, and PHSML-SGB groups (\( P > 0.05 \)). In addition, the IL-2 level in the PHSM was significantly increased compared to that in NML, while SGB treatment reduced the IL-2 concentration in PHSM (\( P < 0.05 \)).

Effects of PHSML-SGB on Autophagy in Splenic CD4 + T Cells Isolated From Normal Rats

Western blotting was used to detect the expression of the LC3 II/I and Beclin1 autophagy markers in CD4 + T cells (Fig. 4A, B) and revealed that PHSM treatment significantly upregulated Beclin-1 expression and increased the conversion of LC3 I to LC3 II compared to the control and DMSO groups (\( P < 0.05 \)), which was obviously inhibited by 3-MA treatment and reversed by PHSML-SGB treatment (\( P < 0.05 \)).

DISCUSSION

The current study demonstrated that SGB pretreatment or blockade of autophagy inhibited the reduction of proliferation and cytokine secretion of splenic CD4 + T cells following hemorrhagic shock. However, intravenous injection of PHSM, or activation of autophagy eliminated the beneficial effects of SGB. Moreover, PHSM decreased the proliferation and cytokine production of CD4 + T cells isolated from normal rats, increased the expression of autophagy marker proteins, and inhibited the phosphorylation of PI3K and AKT proteins, which was also inhibited by SGB pretreatment and blockade of autophagy. The present findings suggest that SGB improves the proliferation and function of CD4 + T cells after hemorrhagic shock by reducing PHSM-induced autophagy.
Previous studies revealed that the capability of CD4+ T cells to proliferate and produce effector cytokines is the key to performing a variety of effector functions and immune responses [21]. The low proliferative response of CD4+ T cells is involved in depressed immune responses after trauma-induced hemorrhagic shock [22]. After T cell activation and polarization into various T helper subsets, CD4+ T lymphocytes can produce IL-2 and IL-4 [23]. In the meantime, TIPE-2 is required for maintaining immune homeostasis [24]. In vivo, the hyposecretion of IL-2, IL-4, and TIPE-2 leads to downregulation of immune function [23]. Therefore, in this study, we measured proliferation and cytokine production in ConA-stimulated CD4+ T lymphocytes and found that hemorrhagic shock decreased the proliferation capacity and levels of cytokines such as IL-2, IL-4, and TIPE-2 in the culture supernatant of CD4+ T cells stimulated by ConA in vitro. The results indicate that downregulated proliferation capacity and cytokine production may be causal factors that lead to immune system dysfunction and MODS.

Neuromodulation is extremely important for internal environmental disturbances caused by hemorrhagic shock. The hypothalamic–pituitary–adrenal (HPA) axis and hypothalamic-sympathetic-adrenal axis are the two key axes for neuromodulation and affect the immune response [25]. In recent years, neuroimmune interactions have received increasing attention [26, 27], and the role of the sympathetic nervous system in the immune response has gradually been elucidated [28]. For example, adrenergic agonists modulate immune responses in vitro, including...
cytokine production, lymphocyte proliferation, and antibody secretion [29, 30, 31], and chemical sympathectomy changes the immune response [32]. Under stress, the body first activates the neuroendocrine regulatory system [33], which regulates immune and endocrine system functions through the HPA axis and hypothalamus-sympathetic nerve-adrenal axis [34]. Studies have shown that SGB changes the distribution of lymphocyte subpopulations by blocking excessive excitability of sympathetic nerves. SGB also increases the proportion of CD4+ T cells [35], and inhibits excessive early inflammatory responses [12], thereby regulating the inflammatory response and immune function homeostasis. Therefore, the implementation of SGB in advance is conducive to reducing the excessive excitement of sympathetic nerves caused by hemorrhagic shock. However, whether SGB affects the function of CD4+ T cells following hemorrhagic shock is unknown. The current study demonstrated that SGB significantly ameliorates the proliferation and cytokine production function of splenic CD4+ T cells in hemorrhagic shock rats, suggesting that SGB is an effective means to restore CD4+ T cell function.

