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Ligation of CD180 contributes to endotoxic shock by regulating the accumulation and immunosuppressive activity of myeloid-derived suppressor cells through STAT3

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ABSTRACT

Myeloid-derived suppressor cells (MDSCs) play an immunosuppressive role in the pathogenesis of inflammatory diseases. CD180, a TLR-like protein, can regulate the proliferation and activation of immune cells. However, the roles of CD180 in regulating the accumulation and function of MDSCs have not been investigated. Here, we found that, compared with non-treated controls, the expression of CD180 was significantly elevated in MDSCs, especially granulocytic MDSCs (G-MDSCs), from mice challenged with lipopolysaccharide (LPS). Ligation of CD180 by the anti-CD180 antibody not only blocked the expansion of MDSCs by preventing the phosphorylation of signal transducer and activator of transcription 3 (STAT3), but also reduced the immunosuppressive activity of MDSCs on M1 macrophage polarization through inhibition of Arg-1 expression *in vitro*. *In vivo* studies showed that injection of anti-CD180 antibody significantly aggravated pathological lesions in mice challenged with LPS. Furthermore, injection of anti-CD180 antibody inhibited the accumulation of G-MDSCs in mice challenged with LPS and reduced the immunosuppressive activity of G-MDSCs on M1 macrophage polarization. Based on these findings, we conclude that ligation of CD180 contributes to the pathogenesis of endotoxic shock by inhibiting the accumulation and immunosuppressive activity of G-MDSCs, thus providing insight into the function of CD180 in inflammatory diseases.

1. Introduction

Inflammation is a physiological response of the immune system to defend the body against injury or infection [1,2]. Generally, inflammation is considered as a protective response to injury or infection, due to its functions in eliminating pathogenic stimuli and maintaining homeostasis of the immune system [3]. However, numerous studies have described a “dual role” of inflammation, as excessive inflammation can be potentially harmful to the body because it can cause host damage [4]. Thus, inflammation can be a double-edged sword that needs to be tightly regulated to avoid excessive tissue damage and inflammatory disease. Macrophages, a highly heterogeneous population derived from the myeloid cell lineage, are the key cells during the inflammatory processes [5,6]. In response to stimuli, macrophages are activated and acquire distinct functional abilities; pro-inflammatory M1 (classically activated macrophages) and anti-inflammatory M2 (alternatively activated macrophages). Dysregulation of macrophage polarization has been shown to contribute to aberrant immune responses and inflammatory disease [7–9]. In the early stage of inflammation, macrophages are activated and polarized to an M1 phenotype, which can produce pro-inflammatory cytokines and lead to tissue damage [10]. Therefore, regulatory mechanisms that control the polarization of macrophages during the inflammatory process require further clarification.

During the past decade, myeloid-derived suppressor cells (MDSCs) have been recognized as a novel heterogeneous population of immature myeloid cells that play a critical role in both innate and adaptive immunity [11,12]. Under pathological conditions, such as acute and

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chronic inflammatory diseases, normal differentiation of immature myeloid cells is blocked to form MDSCs [12]. MDSCs act as an immune suppressor *via* production of high levels of immunosuppressive mediators, including Arginase-1 (Arg-1), inducible nitric oxide synthase (iNOS) and interleukin-10 (IL-10) [12]. In mice, MDSCs can broadly be characterized as $CD11b^+Gr\text{-}1^+$. More specifically, MDSCs can be delineated into two types: granulocytic MDSCs (G-MDSCs) and monocytic MDSCs (M-MDSCs), which are identified as $CD11b^+Ly6G^+Ly6C^{low}$ phenotype and $CD11b^+Ly6G^-Ly6C^{high}$ phenotype, respectively. Notably, we and others have demonstrated that MDSC population are significantly expanded in inflammatory diseases and contribute to control of inappropriate inflammatory activation by inhibiting M1 macrophage polarization [13,14]. Regulation of the expansion and function of MDSCs can significantly affect the activation of immune response and influence the pathogenesis of inflammatory diseases [15–17]. However, the intrinsic regulatory mechanisms of expansion and function of MDSCs in inflammation remains largely unexplored. Thus, it is imperative to clarify the potential regulatory mechanism to enable better control of inflammation.

CD180, also known as RP105, is a TLR-like protein that mainly expressed on B lymphocytes, macrophages, and dendritic cells (DCs) [18]. An increasing body of evidence has emerged to show that CD180 can influence the development, homeostasis, and survival of immune cells, including macrophages, DCs, and B lymphocytes [19–21], indicating a complex association between CD180 and inflammation. CD180 enhances the activation of TLR4 signaling pathways in B cells, but functions as an endogenous inhibitor of TLR4 signaling in macrophages and DCs [22]. Furthermore, CD180 has been reported to enhance TLR2-mediated activation of macrophages [23]. These studies suggest that CD180 plays opposing regulatory roles different cells *via* mechanisms that are not well understood. Thus, in-depth study of the effect of CD180 in inflammatory diseases is compelling.

Considering that MDSCs are a heterogeneous population of cells, generally composed of progenitors and precursors of dendritic cells, macrophages, and granulocytes [12], we hypothesized that CD180 is expressed on MDSCs and plays an immune regulatory role on MDSCs. We investigated the expression of CD180 on MDSCs and its role in regulating the accumulation and immunosuppressive activity of MDSCs in endotoxic shock, which is a life-threatening response caused by disordered immune responses to infection. We found that CD180 is robustly expressed by MDSCs and that mice challenged with LPS exhibited increased CD180 expression on MDSCs derived from spleen compared with control cells. Ligation of CD180 by anti-CD180 antibody not only inhibited accumulation of MDSCs by inhibiting phosphorylation of STAT3, but also inhibited the immunosuppressive activity of MDSCs through inhibition of Arg-1 expression. Moreover, *in vivo* studies showed that ligation of CD180 inhibited the accumulation and immunosuppressive activity of G-MDSCs and attenuated pathological lesions in mice challenged with LPS. Taken together, our studies uncover a significant role of CD180 in regulating the accumulation and functional activity of MDSCs and that CD180 may be a critical pathogenic factor in inflammation.

2. Materials and methods

2.1. Mice

C57BL/6 mice were obtained from Pengyue Experimental Animal Breeding Company (Jinan, China) and maintained under pathogen-free conditions at Jining Medical University. Mice used in the experiments were females, 6–8 weeks old. All experiments were conducted in accordance with institutional guidelines for animal care and used in accordance with the Guide for Animal Care Committee at Jining Medical University.

2.2. Antibodies

The following antibodies were used for immunoblotting: Cell Signaling Technology, anti-p-STAT3 (Cat#: 9145T, diluted at 1:1000), anti-STAT3 (Cat#: 4904T, diluted at 1:1000); Beyotime Institute of Biotechnology, anti-GAPDH (Cat#: AF0006, diluted at 1:1000), HRP-labeled Goat Anti-Rabbit IgG (Cat#: A0208, diluted at 1:3000), HRP-labeled Goat Anti-Mouse IgG (Cat#: A0216, diluted at 1:3000). The following antibodies purchased from Biolegend were used for flow cytometry: anti-CD11b (Cat#: 101206), anti-B220 (Cat#: 103206), anti-NK1.1 (Cat#: 108705), anti-Ly6G (Cat#: 127615 or 127608), anti-CD180 (Cat#: 117706), anti-CD40 (Cat#: 124610), anti-F4/80 (Cat#: 123108), anti-CD86 (Cat#: 105012), anti-Ly6C (Cat#: 128016), anti-CD11c (Cat#: 117310), anti-Gr-1 (Cat#: 108412) and anti-CD3 (Cat#: 100236). All the antibodies used for flow cytometry were diluted at 1:100.

2.3. Preparation of bone marrow-derived macrophages (BMDMs)

BMDMs were obtained as previously described [13]. Briefly, bone marrow cells, isolated from tibias and femurs of C57BL/6 mice, were cultured in complete Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10 ng/ml granulocyte-macrophage colony stimulating factor (GM-CSF, Peprotech), at 37 °C. After four days, the cells received fresh complete DMEM supplemented with GM-CSF (10 ng/ml) and were incubated for three additional days. BMDMs were harvested and then seeded in fresh complete DMEM at a density of 2×10^6 cells/ml for experiments.

2.4. Preparation of bone marrow-derived MDSCs in vitro

Bone marrow cells, isolated from tibias and femurs of C57BL/6 mice, were planted into dishes using complete Roswell Park Memorial Institute 1640 (RPMI 1640, Gibco) medium supplemented with GM-CSF (40 ng/ml, Peprotech) and IL-6 (40 ng/ml, Peprotech). Cultures were maintained at 37 °C in 5% CO_2 humidified atmosphere for four days. To investigate the effect of CD180 on MDSC expansion, anti-CD180 antibody (Biolegend) was added to fresh bone marrow cells or bone marrow-derived MDSCs before supplemented with GM-CSF and IL-6.

