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Septic macrophages induce T cells immunosuppression in a cell-cell contact manner with the involvement of CR3

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ABSTRACT

Background: In addition to excessive inflammation, immunosuppression has been recognized as a contributing factor to poor prognosis of sepsis. Although it has been reported that T cells can become functionally impaired during sepsis, the underlying mechanisms responsible for this phenomenon remain unclear. This study aims to elucidate the mechanisms by which macrophages induce immunosuppression in T cells.

Methods: In an in vivo setting, C57BL-6J mice were subjected to cecal ligation and puncture (CLP) with or without depletion of macrophages, and the functions of T cells were assessed. In vitro experiments involved direct co-culture or separate culture of T cells and septic macrophages using a transwell system, followed by analysis of T cell immunity. Additionally, a siRNA targeting CD18 on macrophages was utilized to investigate the role of complement receptor 3 (CR3).

Results: Both macrophages and T cells exhibited immunosuppression during sepsis. In the in vivo experiments, the absence of macrophages partially alleviated T cell immunosuppression, as evidenced by restored vitality, increased production of TNF-α and IFN-γ, elevated $CD8^+$ T cell levels, and decreased $CD25^+$ T cell levels. In the in vitro experiments, direct co-culture of T cells with septic macrophages resulted in diminished T cell immunity, which was improved when T cells and macrophages were separated by a chamber wall. The expression of CR3 (CD11b/CD18) was upregulated on septic macrophages, and silencing of CD18 led to decreased TNF-α production by T cells, reduced $CD4^+$ T cell numbers, and increased $CD25^+$ T cell numbers.

Conclusion: In sepsis, macrophages induce immunosuppression in T cells through direct cell-cell contact, with the involvement of CR3.

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1. Introduction

Previously, the predominant belief was that excessive inflammation was the main driver of poor outcomes in septic patients. However, accumulating evidence suggests that immunosuppression also plays a significant role in patient mortality [1]. Post-mortem examinations of sepsis patients have revealed widespread leukocyte necrosis [2]. Flow cytometry analysis of peripheral leukocytes has indicated a downregulation of cytokine secretion and phagocytosis [3,4]. Despite various hypotheses being proposed, such as hypoxia, acidosis, or endotoxin toxicity, the underlying mechanisms of immunosuppression in sepsis have remained elusive [5].

In our previous study, we observed pronounced immunosuppression in macrophages and found that restoring macrophage function improved survival in mice [6]. Subsequent experiments revealed that T cells were also immune-paralyzed in sepsis, with the timing paralleling that of septic macrophages. Given the regulatory role of macrophages on T cells [7], we hypothesized that macrophages could induce T cell immunosuppression. Macrophages act as antigen-presenting cells and activate T cells through co-stimulatory molecules such as CD20 and CD28 [8]. T we hypothesized that macrophages could induce T cell immunosuppression. Macrophages act as antigen-presenting cells and activate T cells through co-stimulatory molecules such as CD20 and CD28 [9]. However, extracellular analysis of macrophages did not reveal significant changes in these proteins, suggesting the involvement of an alternative pathway in this induction.

Both cytokines and metabolic products can influence T cell viability and differentiation, indicating that the induction of immunosuppression could occur indirectly through humoral mediation or directly through cell-cell contact. In our study, when T cells and septic macrophages were cultured separately without contact, T cell immunosuppression was significantly alleviated, suggesting a cell contact-mediated induction. β2 integrins, a group of surface proteins expressed on leukocytes, play crucial roles in cell-cell interactions [10]. Our previous research demonstrated a significant increase in CD11b expression on peritoneal macrophages. CD11b, together with CD18, forms complement receptor 3 (CR3) [11], which has been implicated in cell cytoskeleton deformation and migration [12]. Further investigation revealed a substantial upregulation of CD18 on macrophages, suggesting a potential role of CR3 in T cell immunosuppression.

Interestingly, in chronic diseases, β2 integrins often exhibit anti-inflammatory properties [13]. However, the role of β2 integrins in sepsis remains unclear. Furthermore, the mechanism by which macrophages arrest T cell activation remains poorly understood. Growth arrest-specific 6 (Gas6) is involved in cell migration and growth and has been reported to be part of the β2 integrin pathway [14]. In this study, our goal was to elucidate the interaction between septic macrophages and T cells in sepsis, as well as the involvement of CR3 and Gas6 in this induction.

2. Methods

2.1. Animals and cells

Animal study protocols were in strict agreement with international guidelines for the care and use of laboratory animals and were approved by Animal Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University, School of Medicine (No.092). Male 8-10-week-old (24–30 g) C57BL/6J mice were purchased from Lingchang Biology (Beijing, China). All mice were maintained in a temperature-controlled (21–23 °C) and air-conditioned (humidity 30–70 %) environment with a 12/12 h light/dark cycle. Mice had free access to drinking water and chow (LabDiet, St. Louis, MO). n the macrophage depletion trial, the mice received a total of 0.5 ml (i.e., 0.1 ml on the 1st day, 0.2 ml on the 2nd day, and 0.2 ml on the 3rd day) of 1 % clodronate liposomes (Clo-Lip, Solarbio Biology, Beijing, China) via the tail vein three days prior to the examination. For in vitro experiments, macrophages and T cells were isolated from the peripheral blood of mice. The mice were euthanized by cervical spine dissection, and blood was obtained by cardiac puncture. Peripheral blood mononuclear cells (PBMCs) were obtained by centrifuging the peripheral blood at 3000 rpm for 20 min, followed by mixing with 1:1 PBS. The mixture was gently added to 3 ml Ficoll density gradient media (Thermo Fisher, Waltham, MA) for density gradient centrifugation (2000 rpm and 4 °C for 20 min) to isolate PBMCs. Macrophages (F4/80) and T cells (CD3) were isolated from PBMCs using flow cytometry, and the procedure was repeated 2 to 3 times to obtain highly pure cells.