Studies have shown that PHSML return after hemorrhagic shock is a factor that reduces spleen CD4+ T cell function and causes immune dysfunction. Conversely, reducing PHSML return mitigates spleen histological damage and improves immune function [8, 9]. To determine whether the benefits of SGB are achieved through the intestinal lymphatic pathway, we intravenously injected PHSML into SGB-treated hemorrhagic shock rats and found that the infusion of PHSML abolished the beneficial effects of SGB and reduced the proliferation and cytokine secretion of CD4+ T cells. Using splenic CD4+ T cells extracted from normal rats, we also found that PHSML treatment reduced CD4+ T cell proliferation activity and cytokine secretion. In contrast, PHSML-SGB-treated cells

Fig. 2 Proliferation and cytokine production of splenic CD4+ T cells isolated from normal rats following stimulation with various posthemorrhagic shock mesenteric lymph (PHSML) samples. The same number of splenic CD4+ T cells isolated from normal rats was stimulated with ConA (5 μg/mL) for 48 h and PHSML or PHSML isolated from hemorrhagic shock rats treated with stellate ganglion block (PHSML-SGB) or PHSML plus 3-methyladenine (3-MA) for 6 h in vitro. A shows the proliferation of splenic CD4+ T cells determined by CCK-8 analysis following incubation with CCK-8 for 4 h. B, C, and D show the changes in interleukin (IL)-2, IL-4, and tumor necrosis factor-α-induced protein 8-like 2 (TIPE2) in culture supernatants determined by ELISA. Data are presented as the mean ± SE (n = 3). *P < 0.05 vs Control group, #P < 0.05 vs PHSML group.
had significantly increased proliferation and cytokine levels of IL-2, IL-4, and TIEP-2. Meanwhile, the cytokine results in mesenteric lymph indicated that the role of PHSML in reducing IL-2, IL-4, and TIEP-2 was unrelated to the cytokine payload in mesenteric lymph. Hence, the beneficial effects of SGB are related to the reduction or blockade of the intestinal lymphatic pathway. However, the component in PHSML that induces cellular immune dysfunction remains to be further identified.

Autophagy is a highly conserved cellular biological process, and is one of the two major degradation systems in eukaryotic cells. It is involved in cell proliferation and physiological apoptosis. The normal function of autophagy is to protect cells under stress. However, the excessive autophagy leads to cell injury that is involved in the development of sepsis [36] and the immune response [37]. After hemorrhagic shock, splenocyte damage may be associated with excessive cellular autophagy [38]. The present study showed that treatment with 3-MA autophagy inhibitor restored CD4+ T cell proliferation and cytokine secretion following hemorrhagic shock. Conversely, RAPA autophagy agonist counteracted the beneficial effects of SGB. Therefore, inhibition of excessive autophagy is involved in the role of SGB in improving the function of CD4+ T cells.

During autophagy, double-membrane organelles called autophagosomes transfer cytoplasmic material to lysosomes [39]. In mammalian cells, the formation of autophagosomes occurs through the release of ATG6/Beclin-1 from Bcl-2 to form a Vps34-P13K complex containing Beclin-1, which is essential for the production of autophagosomes [40]. Autophagy can be stimulated by starvation and other stressors, various pathological conditions, or drugs such as rapamycin [41, 42]. When autophagy is excessively induced, it will cause autophagic cell death, so-called type II apoptosis [43]. Therefore, in addition to physiological regulatory functions, autophagy is also closely related to inflammation and the onset and development of diseases [44]. Normal levels of autophagy can maintain homeostasis, while excessive autophagy can cause apoptosis [45]. During autophagosome formation, as a classic marker of autophagosomes, an increase in LC3-II represents the beginning of autophagy [46]. Additionally, the expression level of Beclin-1 also reflects the intensity of autophagy and has become an autophagy protein marker [47].