2.5. Isolation of G-MDSCs and $CD4^+$ T cells

Isolation of splenic G-MDSCs from LPS- or LPS plus anti-CD180 antibody-treated C57BL/6 mice was performed using a Myeloid-Derived Suppressor Cell Isolation Kit (Miltenyi Biotec) according to the manufacturer's instructions. $CD4^+$ T cells were isolated from the spleens of C57BL/6 mice using a $CD4^+$ T cell Isolation Kit (Miltenyi Biotec).

2.6. Suppressive assay of MDSCs on M1 macrophage polarization and T cell proliferation

MDSCs, isolated from mouse spleen or generated *in vitro*, were co-cultured with BMDMs (2×10^5 cells/well) at a ratio of 1:1 in Transwell chambers. After 12 h, MDSCs were removed and BMDMs were stimulated with 200 ng/ml LPS (Sigma) plus 10 ng/ml recombinant murine IFN-γ (eBioscience) to detect the polarization of M1 macrophages at different time points. For T cell proliferation assay, $CD4^+$ T cells were labeled with CFSE (Sigma) and co-cultured with purified G-MDSCs at a ratio of 1:1 in the presence of 3 μg/ml anti-CD3 antibody (BioLegend) and 3 μg/ml anti-CD28 antibody (BioLegend). After cultured for three days, proliferation of the cells was analyzed by flow cytometry.

2.7. Flow cytometry

Cells, cultured *in vitro* or isolated from mice, were washed twice

with phosphate buffered saline (PBS) containing 1% fetal bovine serum (FBS, Gibco) and 0.1% NaN_3 followed by blocking. Cells were then surface-stained with mouse antibodies against CD11b, Gr-1, Ly6G, Ly6C, F4/80, CD86, CD3, CD40, NK1.1, B220, CD11c and CD180 for 30 min at 4 °C. Cells were washed twice with PBS and then analyzed by fluorescence activated cell sorting (FACS, FACS Calibur, Becton Dickinson). An isotype control was used for each antibody. All the FACS data were analyzed on FlowJo software.

2.8. Histological analyses

Histological analyses were performed as described previously [13]. Briefly, 4-μm-thick sections were cut from paraffin-embedded lung and liver tissue, fixed in paraformaldehyde (Sigma), and stained with hematoxylin and eosin. Degree of lung injury was evaluated by an independent pathologist to objectively quantify the score based on the following histological features: Edema; hyperemia and congestion; neutrophil margination and tissue infiltration; intra-alveolar hemorrhage and debris. Each item was graded according to a five-point scale: 0, normal, 1, mild severity, 2, moderate severity, 3, severe severity. The total lung injury score was calculated by adding up the individual scores of each category. A total of 6 slides were randomly selected from each group. The histological scoring analysis was performed in a blinded fashion.

The degree of liver injury was evaluated by a histological scoring analysis. Lobular inflammation (0, normal; 1, mild severity; 2, moderate severity; 3, severe severity; 4, maximum severity), portal inflammation (0, normal; 1, mild severity; 2, moderate severity; 3, severe severity; 4, maximum severity) and necrosis (0, no necrosis; 1, ≤10% of liver parenchyma; 2, 11–20% of liver parenchyma; 3, 21–30% of liver parenchyma; 4, >30% of liver parenchyma) were scored and summed up to determine the overall histopathology score. The histological scoring analysis was performed in a blinded fashion.

2.9. TUNEL assay

Terminal deoxynucleotidyl transferase-mediated nucleotide nick-end labeling (TUNEL) assay was performed using an *In Situ* Apoptosis Detection Kit (Roche) according to the manufacturer's protocol. Briefly, tissue sections were dewaxed and rehydrated using a series of xylene and ethanol. After incubation with proteinase K working solution at 37 °C for 30 min, tissue sections were incubated with TUNEL reaction mixture at 37 °C for 60 min. After washing with PBS three times, sections were analyzed under a fluorescence microscope (Olympus, Japan). For each liver section per mouse, the number of TUNEL-positive cells in three randomly selected high power fields (×200) was counted under the fluorescence microscopy.

2.10. Enzyme-linked immunosorbent assay (ELISA)

The concentrations of serum IL-6 and IL-12 in mice were determined using a mouse IL-6 and IL-12 ELISA kits (Biolegend) according to the manufacturer's instructions. All samples were assayed in duplicate.

2.11. RNA isolation and quantitative real-time RT–PCR (Q-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions and then reverse-transcribed with a RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific). Q-PCR assays for mRNA were performed using SYBR Green PCR Master Mix (Vazyme Biotech). The $2^{-\Delta\Delta Ct}$ method was used for gene expression analysis. All the expression levels of target genes were normalized to *GAPDH* expression.

2.12. Immunoblotting analysis

Cells were collected and homogenized in lysis buffer followed by centrifugation for 15 min at 12,000*g*. Protein concentration was measured using a BCA protein assay kit (Beyotime) according to the manufacturer's instructions. Proteins were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to 0.45 μm polyvinylidene difluoride (PVDF) membranes (Millipore). Membranes were blocked in tris-buffered saline-Tween 20 (TBST) containing 3% bovine serum albumin (BSA) for 2 h at room temperature and then incubated with primary antibodies overnight at 4 °C. After washing, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (dilution at 1:3000, Beyotime) for 2 h at room temperature. Enhanced luminol-based chemiluminescent (ECL) Plus Western Blotting Detection substrate (ThermoFisher Scientific) was used to visualize protein expression. GAPDH was used as an internal control. Quantity One (Bio-Rad) was used to quantify the phosphorylation of STAT3.

2.13. Adoptive transfer and endotoxic shock

MDSCs, treated with anti-CD180 antibody for 6 h, were transferred into C57BL/6 mice (i.v., 2×10^6 cells/mouse). Mice were rested for 12 h and endotoxic shock was induced by an intraperitoneal injection of *Escherichia coli*-derived ultrapure LPS (40 μg/g or 20 μg/g). Survival after administration of LPS was monitored. For pathological analysis, mice were injected with LPS (10 μg/g). Serum samples were collected after 6 h and livers, lungs, and spleens were collected after 12 h.

2.14. Statistical analysis

All values in the graphs were given as means plus or minus standard error of the mean (SEM). Significance was assessed by using the ANOVA; Kaplan–Meier method was used to estimate overall survival and the Log-rank test was applied to determine the difference of survival rate. p values < 0.05 were considered significant.

3. Results

3.1. CD180 is expressed on multiple immune cells in the early phase of endotoxic shock

Studies have demonstrated that CD180 is expressed on B cells, macrophages, and dendritic cells [19–21]. We examined the expression of CD180 on different types of immune cells in the early phase of endotoxic shock. There was no difference in the expression of CD180 on splenic $B220^+$ cells between mice challenged with LPS and control mice (Fig. 1A). However, the expression of CD180 on both splenic NK cells and dendritic cells from mice treated with LPS were higher than that from controls (Fig. 1B and D). In addition, macrophages from mice challenged with LPS showed lower CD180 expression compared with controls (Fig. 1C). These results demonstrate that LPS can regulate the expression of CD180 on different types of immune cells, *in vivo*.

Our previous study showed that MDSCs, especially G-MDSCs, accumulate and play a protective role in the early phase of endotoxic shock [13]. Considering that MDSCs are immature myeloid progenitors, we investigated whether CD180 is expressed on MDSCs and whether its expression changes on MDSCs in the early phase of endotoxic shock. Mice were challenged with LPS or vehicle and the expression of CD180 on splenic MDSCs was examined. Intriguingly, the expression of CD180 on MDSCs from mice challenged with LPS was significantly higher compared with controls (Fig. 1E). Moreover, analysis of MDSCs subsets revealed that both G-MDSCs (Fig. 1F) and M-MDSCs (Fig. 1G) from mice challenged with LPS showed higher expression levels of CD180 compared with controls. These data demonstrate that MDSCs from mice with endotoxic shock exhibit higher expression level of CD180.