2.2. CLP model and LPS stimulation

Mice were anesthetized by intraperitoneal administration of ketamine (75 mg/kg) and xylazine (15 mg/kg), and cecal ligation and puncture (CLP) were conducted as previously described [15]. SHAM mice received only cecal exposure without ligation or puncture. All mice were subcutaneously injected with 1 ml of sterile 0.9 % saline for fluid resuscitation and had unlimited access to food and water. No antibiotics were administered to the mice. In vitro, cells were seeded in a 6-well plate at a concentration of 6 × 10ˆ5 cells/well and stimulated with 100 ng/ml lipopolysaccharide (LPS) (O111:B4, Sigma-Aldrich, St. Louis, MO) for 2 h.

2.3. Phagocytosis, co-culture procedures and cell viability

Macrophages and T cells were isolated from the peripheral blood of healthy or pre-treated mice as described above. DMEM culture medium (Thermo Fisher, Waltham, MA) supplemented with fetal bovine serum (Thermo Fisher, Waltham, MA) was used for cell growth. Macrophages were seeded at a density of ~10ˆ5 cells in 12-well plates and incubated with an excess of GFP-labeled microspheres (~10ˆ6) (Thermo Fisher, Waltham, MA) at 37 °C for 2 h. The cells were then washed with PBS three times and stained with DAPI. Cell visualization was performed using a fluorescence microscope (BX53, Olympus), and intracellular fluorescence intensity was

quantified by flow cytometry. In co-culture experiments, macrophages and T cells were directly co-cultured in the same culture medium or cultured in both halves of the transwell chamber with a 3.0 μm pore polycarbonate membrane inserted (Corning, NY, USA). To assess the intracellular cytokine levels of T cells, the co-cultured cells were re-suspended and incubated for 1.5 h to allow macrophages to attach to the walls. Subsequently, the T cells were collected from the supernatant. Cell viability was assessed using a CCK8 kit purchased from Solarbio Biology (Beijing, China), following the manufacturer's instructions.

2.4. Flow cytometry

Before flow cytometry analysis, macrophages and T cells were incubated with 7-Amino Actinomycin D (7-AAD, eBioscience, San Diego, CA) or Fixable Viability Dye eFluor™ 780 (FVD, eBioscience, San Diego, CA) to distinguish live cells from dead cells. Only live cells were gated for further evaluation. Macrophages were stained with PE-F4/80, and T cells were stained with APC-CD3, Qdot 605-CD4 or APC Cy7-CD4, PerCP-Cy5.5-CD8, FITC-CD25, and their respective isotypes (all antibodies were purchased from BD Bioscience, San Diego, CA) at 4 °C for 30 min. To assess the cytokine levels of T cells, PE-TNF-α and APC–IFN–γ were also used for T cell staining. Stained samples were fixed with 2 % paraformaldehyde and analyzed on an LSRFortessaTM X20 flow cytometer (BD Biosciences, San Diego, CA).

2.5. CD18 silencing

To verify the role of CR3 in macrophage-T cell interaction, macrophage CD18 was silenced using siRNA. Three siRNA sequences were designed according to the mRNA coding area, respectively labeled CD18-360 (5′-3': AUUCACAUAAGUAUUCUCCTT), CD18-1199 (5′-3': UAUCCUUCGAUGUAUACAGTT) and CD18-3413 (5′-3': UUAAACAGGAUCUCAGUGCTT). Macrophages were transfected with these siRNA sequences using the transfection reagent Lipofectamine 2000 (Invitrogen, Carlsbad, CA) for 24 h, and CD18 expression was assessed using western-blot. The results indicated that CD18-1199 was the most effective siRNA sequence. In subsequent experiments, macrophages were transfected with CD18-1199 in vitro for 24 h prior to the co-culture experiments.

2.6. Western blotting

Macrophages and T cells were homogenized in a radioimmunoprecipitation assay (RIPA) buffer containing a complete protease inhibitor cocktail (Life Technologies, Grand Island, NY) and phosphatase inhibitors (Sigma-Aldrich, St. Louis, MO), and separated on 12.5 % SDS polyacrylamide mini gels. Membranes were incubated with rabbit anti-mouse antibodies GAPDH, Tublin, CD18, and Gas6 (all antibodies were purchased from Cell Signaling Technology, MA), and proteins were visualized using a Tanon-MP image analyzer (Shanghai, China).