To further clarify the mechanism of cell autophagy, in this study, splenic CD4+ T cells from normal rats were cultured with ConA for 48 h in vitro and then incubated with PHSML. We found that PHSML increased the protein expression of LC3 II/I and Beclin-1 in CD4+ T cells. In addition, the 3-MA autophagy inhibitor significantly reduced the conversion of LC3 I to LC3 II and the expression of Beclin-1 in CD4+ T cells, suggesting that PHSML activated autophagy in CD4+ T cells. Furthermore, PHSML-SGB treatment significantly reduced the expression of autophagy marker proteins, suggesting that SGB reduced the autophagy of CD4+ T cells through the intestinal lymphatic pathway.

The PI3K/AKT pathway is the classic pathway of autophagy. Recent studies have shown that the PI3K/Akt signaling pathway is a primary pathway that inhibits autophagy...
The present study further confirmed that PHSML inhibits the phosphorylation of PI3K and Akt in CD4+ T cells, which was reversed by PHSML-SGB and PHSML + 3-MA treatments. These results indicate that the effects of PHSML in inducing autophagy and SGB in reducing autophagy are related to the PI3K/AKT signaling pathway.

There were some limitations in this study. First, the current study demonstrated the beneficial effects of SGB on immune inflammation, focusing on CD4+ T cells following hemorrhagic shock, but we did not examine the effects of SGB on other immunocytes, such as CD8+ T cells, NK cells, DCs, and macrophages. Therefore, the potential role of SGB on other immunocytes should be investigated in the future. Second, the reduction in the number of CD4+ T cells and the loss of cell function may be related to autophagy-mediated proliferation dysfunction and apoptosis. The present study examined proliferation dysfunction but not apoptosis, which should be clarified in subsequent studies. In addition, since there were not enough CD4+ T cells isolated from shocked rats to extract proteins, the present study did not determine the protein expression of autophagy markers in vivo.

In summary, our experimental data demonstrate that SGB improves the function of CD4+ T cells after

**Fig. 4** Expression of LC3 II/I, Beclin-1, p-PI3K, and p-AKT in splenic CD4+T cells isolated from normal rats following stimulation with various posthemorrhagic shock mesenteric lymph (PHSML) samples. Splenic CD4+ T cells were isolated from normal rats and stimulated with PHSML or PHSML isolated from hemorrhagic shock rats treated with stellate ganglion block (PHSML-SGB) or PHSML plus 3-methyladenine (3-MA) for 6 h in vitro. The expression of LC3 II/I and Beclin-1 autophagy marker proteins and the signaling molecules PI3K, p-PI3K, AKT, and p-AKT was examined by western blot analysis. Data are presented as the mean ± SE (n=3). *P < 0.05 vs Control group, #P < 0.05 vs PHSML group.
hemorrhagic shock by regulating the intestinal lymphatic pathway and PI3K/AKT pathway and by reducing cell autophagy, which represents an effective means to maintain immune homeostasis. This study focusing on CD4+ T cells provides a basis for understanding the pathogenesis of hemorrhagic shock–induced immune dysfunction and may represent a new therapeutic target for the prevention and treatment of severe hemorrhagic shock.

SUPPLEMENTARY INFORMATION

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AUTHOR CONTRIBUTION

Y.L., H.D., L.J., C.W., M.Y., L.Z., H.Z., Z.Z., and Z.L. performed majority of the animal experiment and laboratory work; Y.L. acquired and analyzed the data; C.N. and Z.Z. were involved in the conception and design of the study and data interpretation and critically revised the manuscript.

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AVAILABILITY OF DATA AND MATERIALS

The data generated for this study are available on request to the corresponding author.

DECLARATIONS

Ethics Approval and Consent to Participate All the protocols were approved by the Animal Care Committee of Hebei North University (Zhangjiakou, China).

Consent for Publication All the authors agree to publish this manuscript.

Conflict of Interest No benefits in any form have been received or will be received from a commercial association related directly or indirectly to the subject of this article. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Stellate Ganglion Block Improves the Proliferation and Function of Splenic CD4+ T Cells Through…

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