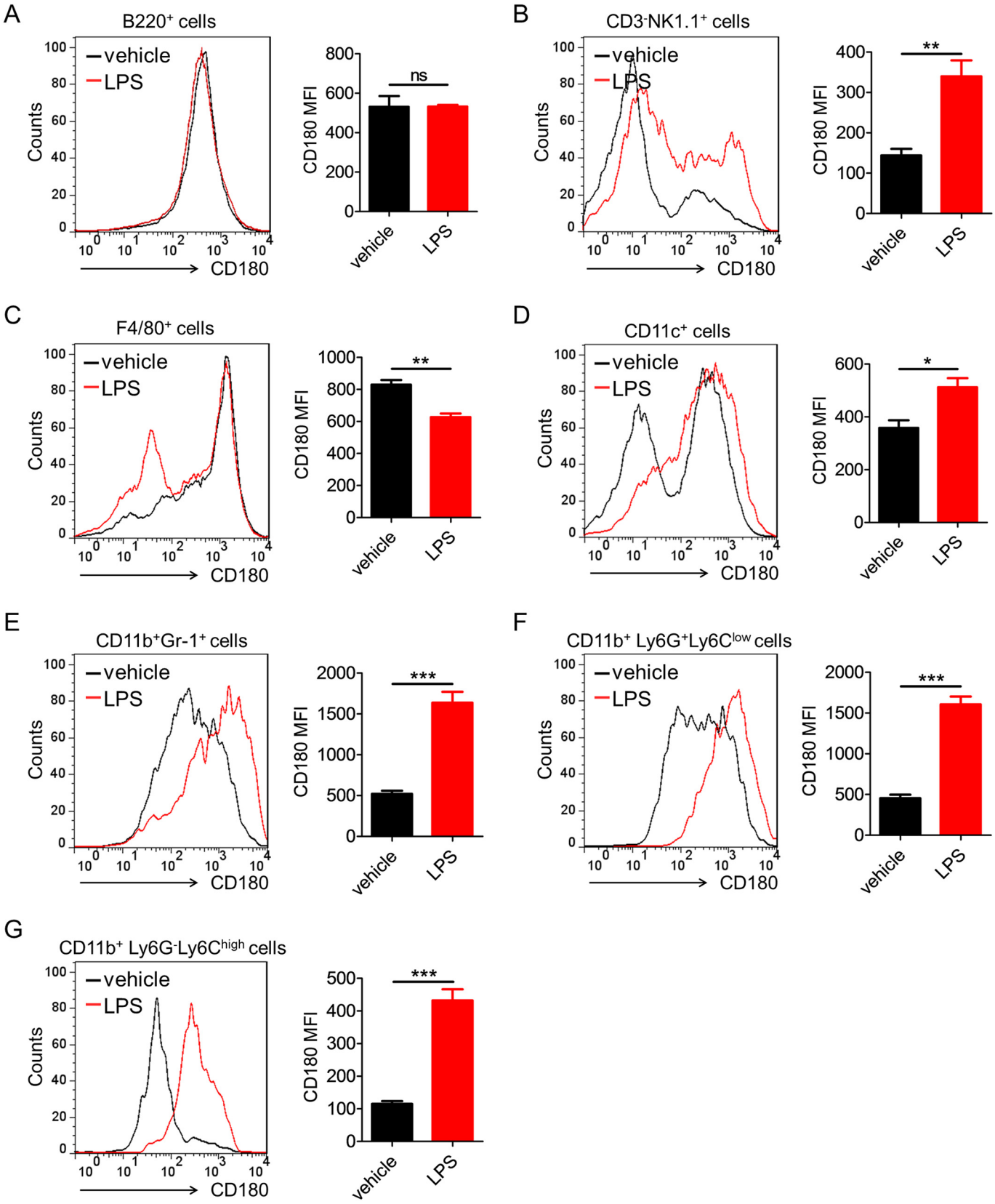


Fig. 1. CD180 expression on immune cells in the early phase of endotoxic shock. C57BL/6 mice were injected with LPS (10 μg/g) or vehicle for 12 h and the expression of CD180 on splenic $B220^+$ cells (A), $CD3^-NK1.1^+$ cells (B), $F4/80^+$ cells (C), $CD11c^+$ cells (D), $CD11b^+Gr1^+$ cells (E), $CD11b^+Ly6G^+Ly6C^{low}$ cells (F) and $CD11b^+Ly6G^-Ly6C^{high}$ cells (G) were detected by FACS. The data are representative of three independent experiments (n = 6 mice/group). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, as determined by ANOVA; ns denotes $p > 0.05$.

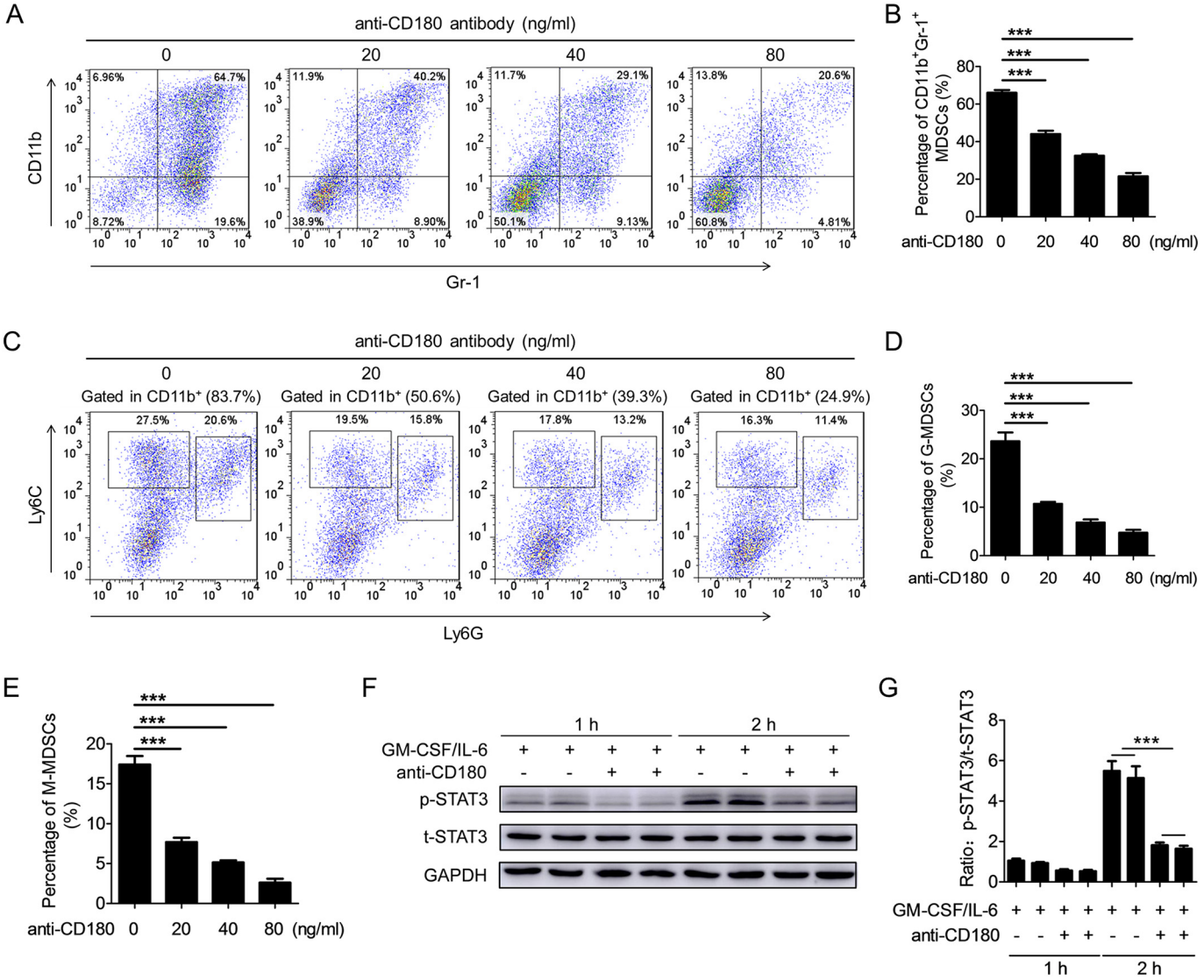


Fig. 2. Effect of anti-CD180 antibody on the expansion of MDSCs induced by GM-CSF plus IL-6 *in vitro*. (A–E) Bone marrow cells pretreated with different concentrations of anti-CD180 antibody (20, 40 and 80 ng/ml) or vehicle were cultured with murine GM-CSF (40 ng/ml) and IL-6 (40 ng/ml). After four days, percentages of $CD11b^+Gr\text{-}1^+$ MDSCs (A, B), $CD11b^+Ly6G^+Ly6C^{low}$ G-MDSCs (C, D), and $CD11b^+Ly6G^-Ly6C^{high}$ M-MDSCs (C, E) were analyzed by FACS. (F, G) Bone marrow cells pretreated with anti-CD180 antibody (80 ng/ml) or vehicle were cultured with GM-CSF (40 ng/ml) and IL-6 (40 ng/ml). Phosphorylation of STAT3 was detected by western blot at 1 and 2 h. Data are representative of three biological replicates, each with three technical replicates. Error bars represent SEM $^{***}p < 0.001$, as determined by ANOVA; ns denotes $p > 0.05$.