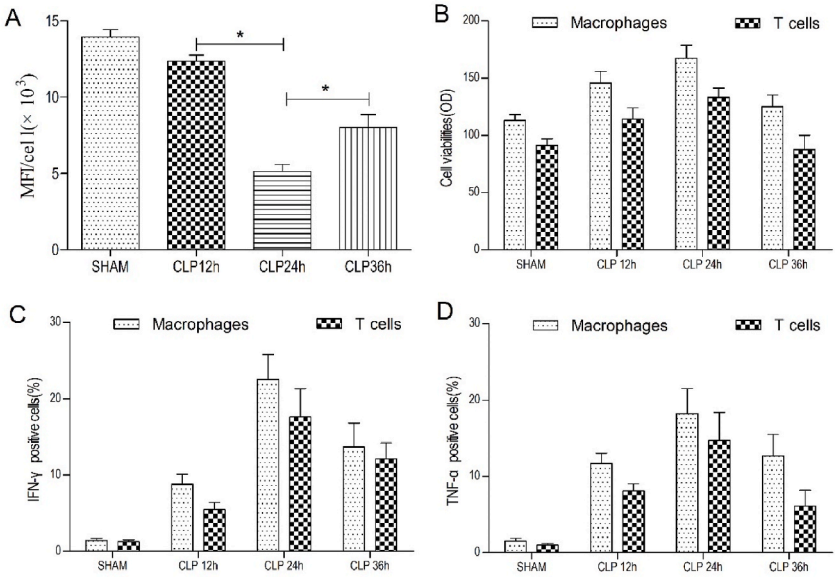

Fig. 1. Immune depression of macrophages and T Cells in sepsis.
Note: **A.** Phagocytic capacity of macrophages from SHAM mice or CLP mice. Macrophages were co-cultured with an excess of fluorescence-labeled microspheres. After a 2-h incubation period, the macrophages were collected, and the intracellular fluorescence was evaluated using flow cytometry. **B.** Cell viabilities assessed using the CCK8 test. **C and D.** The secretions of IFN-γ and TNF-α in macrophages and T cells were evaluated following sepsis. n = 4 for each group. The data presented are means with standard deviations. MFI: mean fluorescence intensity.

2.7. Statistics

All statistical analyses were performed using GraphPad Prism software version 5.0.1 (GraphPad Software, San Diego, CA) or SAS 9.1.0 (SAS Institute, Cary, NC). Data were expressed as means with standard deviations or medians with quartiles. Comparisons among groups were performed using one-way ANOVA or the Mann-Whitney test. A p-value <0.05 (two-tailed) was considered statistically significant.

3. Results

3.1. Both macrophages and T cells undergo immunosuppression in sepsis

To determine the specific time when immunodepression occurs, cell functions were tested at 12 h, 24 h, and 36 h after the onset of sepsis. Macrophages demonstrated impaired phagocytosis capacity starting from 12 h after cecal ligation and puncture (CLP), reaching their lowest level at 24 h, and partially restoring at 36 h (Fig. 1A). According to the CCK-8 test results (Fig. 1B), both macrophages and T cells exhibited increased viability over time but started to decrease at 24 h after sepsis. In line with the impaired viabilities, cytokine secretions were also downregulated at 24 h after sepsis (Fig. 1C and **D**).

3.2. Macrophages induce T cell immunosuppression in sepsis

To investigate the immunoregulation of T cells by macrophages, a macrophage null model was established to confirm the induction of T cell immunosuppression by septic macrophages. Fig. 2A demonstrated that 72 h after the infusion of clodronate liposomes (Clo-Lip), macrophages were substantially eliminated in vivo, as evidenced by the unimodal distribution of F4/80 in flow cytometry. With the successful depletion of macrophages, both normal control (NC) mice and macrophage-depleted mice were subjected to CLP to induce sepsis. Following the sepsis insult, T cells from the NC mice showed significantly greater downregulation of viability compared to cells from the macrophage-depleted mice (Fig. 2B). In another experiment, T cells were co-cultured with macrophages that had been exposed to sepsis for 12 h, 24 h, or 36 h before the in-vitro culture. As expected, naive T cells or T cells co-cultured with naive macrophages exhibited minimal activation, as shown in Fig. 2C. Interestingly, when T cells were stimulated by septic macrophages, as indicated by demonstrable viabilities, this activation gradually attenuated in a time-dependent manner. The longer macrophages endured the sepsis insult, the more impaired the viability of T cells became. Next, the T cell population was analyzed in vivo in the presence or absence of macrophages. Upon CLP in the absence of macrophages, T cells exhibited a skew towards more Teff

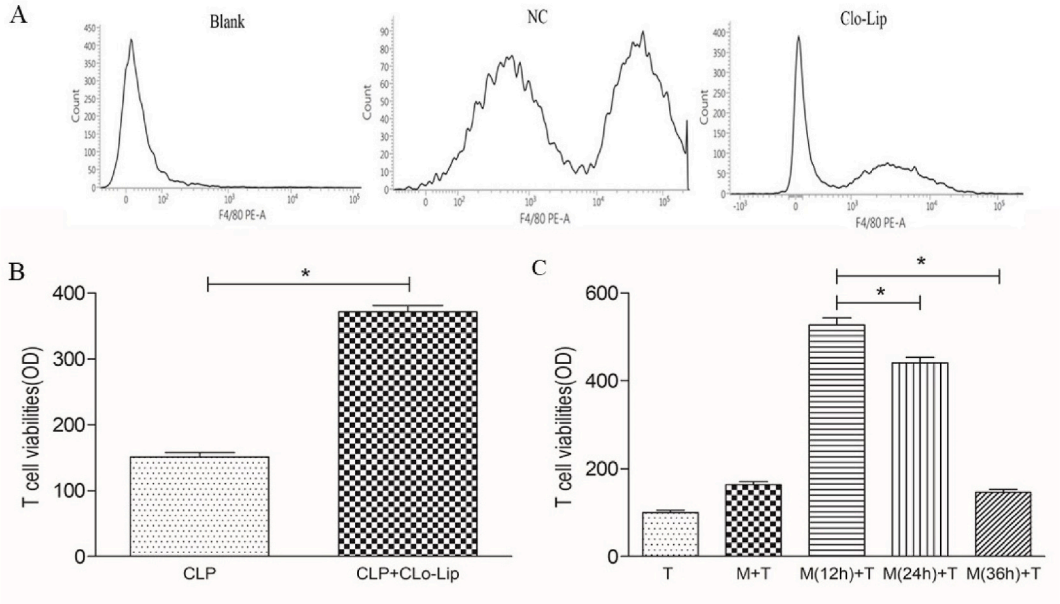

Fig. 2. The presence of septic macrophages resulte in a dampened activity of T cells in both in vivo and in vitro settings.
Note: **A.** In-vivo elimination of macrophages. PE-F4/80 indicated active macrophages.
B. T cell viabilities upon macrophages depletion in a in-vivo setting. Macrophages were globally depleted in-vivo using Clo-Lip injection as described above and then the mice were subjected to CLP procedure. T cells were collected and their activities were quantified by the CCK8 method.
C. Macrophages were stimulated with 100 ng/ml LPS for different periods in vitro (12 h, 24 h, 36 h) and naïve T cells were co-cultured in the presence or absence of the stimulated macrophages. Macrophages were then removed from the mixture through adherence. T cells alone were re-stimulated with 100 ng/ml LPS for 2 h and their activities were quantified by the CCK8 method. n = 4 for each group. $^*P < 0.05$.

($CD3^+CD8^+$) and fewer Tregs ($CD4^+CD25^+$) compared to the NC mice, while the proportion of $CD4^+$ T cells appeared relatively unchanged (Fig. 3A and **B**). The effect of macrophage depletion on T cell cytokine secretions was also investigated. Interestingly, the levels of IFN-γ and TNF-α were restored in T cells upon macrophage depletion in septic mice (Fig. 3C).

3.3. Macrophages induce immunosuppression in T cells through direct cell-cell contact

The in vivo and in vitro experiments provided evidence of a relationship between macrophages immunosuppression and T cells depression, suggesting that macrophages can induce immunosuppression in T cells. However, the exact mechanism underlying this induction is still unclear based on the available data. Cytokines are expressed by a wide range of cell types and play a crucial role in regulating various cellular functions. Therefore, their significance in cell regulation should not be overlooked. Additionally, the products generated during cellular metabolism can also have profound effects on cell functions. Our next task was to determine whether the induction was mediated by humoral factors or by cellular mechanisms. To disrupt cell-cell contacts, a piece of transwell equipment was utilized, allowing free access to cytokines and metabolic products but not to cells (Supplementary Fig. 1). When septic macrophages and naive T cells were directly co-cultured in the same chamber of the transwell equipment, T cells exhibited a similar suppression of cell function as observed in vivo. Conversely, when macrophages and T cells were cultured separately in individual chambers, the absence of cell-cell contacts prevented T cell immunosuppression, resulting in more active cells in the Transwell group, as depicted in Fig. 4A. T cells also displayed a greater inclination to produce IFN-γ and TNF-α when cultured without direct contact with septic macrophages (Fig. 4B). Transwell culture also disrupted the in-vitro differentiation pattern of T cells. As illustrated in Fig. 5, when cells were co-cultured without direct contact, T cells exhibited a greater tendency to differentiate into $CD8^+$ rather than $CD4^+CD25^+$ cells, while the ratio of $CD4^+$ T cells appeared unaffected. These findings suggest that septic macrophages induce T cell immunosuppression through cell-cell contact.

3.4. CR3 and Gas6 are involved in the induction of immunosuppression

In our previous studies, we observed an up-regulation of CD11b on macrophages in a time-dependent manner (Fig. 6A). As CD11b forms a complex with CD18, we further conducted an immune blot analysis to examine the expression of CD18. Consistent with the findings for CD11b, CD18 levels also increased in macrophages, particularly at 24 h or later after the onset of sepsis (Fig. 6B). In comparison, macrophages from SHAM mice showed only a slight increase in CD18 expression, suggesting that the overexpression of CR3 (CD11b/CD18) was likely infection-related. To further investigate the role of CR3 in intercellular communication, we designed a CD18-targeted siRNA and transfected it into macrophages to silence CD18 expression. Fig. 6C demonstrates the significant inhibitory effect of CD18-1199 on CD18 expression in macrophages. Interestingly, the differentiation of T cells upon CD18 silencing differed from that observed in the transwell experiments. Overall, the lack of CD18 on macrophages led to a significant increase in $CD4^+CD25^+$ T cells and a decrease in $CD4^+$ T cells, while it had no apparent impact on the ratio of $CD8^+$ cells (Fig. 7A and **B**). Cytokine secretion was also assessed using flow cytometry. The results demonstrated a reduction in TNF-α+ T cells in the siCD18 group, while CD18 silencing had little effect on the ratio of IFN-γ+ cells (Fig. 7C). Since cell-cell contact was required for the induction and CR3 was implicated in