3.2. Ligation of CD180 blocks MDSC expansion, in vitro, by inhibiting STAT3 phosphorylation

MDSCs act as an immune suppressor *via* production of high levels of immunosuppressive mediators, including Arg-1, iNOS and IL-10 [12]. To investigate the immunoregulatory effect of CD180 on MDSCs *in vitro*, bone marrow cells were treated with different doses of anti-CD180 antibody, cultured with GM-CSF plus IL-6 and the expression of Arg-1, iNOS, Nox1 and IL-10 measured by Q-PCR. Ligation of CD180 by anti-CD180 antibody markedly inhibited the expression of Arg-1 and iNOS in bone marrow derived MDSCs (Fig. S1). To investigate whether the reduced expression of Arg-1 and iNOS in MDSCs was due to impaired expansion of MDSCs, the percentage of MDSCs was determined by FACS. Strikingly, ligation of CD180 by anti-CD180 antibody markedly inhibited GM-CSF plus IL-6-induced expansion of MDSCs in a dose-dependent manner (Fig. 2A and B). The percentage of both G-MDSCs ($CD11b^+Ly6G^+Ly6C^{low}$) and M-MDSCs ($CD11b^+Ly6G^-Ly6C^{high}$) was significantly reduced (Fig. 2C–E). These results demonstrate that ligation of CD180 significantly blocks the expansion of MDSCs *in vitro*.

STAT3 plays a critical role in the expansion of MDSCs under pathological conditions [12,24]. We previously reported that ligation of CD180 inhibited IFN-α-induced phosphorylation of STAT2 in B cells [25], indicating that CD180 may play an inhibitory role in activation of STATs. Accordingly, we hypothesized that ligation of CD180 blocks the expansion of MDSCs by inhibiting STAT3 activation. To investigate this possibility, bone marrow cells were pretreated with anti-CD180 antibody and then cultured with murine GM-CSF plus IL-6. Consistent with our previous findings, ligation of CD180 significantly inhibited GM-CSF plus IL-6-induced phosphorylation of STAT3 (Fig. 2F and G). Together with previous reports, these data suggest that CD180 negatively regulates the expansion of MDSCs, *in vitro*, by inhibiting phosphorylation of STAT3.

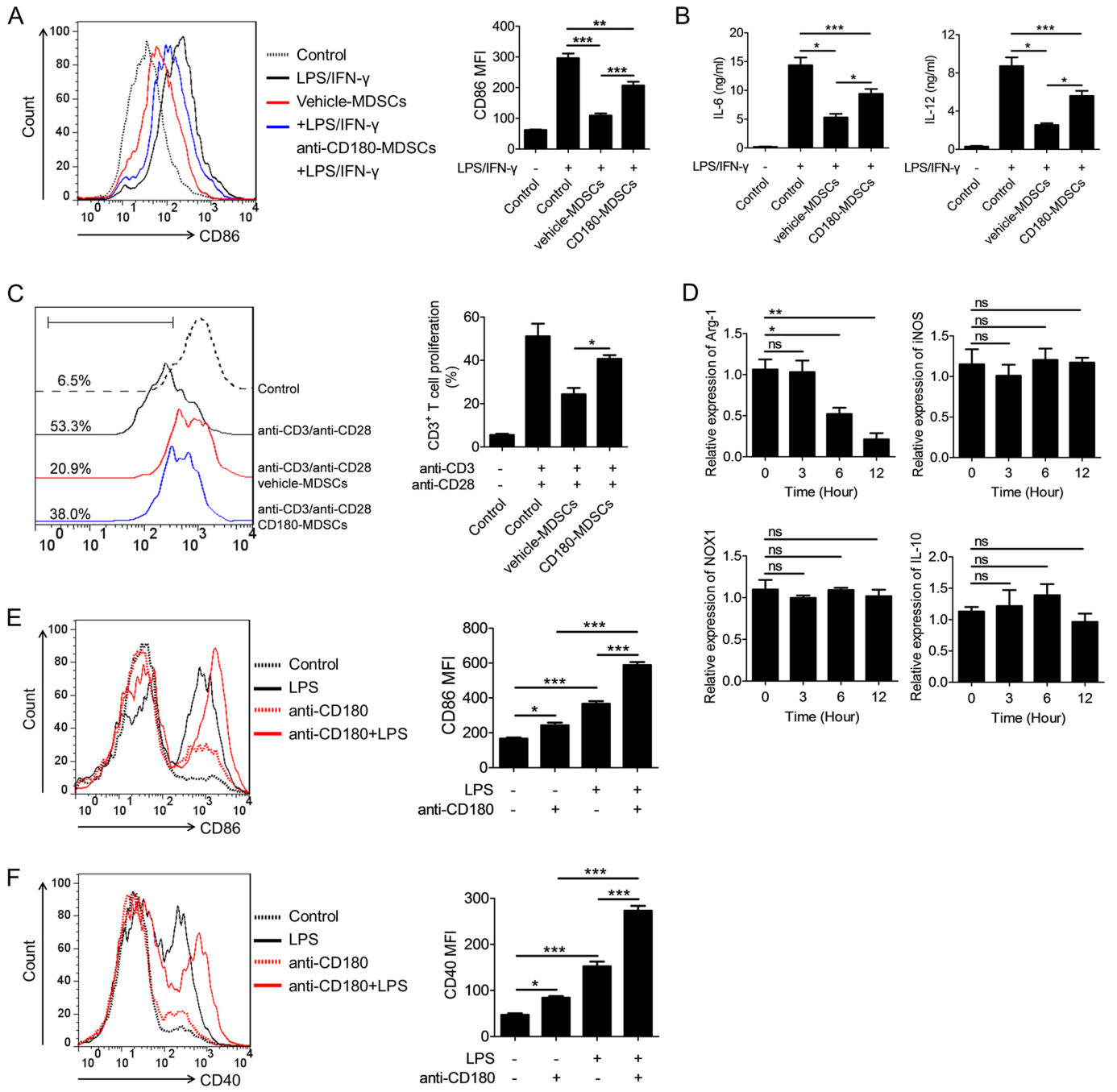


Fig. 3. Effect of CD180 ligation on MDSC inhibition of M1 macrophage polarization. (A, B) MDSCs, generated by GM-CSF plus IL-6 *in vitro*, were stimulated with anti-CD180 antibody (80 ng/ml) or vehicle for 6 h and then co-cultured with BMDMs. After 12 h, BMDMs were stimulated with LPS (200 ng/ml) plus IFN-γ (10 ng/ml). FACS analysis of CD86 expression at 24 h (A). Levels of IL-6 and IL-12 in supernatant by ELISA at 6 h (B). (C) MDSCs, generated by GM-CSF plus IL-6 *in vitro*, were stimulated with anti-CD180 antibody (80 ng/ml) or vehicle for 6 h and then co-cultured with freshly isolated $CD4^+$ T cells which are labeled with CFSE in the presence of anti-CD3 (3 μg/ml) and anti-CD28 (3 μg/ml) antibodies for 72 h. At the end of incubation, cells were harvested and analyzed by flow cytometry. (D) MDSCs, generated by GM-CSF plus IL-6 *in vitro*, were stimulated with anti-CD180 antibody (80 ng/ml) for 0, 3, 6 and 12 h. mRNA levels of Arg-1, iNOS, Nox1 and IL-10 were quantitated using Q-PCR. (E, F) MDSCs, generated by GM-CSF plus IL-6 *in vitro*, were pretreated with anti-CD180 antibody (80 ng/ml) or vehicle and treated with LPS. FACS analysis of CD86 (E) and CD40 (F) expression at 24 h. Data are representative of three biological replicates, each with three technical replicates. Error bars represent SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, as determined by ANOVA; ns denotes $p > 0.05$.

3.3. Ligation of CD180 eliminates the immunosuppressive activity of MDSCs

Previously, we and others have demonstrated that MDSCs can suppress the polarization of M1 macrophages and the proliferation of T cells [13,17], *in vitro* studies were carried out to examine the effect of CD180 on the immunosuppressive activity of MDSCs. MDSCs, pretreated with anti-CD180 antibody or vehicle, were co-cultured with BMDMs. BMDMs were then stimulated with LPS plus IFN-γ. As expected, vehicle-treated MDSCs (vehicle-MDSCs) exerted a strong inhibitory effect on LPS plus IFN-γ-induced expression of CD86 (Fig. 3A), as well as the secretion of IL-6 and IL-12 (Fig. 3B) in BMDMs. Compared with vehicle-MDSCs, anti-CD180 antibody-treated MDSCs (CD180-MDSCs) had a significantly reduced ability to inhibit LPS plus IFN-γ-

induced expression of CD86 (Fig. 3A), as well as the secretion of IL-6 and IL-12 (Fig. 3B) in BMDMs. For T cell proliferation assay, isolated $CD4^+$ T cells were stained with CFSE and co-cultured with vehicle-MDSCs or CD180-MDSCs in the presence of anti-CD3/anti-CD28 antibodies for 72 h. At the end of incubation, cells were harvested and analyzed by flow cytometry. As shown in Fig. 3C, vehicle-MDSCs exerted a strong inhibitory effect on T cell proliferation. Compared with vehicle-MDSCs, CD180-MDSCs showed a significantly reduced ability to inhibit T cell proliferation. To identify the mechanism underlying CD180-mediated inhibition of MDSC activity, the expression of immunosuppression mediators was examined. Ligation of CD180 markedly reduced the expression of Arg-1, while no alterations in the expression of iNOS, Nox1 and IL-10 were seen (Fig. 3D). These data indicate that ligation of CD180 may reduce the ability of MDSCs to inhibit M1 macrophage polarization through inhibition of Arg-1 expression.

MDSCs are a heterogeneous population of cells that resemble an immature or undifferentiated phenotype. It has been demonstrated that TLR agonists, such as LPS and R848, can induce MDSC differentiation into M1-like macrophages. Consistent with this, we found that adoptive transfer of LPS-treated MDSCs had no effect on the mortality of mice challenged with LPS, while mock-treated MDSCs had a marked protective effect against mortality in mice challenged with LPS (Fig. S2). To ascertain whether anti-CD180 antibody inhibition of Arg-1 expression in MDSCs was due to maturation of MDSCs, MDSCs were pretreated with anti-CD180 antibody prior to LPS stimulation and measurement of CD86 and CD40 expression by FACS. Anti-CD180 antibody lonely promoted expression of CD86 and CD40 on MDSCs, and also appeared to have a synergistic effect with LPS on the expression of CD86 (Fig. 3E) and CD40 (Fig. 3F) on MDSCs. These data show that ligation of CD180 promotes maturation of MDSCs from an immature or undifferentiated phenotype to a mature or differentiated phenotype, which subsequently weakened the immunosuppressive activity of MDSCs.

3.4. Ligation of CD180 reverses the protective effect of MDSCs in early endotoxic shock

Since ligation of CD180 reduced the ability of MDSCs to inhibit M1 macrophage polarization *in vitro*, we predicted that CD180 could affect the protective effects of MDSCs in endotoxic shock. MDSCs, generated *in vitro*, were treated with anti-CD180 antibody (CD180-MDSCs) or vehicle (vehicle-MDSCs) before being transferred into mice. Mice were then challenged with LPS. Adoptive transfer of mock-MDSCs significantly reduced mortality, while adoptive transfer of CD180-MDSCs had no effect on mortality in mice challenged with LPS compared to LPS-challenged mice that did not receive adoptive transfer (Fig. 4A). Furthermore, adoptively transferred mock-MDSCs had a protective effect against LPS-induced serum levels of IL-6 and IL-12 (Fig. 4B), damage to lung and liver (Fig. 4C and D), and apoptosis of liver cells (Fig. 4E and F) that was not seen in mice that received adoptive transfer of CD180-MDSCs. We also investigated the polarization of M1 macrophages in mice that received adoptive transfer of CD180-MDSCs or mock-MDSCs. Compared to mice without adoptive transfer, mice that received adoptive transfer of mock-MDSCs showed significantly reduced expression of CD86 and CD40 on splenic macrophages, while adoptive transfer of CD180-MDSCs had no effect (Fig. S3). Together, these findings demonstrate that ligation of CD180 reverses the protective effect of MDSCs in the early phase of endotoxic shock.

3.5. Ligation of CD180 aggravates inflammation in the early phase of endotoxic shock

Since ligation of CD180 inhibits the accumulation and immunosuppressive activity of MDSCs, we predicted that ligation of CD180 could promote the pathogenesis of endotoxic shock. Mice were injected with anti-CD180 antibody followed by LPS challenge and mortality, serum inflammatory cytokines and damage to lung and liver were observed. Compared with control mice, anti-CD180 antibody-treated mice showed significantly elevated mortality (Fig. 5A). Moreover, anti-CD180 antibody treated mice had significantly elevated the levels of IL-6 and IL-12 in serum (Fig. 5B), as well as the damage to lung and liver (Fig. 5C and D). The TUNEL assay also showed that in anti-CD180 antibody treated mice, apoptosis of liver cells was increased (Fig. 5E and F). Furthermore, compared with controls, anti-CD180 antibody-treated mice showed a higher expression of CD86 and CD40 in spleen (Figs. 5G, S4A) and in the peritoneal cavity (Figs. 5H, S4B), indicating that ligation of CD180 promotes M1 macrophage polarization *in vivo*. Collectively, these data strongly suggest that ligation of CD180 aggravates inflammation during endotoxic shock.

3.6. Ligation of CD180 reduces the accumulation and immunosuppressive activity of MDSCs in the early phase of endotoxic shock

We next asked whether CD180 could regulate the accumulation and immunosuppressive activity of MDSCs *in vivo*. Mice were injected with anti-CD180 antibody prior to LPS challenge and the percentage of splenic MDSCs was examined. Although anti-CD180 antibody alone showed no effect on accumulation of MDSCs, the percentage of MDSCs in anti-CD180 antibody-treated mice was significantly lower than that in control mice after LPS challenge (Fig. 6A and B). Moreover, the percentages of G-MDSCs, but not M-MDSCs, in anti-CD180 antibody-treated mice were significantly lower than that in control mice after LPS challenge (Fig. 6C–E). Thus, ligation of CD180 reduces the accumulation of MDSCs in early endotoxic shock.

To ascertain whether ligation of CD180 affected the immunosuppressive activity of MDSCs *in vivo*, we examined the expression of immunosuppressive mediators in G-MDSCs isolated from anti-CD180 antibody plus LPS-treated mice (LPS/CD180-G-MDSCs) and control mice (LPS-G-MDSCs). Reduced expression of both Arg-1 and Nox1 genes was observed in LPS/CD180-G-MDSCs compared with LPS-G-MDSCs (Fig. 6F), indicating that ligation of CD180 may affect the immunosuppressive activity of MDSCs *in vivo*. To further confirm the effect of CD180 on the immunosuppressive activity of MDSCs, LPS-G-MDSCs and LPS/CD180-G-MDSCs were co-cultured with BMDMs. BMDMs were then stimulated with LPS plus IFN-γ. Control LPS-G-MDSCs inhibited LPS plus IFN-γ-induced CD86 expression in BMDMs, as well as the secretion of IL-6 and IL-12 (Fig. 6G and H). Compared with LPS-G-MDSCs, LPS/CD180-G-MDSCs showed a reduced ability to inhibit CD86 expression (Fig. 6G), as well as the secretion of IL-6 and IL-12 (Fig. 6H). In addition, the effects of LPS-G-MDSCs and LPS/CD180-G-MDSCs on T cell proliferation were examined. Isolated $CD4^+$ T cells were stained with CFSE and co-cultured with LPS-G-MDSCs or LPS/CD180-G-MDSCs in the presence of anti-CD3/anti-CD28 antibodies for 72 h. At the end of incubation, cells were harvested and analyzed by flow cytometry. As shown in Fig. 6I, vehicle-MDSCs exerted a strong inhibitory effect on T cell proliferation. Compared with vehicle-MDSCs, CD180-MDSCs showed a significantly reduced ability to inhibit T cell proliferation. These findings reveal an important role of CD180 in regulating the accumulation and immunosuppressive activity of MDSCs *in vivo*, which advanced M1 macrophage polarization and enhanced inflammation.

4. Discussion

MDSCs were originally identified as a population of cells with immunosuppressive activity in cancer [26–28]. Recent studies show that inflammation has a key role in inducing MDSC development and accumulation, and that MDSCs participate in regulating activation of inflammatory responses [29,30]. There is convincing evidence that many cancers and inflammatory diseases are caused by the inhibition of the immunosuppressive activities of MDSCs. We and others have