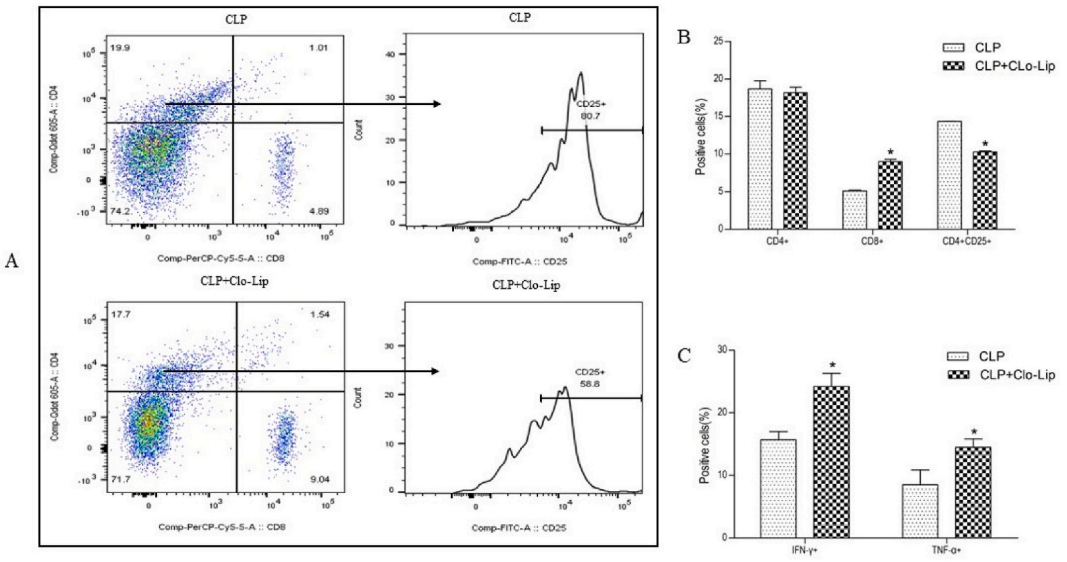

Fig. 3. Septic macrophages alter the differentiation of T cells and the production of cytokines
Note: Macrophages were pre-depleted in vivo using Clo-Lip injections, and then the mice underwent CLP for 24 h. T cells were collected from peripheral blood and analyzed using flow cytometry. Subgroups were identified based on the expression of CD4, CD8, and CD25 (**A** and **B**) and the levels of IFN-γ and tumor TNF-α were analyzed using flow cytometry (**C**). n = 4 for each group. $^*P < 0.05$ compared with the CLP group.

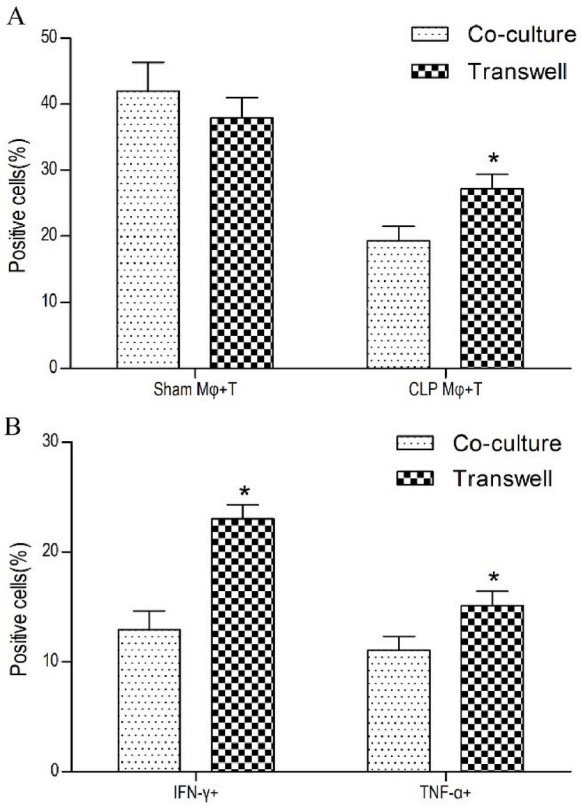

Fig. 4. Septic macrophages impair T cell viability and cytokine production through cell-cell contact
Note: Macrophages and serum were obtained from septic mice that experienced sepsis for 24 h. The septic serum was added to the DMEM culture at a concentration of 2 %. Septic macrophages and naïve T cells were either directly co-cultured or cultured separately in chambers of a transwell system for 24 h. T cells were then collected and analyzed using flow cytometry. Cell viabilities were quantified as depicted in **A**, while levels of IFN-γ and TNF-α were measured as shown in **B**. n = 4 for each group. $^*P < 0.05$ compared with the Co-culture group.

this process, we proceeded to determine the level of Gas6 in T cells. Gas6 expression was significantly upregulated during sepsis, but its concentration was downregulated in the presence of macrophages (Fig. 8).

4. Discussion

Over the past two decades, advancements in early-stage treatment, such as antibiotics and fluid resuscitation, have led to a decrease in fatality rates during the initial phase of sepsis [2]. However, emerging evidence highlights a concerning prospect for critically ill septic patients who survive the primary hyperinflammation stage but experience impaired immunity [16]. This immunosuppression leaves them vulnerable to secondary infections, which are often more severe and resistant to treatment [17]. In a previous study, it was discovered that improving the immunosuppression of macrophages could enhance survival and organ function [6]. Further experiments revealed that T cells' viabilities were also improved after macrophages' salvation whereas the exact mechanisms remained unclear. In this study, it was further revealed that immune-paralyzed macrophages can induce immunosuppression in T cells. This induction depends on cell-cell contacts, and CR3 is probably involved in the process. This finding sheds light on a possible explanation for the limited efficacy of cytokine blockade as a therapeutic approach for sepsis, as direct cell-cell contacts are unavoidable in this condition [18]. This study uncovers a potential mechanism by which immunosuppression is transmitted from the innate immune system to the adaptive immune system.

The pathogenesis of sepsis is heterogeneous and depends on various factors such as infection sites, pathogens, complications, immune status, age, and sex [17]. Consequently, there is no ideal animal model that can fully mimic the entire course of sepsis. The timing of the onset of immunosuppression in sepsis can vary from hours to days. In our study, we observed a decrease in macrophage phagocytosis during the initial 24 h of sepsis, which was partially restored at 36 h. However, the viabilities and cytokine secretions of both macrophages and T cells followed a reverse V-shaped trend, as depicted in Fig. 1. It appears that macrophage phagocytosis is more easily affected compared to cytokine production. The divergent patterns observed in macrophages and T cells suggest different moments of damage during sepsis. A later point in the course of sepsis may lead to a more dysregulated immune status, as demonstrated in our study, but it may also result in a substantial loss of antigen presentation by macrophages. Taking into consideration human reports and animal studies, we sampled macrophages and T cells at 24 h after the onset of sepsis.

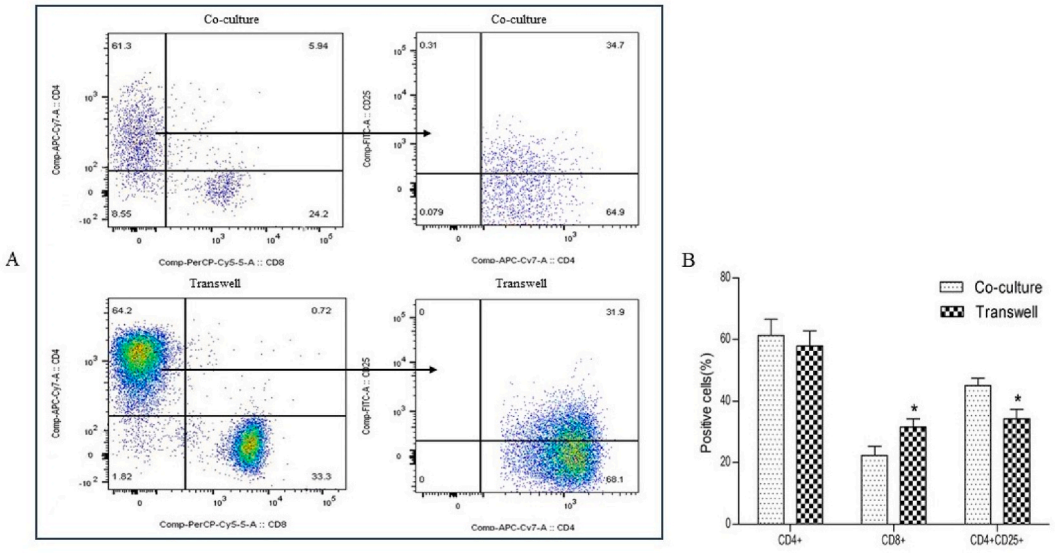

Fig. 5. Septic macrophages modulate T cell differentiation through cell-cell contact
Note: Macrophages and serum were obtained from septic mice that experienced sepsis for 24 h. The septic serum was added to the DMEM culture at a concentration of 2 %. Septic macrophages and naïve T cells were either directly co-cultured or cultured separately in chambers of a *trans*-well system for 24 h. T cells were then collected and analyzed using flow cytometry. Subgroups were defined based on CD4, CD8, and CD25 markers. n = 3 for each group. $*P < 0.05$ compared with the Co-culture group.

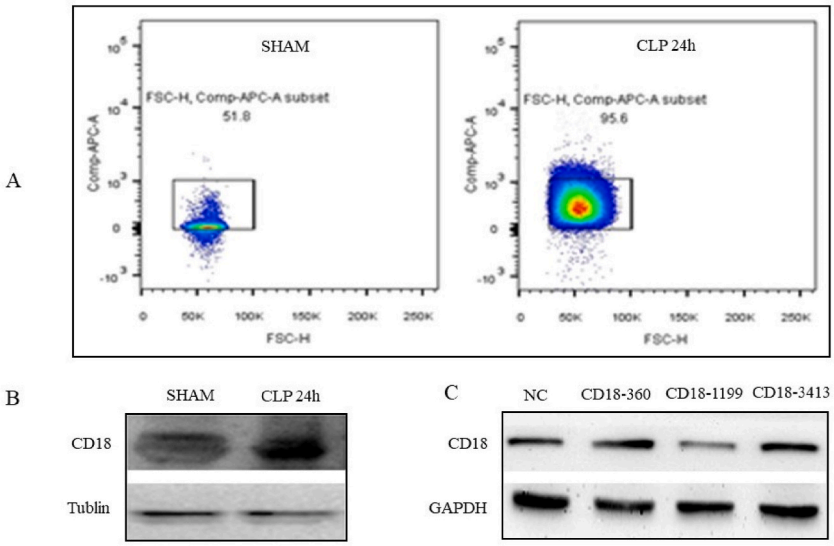

Fig. 6. Expression of CR3 on macrophages during sepsis.
Note: Mice were subjected to CLP for 24 h, and peripheral macrophages were collected. The expression of CD11b was analyzed using flow cytometry (**A**), while CD18 expression was assessed using Western blot (**B**). The effects of three different siRNAs on CD18 expression in macrophages are depicted in **C**. n = 4 for each group.