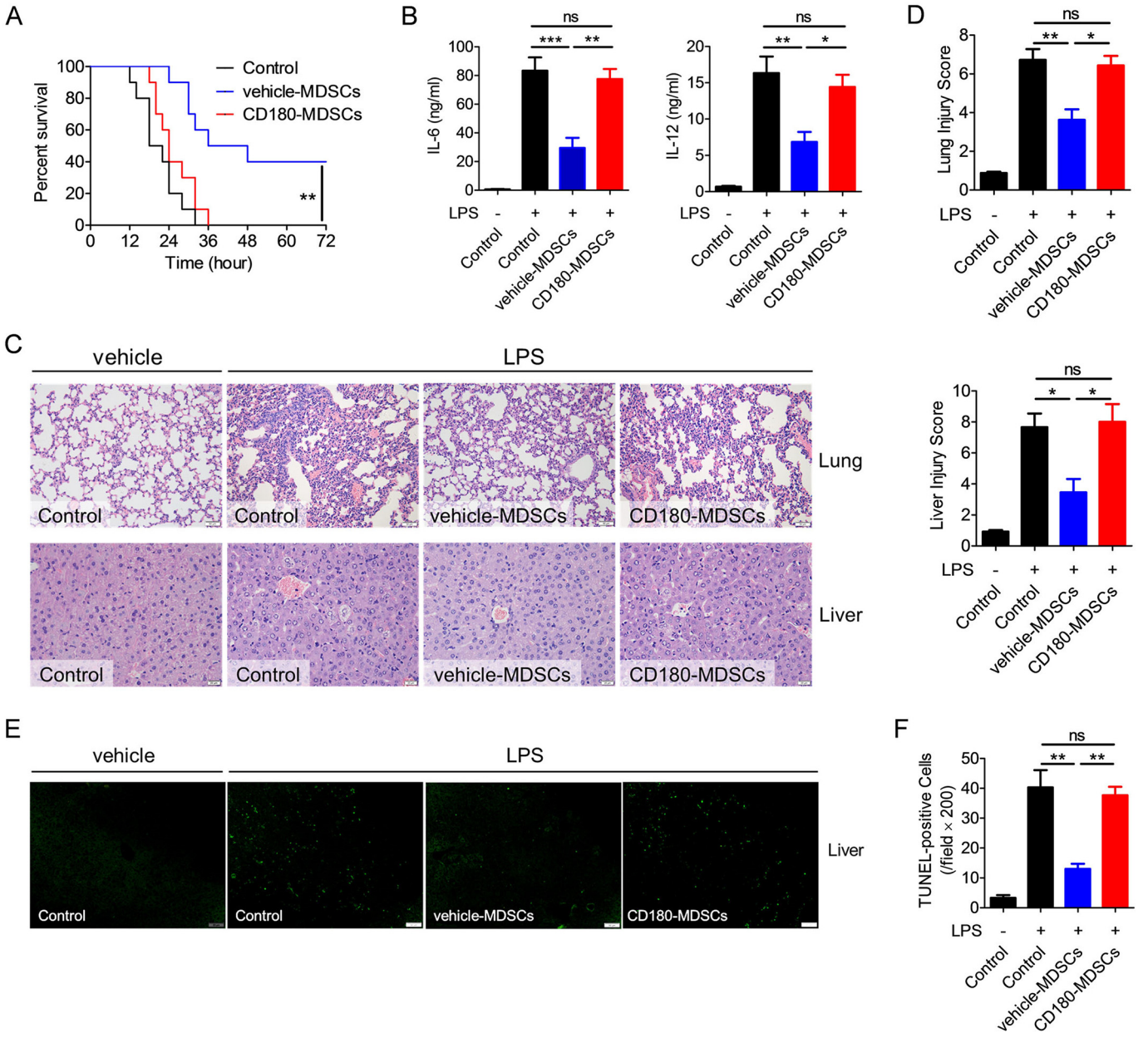


Fig. 4. Treatment effects of anti-CD180 antibody-treated MDSCs or mock-treated MDSCs on endotoxic shock. MDSCs, generated by GM-CSF plus IL-6 *in vitro*, were stimulated with anti-CD180 antibody (80 ng/ml) or vehicle for 6 h and then transferred to C57BL/6 recipient mice. (A) Kaplan–Meier method was used to estimate overall survival of recipient mice challenged with LPS (40 μg/g) 12 h after adoptive transfer. Log-rank test was applied to determine the difference in survival rate (n = 10 mice/group). (B–F) Recipient mice challenged with LPS (10 μg/g) 12 h after adoptive transfer. Serum levels of IL-6 and IL-12 were determined by ELISA at 6 h (B). Paraffin-embedded lung and liver sections were stained with H&E at 12 h post adoptive transfer (C, D). Apoptosis of liver cells were detected by TUNEL assay at 12 h post adoptive transfer (E, F). The data are presented as the mean ± SEM (n = 6 mice/group) and are representative of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as determined by ANOVA; ns denotes $p > 0.05$.

demonstrated that MDSCs are involved in the early phase of endotoxic shock [13,31]. However, the intrinsic mechanisms of MDSC generation and/or regulation requires further investigation, and understanding these mechanisms will further our understanding of the pathogenesis of inflammatory diseases.

CD180, a TLR-like molecule, was originally identified as a 105 kDa protein (RP105) expressed on antigen presenting cells, such as macrophages, myeloid dendritic cells and B cells [18]. MDSCs are immature myeloid progenitors, thus it is reasonable to assume that CD180 might be expressed on MDSCs where it can modulate the accumulation and immunosuppressive activity of MDSCs. In the present study, we have demonstrated that MDSCs from mice challenged with LPS express elevated levels of CD180. Moreover, ligation of CD180 by anti-CD180 antibody significantly inhibited the accumulation and immunosuppressive activity of MDSCs, both *in vitro* and *in vivo*. Injection of anti-CD180 antibody attenuated LPS-induced endotoxic shock. These data indicate that CD180 is involved in the pathogenesis of endotoxic shock by regulating the accumulation and immunosuppressive activity of MDSCs.

Previously, we and others have demonstrated that demonstrated that Gr-1^{+}CD11b^{+} MDSCs were accumulated in mice challenged with LPS compared with controls [13,32,33]. We found that the percentage of G-MDSCs was significantly increased, while the percentage of M-MDSCs was reduced in mice challenged with LPS compared with controls. In fact, many studies have found that the changes of the two subsets, M-MDSCs and G-MDSCs, are not synchronized under

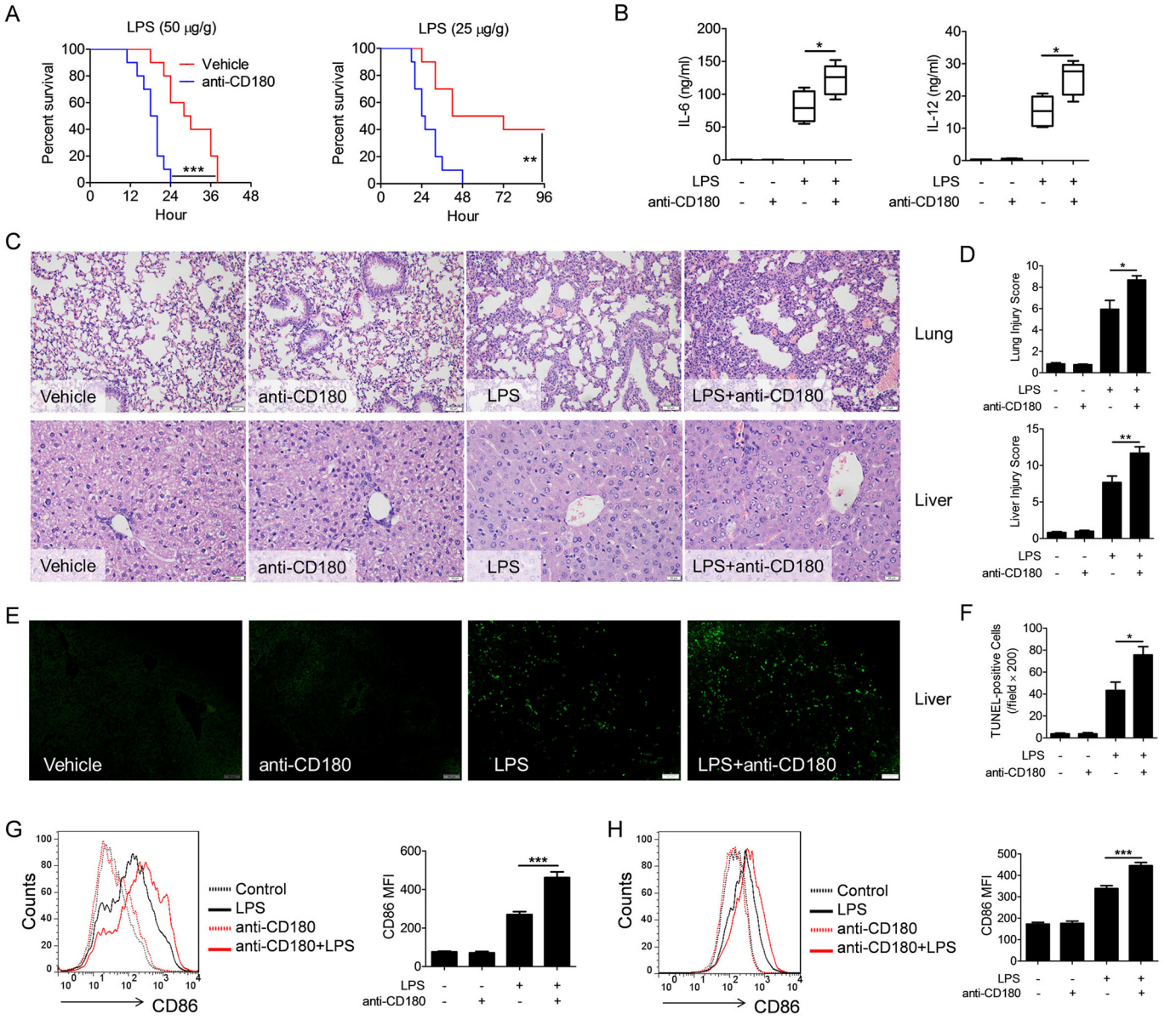
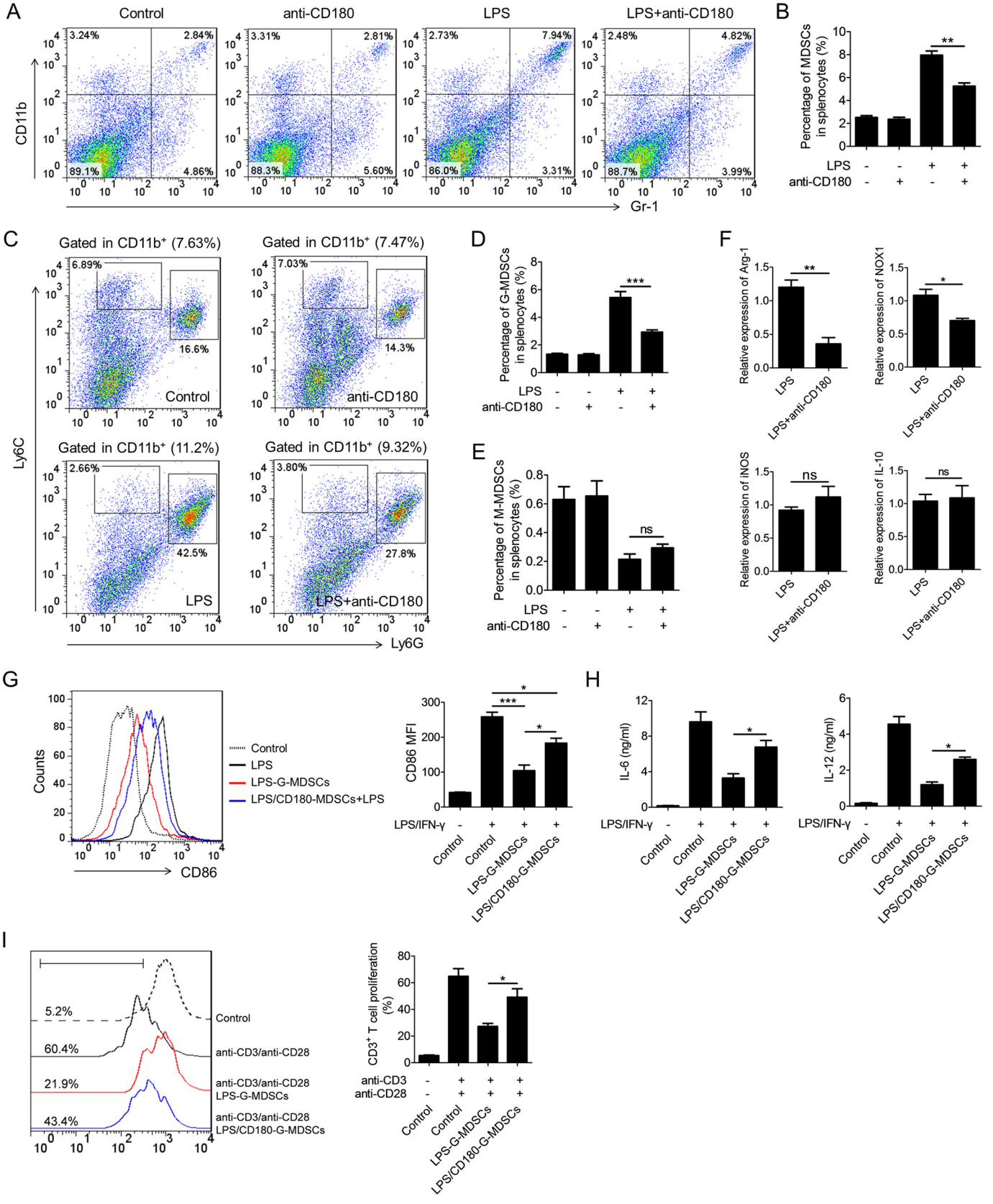


Fig. 5. Treatment effects of anti-CD180 antibody on endotoxic shock. (A) C57BL/6 mice were injected with anti-CD180 antibody (10 μg/g, i.p.) or vehicle then challenged with LPS (20 μg/g or 40 μg/g, i.p.). Kaplan–Meier method was used to estimate overall survival and the Log-rank test was applied to determine the difference in survival rates (n = 10 mice/group). (B–H) C57BL/6 mice were injected with anti-CD180 antibody (10 μg/g, i.p.) or vehicle then challenged with LPS (10 μg/g, i.p.). Serum levels of IL-6 and IL-12 were determined by ELISA at 6 h post LPS challenge (B). Paraffin-embedded lung and liver sections were stained with H &E at 12 h post LPS challenge (C, D). Apoptosis of liver cells was detected by TUNEL assay at 12 h (E, F). FACS analysis of CD86 expression on macrophages from spleen (G) and peritoneal cavity (H) at 12 h post LPS challenge. The data are presented as the mean ± SEM (n = 6 mice/group) and are representative of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as determined by ANOVA; ns denotes $p > 0.05$.

pathological conditions. For example, MDSCs were significantly accumulated in both the spleen and liver from mice infected with *T. congolense* and the increased numbers of MDSCs were due mainly to an increase in the numbers of M-MDSC subtype. In contrast, there was a profound reduction in the numbers of G-MDSC subtype [34]. The percentage and absolute numbers of MDSCs in *S. japonicum* infected mice were higher than that in normal mice. However, the percentage of G-MDSCs increased significantly, while the change of M-MDSCs was not significant [35]. We guess that there may be some factors which can affect the differentiation of M-MDSCs and reduce the proportion of M-MDSCs. However, to date, it is still unknown the specific mechanism that lead to the decrease of MDSCs. It's worth noting that MDSCs are a heterogeneous population of cells that resemble an immature or undifferentiated phenotype. LPS can induce MDSCs from an immature or undifferentiated phenotype to a mature or differentiated phenotype. We guess that the reduction of M-MDSCs maybe related with LPS in mice challenged with LPS. In further studies, we will pay attention to this problem and try to explore the mechanism.

Numerous studies have shown that MDSCs participate in the pathogenesis of other diseases, including pathogen infection, autoimmune diseases, and cancer [11]. Since CD180 can regulate the accumulation and immunosuppressive function of MDSCs in endotoxic shock, we anticipate that CD180 may also participate in the pathogenesis of other diseases involving MDSCs *via* the same mechanisms. It is worth exploring the role of CD180 in the pathogenesis of these diseases. We have previously shown that ligation of CD180 on B cells inhibits activation of IFN-α signaling by inhibiting tyrosine phosphorylation of STAT2 [25]. This suggested that CD180 may negatively regulate activation of the



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JAK-STAT signaling pathway. Consistent with this, the results presented here show that ligation of CD180 significantly inhibits the expansion of MDSCs induced by GM-CSF plus IL-6 *via* inhibition of STAT3 phosphorylation. Indeed, further investigations are required to clarify the mechanisms of CD180-mediated inhibition of STAT3 phosphorylation.

Fig. 6. Effects of anti-CD180 antibody on the accumulation and immunosuppressive activity of MDSCs in endotoxic shock. C57BL/6 mice were injected with anti-CD180 antibody (10 μg/g, i.p.) or vehicle, rested for 6 h and then challenged with LPS (10 μg/g, i.p.). Mice were sacrificed at 12 h post LPS challenge (A, B) FACS analysis of the percentages of MDSCs in splenocytes. (C-E) FACS analysis of the percentages of G-MDSCs and M-MDSCs in splenocytes. (F) Splenic G-MDSCs were isolated and the mRNA levels of Arg-1, iNOS, Nox1 and IL-10 were performed using Q-PCR. The data are shown as the means ± SEM (n = 6 mice/group) and are representative of three independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$ as determined by ANOVA; ns denotes $p > 0.05$. (G, H) Splenic G-MDSCs were isolated and then co-cultured with BMDMs. After 12 h, BMDMs were stimulated with LPS (200 ng/ml) plus IFN-γ (10 ng/ml). FACS analysis of CD86 expression at 24 h post stimulation (G). Levels of IL-6 and IL-12 in supernatant were determined by ELISA at 6 h post stimulation (H). (I) Splenic G-MDSCs were isolated and co-cultured with freshly isolated $CD4^+$ T cells which are labeled with CFSE in the presence of anti-CD3 (3 μg/ml) and anti-CD28 (3 μg/ml) antibodies for 72 h. At the end of incubation, cells were harvested and analyzed by flow cytometry. Data are representative of three biological replicates, each with three technical replicates. Error bars represent SEM $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, as determined by ANOVA; ns denotes $p > 0.05$.