In a normal physiological condition, macrophages serve as antigen-presenting cells (APCs) and activate T cells through the processing of infectious agents and immunological stimuli, leading to the generation of various types of T cells [19]. This process involves several molecules, including the major histocompatibility complex (MHC), CD20, and CD28 [20]. However, in sepsis, macrophages undergo MHC loss and endotoxin tolerance, which are believed to passively dampen T cell stimulation both quantitatively and qualitatively [9]. In this study, the researchers aimed to investigate the role of macrophages in T cell immunosuppression by globally eliminating the antigen presentation function of macrophages using Clo-Lip, a method that depletes macrophages. We expected that T cells would be inactivated as a result. However, contrary to our expectations, the results depicted in Figs. 3–5 showed the opposite effect. It is possible that the lack of co-stimulatory molecules on macrophages may be partially compensated by other APCs such as

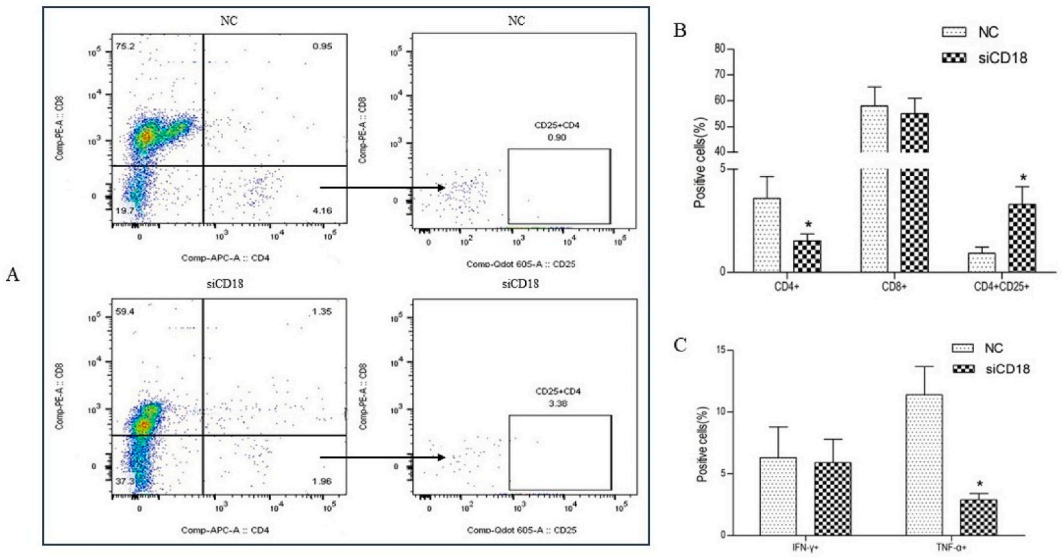

Fig. 7. Septic macrophages modulate T cell differentiation and cytokine production involving CD18
Note: CD18 expression was silenced in macrophages using siRNA CD18-1199 specifically targeting CD18. Following stimulation with 100 ng/ml LPS for 24 h, NC macrophages and siCD18 macrophages were co-cultured with T cells in the presence of 100 ng/ml LPS for an additional 24 h. T cells were collected and analyzed using flow cytometry. Subgroups were defined based on CD4, CD8, and CD25 markers (**B**). Levels of IFN-γ and TNF-α were measured (**C**). n = 3 for each group. $*P < 0.05$ compared with the NC group.

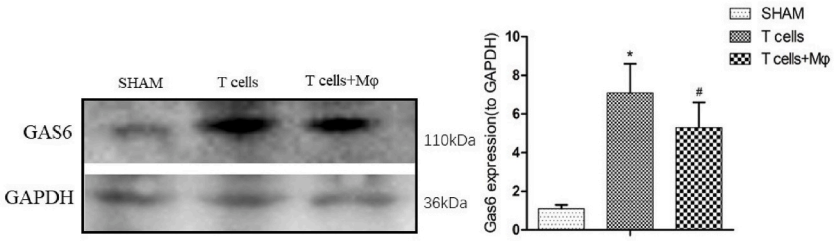

Fig. 8. Gas6 expression in septic T cells.
Note: Peripheral T cells from mice were stimulated with 100 ng/ml LPS in the presence or absence of macrophages for 24 h. Gas6 levels in T cells were assessed using Western blot analysis. n = 4 for each group. $*P < 0.05$ compared with the SHAM group. $\#P < 0.05$ compared with the T cells group.

dendritic cells (DCs) [21]. However, even if such compensation occurs, it should not completely negate the loss of macrophages. Taking into consideration that T cells demonstrated improved viability in the absence of macrophages during sepsis, we posit that negative signals are actively conveyed from macrophages to T cells, potentially serving as the underlying mechanism.

The study suggests that septic macrophages are responsible for the immunosuppression observed in T cells. The potential pathways linking macrophages and T cells could be mediated through humoral factors as well as direct cell-to-cell interactions. Sepsis is associated with the production of numerous cytokines that influence the differentiation of T cells into different phenotypes [21]. For example, IL-12 is essential for Th1 induction, while IL-4 is required for Th2 differentiation [22]. The metabolic bias of T cells is also observed in the tissue environment [23]. Blocking cytokines or isolating cell contact are two methods to study the induction pathways. However, the complex network of cytokines in vivo makes it impractical to block all relevant cytokines or metabolic products. As an alternative approach, we chose cell isolation using a transwell system. This setup eliminates direct cell-cell contacts while allowing the exchange of soluble factors. The results from this experiment showed a similar pattern of variation in T cells as observed in the macrophage depletion experiments. Since the diaphragm of the transwell system does not restrict the diffusion of molecules in the septic serum-like culture, these findings strongly suggest that macrophages induce immunosuppression in T cells through direct cell-cell contact.

Integrin families, consisting of an α-subunit and a β-subunit, are widely expressed on immune cells and mediate processes such as recruitment to infection sites, cell-cell contact, and cellular signaling [10]. For β2 integrins, 4 α-subunit (CD11a, CD11b, CD11c, and CD11d) and 1 β-subunit (CD18) have been identified [10]. Normally, β2 integrin remains in an inactive state with a bent closed headpiece until "inside-out" or "outside-in" signaling triggers integrin-mediated adhesion and extravasation into tissues [24]. It is worth

noting that CD18 is abundantly expressed, and the levels of different α-subunits determine the amount of corresponding β2 integrin on the cell surface [25]. Complement receptor 3 (CR3), also known as macrophage-1 antigen (MAC-1), is a dimeric receptor composed of CD11b and CD18, and it plays a crucial role in intercellular communication. In our previous study, we observed significantly increased CD11b expression on macrophages during sepsis, indicating upregulation of CR3 [6]. Based on these findings, we hypothesize that CR3 plays a role in regulating the interaction between macrophages and T cells.

Several studies have reported conflicting findings regarding the pro-inflammatory role of β2 integrins and their interaction with the TLR4 signaling pathway [26,27]. However, regardless of their precise roles in the cellular response to pathogen stimuli, all four β2 integrins can bind proteins of the extracellular matrix (ECM) and surface receptors involved in intercellular interactions [28]. Consequently, β2 integrins enable cells to adhere to the endothelium and extravasate from blood vessels at sites of inflammation. Macrophage adhesion to inflamed sites facilitates contact with pathogens and T cells. The term "immunological synapse" (IS) refers to the contact region between an antigen-presenting cell (APC) and a T cell [29]. Extensive research has been conducted on the structure of the IS in dendritic cells (DCs) and $CD4^+$ T cells, while limited studies have focused on the role of CR3, particularly in T cell differentiation and cytoskeletal rearrangements. Verga's work revealed that active MAC-1 on DCs inhibits full T-cell activation independently of antigen presentation [30]. Surprisingly, according to our study, the deficiency of CD18 in macrophages did not affect the proportion of $CD8^+$ T cells, but it did result in decreased $CD4^+$ T cells and increased $CD4^+CD25^+$ cells when T cells were pretreated with siCD18 macrophages. Furthermore, silencing CD18 led to a restriction in TNF-α production, while IFN-γ production appeared unaffected. These findings suggest that macrophage CR3 is necessary for T cell TNF-α production but not for IFN-γ production, and it may play a pro-inflammatory role in cell differentiation. This is partially supported by the work of Kragstrup et al., who demonstrated that soluble CD18 competitively inhibits the adhesion of iC3d to CD18 on APCs, resulting in an anti-inflammatory effect [31].

The receptor for CR3 on T cells has not been clearly identified. It is known that β2 integrin can bind to complement molecules, facilitating phagocytosis [32]. Complement molecules are extensively produced during sepsis [33]. A previous study revealed an interaction between C1q and Gas6 [34]. Gas6, which plays a crucial role in tumor proliferation, survival, migration, and invasion, was found to increase in septic T cells but decrease in T cells after co-culture with septic macrophages. Our data suggest that Gas6 is involved in the induction of T cell immunosuppression by CR3, possibly mediated by complement C1. However, due to the limited depth of our study, the detailed mechanism remains unclear.

The current study has several limitations that need to be addressed. One major concern is the heterogeneity of both macrophages and T cells. Macrophages can polarize into M1 or M2 phenotypes under different conditions, especially in sepsis. However, the polarization status of macrophages in this study was not examined or visualized. It is possible that the induction of immunosuppression is specific to a certain subtype of macrophages rather than a universal potential for all cells. Furthermore, despite the identification of T cell differentiation changes, corresponding functional assessments of the cell population were not performed, highlighting a limitation in this study. Additionally, CD18 silencing was achieved using siCD18 interference rather than gene knockdown. The efficiency of siCD18 is limited, and it may not completely silence CD18 expression. The exact role of CD18 in the pathway remains uncertain and requires further investigation.

In summary, this study uncovers a novel pathway through which immunosuppression is transmitted from macrophages to T cells, facilitated by direct cell-to-cell contact. The involvement of CR3 and Gas6 in this process has been identified, although the precise mechanism still requires further investigation. The study provides insights into the mechanism of persistent immunodepression in sepsis and suggests a potential approach to restore immune function.

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Availability of data and materials

Data will be made available on request.

CRediT authorship contribution statement

Shunwei Huang: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Ying Chen:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing, Funding acquisition. **Fangchen Gong:** Methodology, Project administration. **Weiwei Chen:** Investigation, Methodology. **Yanjun Zheng:** Data curation, Formal analysis. **Bing Zhao:** Data curation, Formal analysis. **Wen Shi:** Data curation, Formal analysis. **Zhitao Yang:** Formal analysis, Resources, Software, Writing – original draft, Writing – review & editing. **Hongping Qu:** Resources, Software. **Enqiang Mao:** Resources, Supervision, Validation, Funding acquisition. **Erzhen Chen:** Conceptualization, Funding acquisition, Resources, Supervision, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23266.

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