Numerous studies indicate that multiple immune cells, such as macrophages, dendritic cells, MDSCs and neutrophils, play pivotal roles in the pathogenesis of endotoxic shock [36]. As is known, CD180 mainly expresses on macrophages, DCs and B cells [18]. CD180 can influence the development, homeostasis and survival of these cells, suggesting a complex association between CD180 and immune response. Interestingly, CD180 plays opposing regulatory roles in different cells *via* mechanisms that are not well understood [22,23]. It is important to note that the immunoregulatory functions of CD180 in different types of immune cells are distinct. For example, CD180 plays a major role in promoting TLR4 signaling-mediated activation and proliferation of B cells, while inhibiting the activation of TLR4 signaling in macrophages and dendritic cells [22]. Here, we found that ligation of CD180 reduces the accumulation and immunosuppressive activity of MDSCs and promotes M1 macrophage polarization in the early phase of endotoxic shock. However, the effects of CD180 ligation on other immune cell types are still largely unknown in the early phase of endotoxic shock and we thought more in-depth studies are needed to do to address this issue.

In this study, we demonstrate that ligation of CD180 aggravates inflammation in early phase of endotoxic shock, indicating that CD180 might be a potential therapeutic target in the treatment of endotoxic shock. However, to really make the point that whether CD180 can be considered as a potential therapeutic target in the treatment of endotoxic shock, it may be more conducive to study the role of CD180 in the early phase of endotoxic shock by constructing a CD180-knockout or a CD180-transgenic mouse model. Considering that CD180 can affect the differentiation and activation of a variety of immune cells and may play opposite roles in different types of cells, the treatment effect of targeting CD180 may be unpredictable. More in-depth studies are needed to confirm that whether CD180 can be a potential therapeutic target in the treatment of endotoxic shock.

In conclusion, the work presented here demonstrates that ligation of CD180 contributes to the pathogenesis of LPS-induced endotoxic shock by inhibiting the accumulation and immunosuppressive activity of MDSCs. Taken together, these findings demonstrate a novel role of CD180 in regulating inflammatory activation and implicate CD180 as a potential therapeutic target for control of inflammatory diseases.

Competing financial interests

All the authors declare they have no competing interests.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2018.12.013.

References

[1] R. Medzhitov, Origin and physiological roles of inflammation, Nature 454 (7203) (2008) 428–435.
[2] D.E. Rothschild, D.K. McDaniel, V.M. Ringel-Scaia, I.C. Allen, Modulating inflammation through the negative regulation of NF-kappaB signaling, J. Leukoc. Biol. (Feb 1 2018).
[3] M.G. Netea, F. Balkwill, M. Chonchol, F. Cominelli, M.Y. Donath, E.J. Giamarellos-Bourboulis, et al., A guiding map for inflammation, Nat. Immunol. 18 (8) (2017) 826–831.
[4] W. Huang, C.K. Glass, Nuclear receptors and inflammation control: molecular mechanisms and pathophysiological relevance, Arterioscler. Thromb. Vasc. Biol. 30 (8) (2010) 1542–1549.
[5] G.J. Koelwyn, E.M. Corr, E. Erbay, K.J. Moore, Regulation of macrophage immunometabolism in atherosclerosis, Nat. Immunol. 19 (6) (2018) 526–537.
[6] A. Shapouri-Moghaddam, S. Mohammadian, H. Vazini, M. Taghadosi, S.A. Esmaeili, F. Mardani, et al., Macrophage plasticity, polarization, and function in health and disease, J. Cell. Physiol. 233 (9) (2018) 6425–6440.
[7] W.K.E. Ip, N. Hoshi, D.S. Shouval, S. Snapper, R. Medzhitov, Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages, Science 356 (6337) (2017) 513–519.
[8] C.Z. Han, I.J. Juncadella, J.M. Kinchen, M.W. Buckley, A.L. Klibanov, K. Dryden, et al., Macrophages redirect phagocytosis by non-professional phagocytes and influence inflammation, Nature 539 (7630) (2016) 570–574.
[9] L.D. Orozco, B.J. Bennett, C.R. Farber, A. Ghazalpour, C. Pan, N. Che, et al., Unraveling inflammatory responses using systems genetics and gene-environment interactions in macrophages, Cell 151 (3) (2012) 658–670.
[10] V. Kain, S.D. Prabhu, G.V. Halade, Inflammation revisited: inflammation versus resolution of inflammation following myocardial infarction, Basic Res. Cardiol. 109 (6) (2014) 444.
[11] K. Ben-Meir, N. Twaik, M. Baniyash, Plasticity and biological diversity of myeloid derived suppressor cells, Curr. Opin. Immunol. 51 (2018) 154–161.
[12] F. Veglia, M. Perego, D. Gabrilovich, Myeloid-derived suppressor cells coming of age, Nat. Immunol. 19 (2) (2018) 108–119.
[13] G. Dong, C. Si, Q. Zhang, F. Yan, C. Li, H. Zhang, et al., Autophagy regulates accumulation and functional activity of granulocytic myeloid-derived suppressor cells via STAT3 signaling in endotoxin shock, Biochim. Biophys. Acta 1863 (11) (2017) 2796–2807.
[14] Y.M. He, X. Li, M. Perego, Y. Nefedova, A.V. Kossenkov, E.A. Jensen, et al., Transitory presence of myeloid-derived suppressor cells in neonates is critical for control of inflammation, Nat. Med. 24 (2) (2018) 224–231.
[15] C. Onyilagha, S. Kuriakose, N. Ikeogu, P. Jia, J. Uzonna, Myeloid-derived suppressor cells contribute to susceptibility to *Trypanosoma congolense* infection by suppressing CD4(+) T cell proliferation and IFN-gamma production, J. Immunol. 201 (2) (Jun 13 2018) 507–515.
[16] H. Namkoong, M. Ishii, H. Fujii, K. Yagi, T. Asami, T. Asakura, et al., Clarithromycin expands CD11b+Gr-1+ cells via the STAT3/Bv8 axis to ameliorate lethal endotoxic shock and post-influenza bacterial pneumonia, PLoS Pathog. 14 (4) (2018) e1006955.
[17] Y. Gao, T. Wang, Y. Li, Y. Zhang, R. Yang, Lnc-chop promotes immunosuppressive function of myeloid-derived suppressor cells in tumor and inflammatory environments, J. Immunol. 200 (8) (2018) 2603–2614.
[18] T.E. Schultz, A. Blumenthal, The RP105/MD-1 complex: molecular signaling mechanisms and pathophysiological implications, J. Leukoc. Biol. 101 (1) (2017) 183–192.
[19] A. Blumenthal, T. Kobayashi, L.M. Pierini, N. Banaei, J.D. Ernst, K. Miyake, et al., RP105 facilitates macrophage activation by *Mycobacterium tuberculosis* lipoproteins, Cell Host Microbe 5 (1) (2009) 35–46.
[20] H. Ogata, I. Su, K. Miyake, Y. Nagai, S. Akashi, I. Mecklenbrauker, et al., The toll-like receptor protein RP105 regulates lipopolysaccharide signaling in B cells, J. Exp.

Med. 192 (1) (2000) 23–29.
[21] K. Miyake, Y. Yamashita, M. Ogata, T. Sudo, M. Kimoto, RP105, a novel B cell surface molecule implicated in B cell activation, is a member of the leucine-rich repeat protein family, J. Immunol. 154 (7) (1995) 3333–3340.
[22] S. Divanovic, A. Trompette, S.F. Atabani, R. Madan, D.T. Golenbock, A. Visintin, et al., Negative regulation of Toll-like receptor 4 signaling by the Toll-like receptor homolog RP105, Nat. Immunol. 6 (6) (2005) 571–578.
[23] B. Liu, N. Zhang, Z. Liu, Y. Fu, S. Feng, S. Wang, et al., RP105 involved in activation of mouse macrophages via TLR2 and TLR4 signaling, Mol. Cell. Biochem. 378 (1–2) (2013) 183–193.
[24] J. Xin, Z. Zhang, X. Su, L. Wang, Y. Zhang, R. Yang, Epigenetic component p66a modulates myeloid-derived suppressor cells by modifying STAT3, J. Immunol. 198 (7) (2017) 2712–2720.
[25] M. You, G. Dong, F. Li, F. Ma, J. Ren, Y. Xu, et al., Ligation of CD180 inhibits IFN-alpha signaling in a Lyn-PI3K-BTK-dependent manner in B cells, Cell. Mol. Immunol. 14 (2) (2017) 192–202.
[26] M. Taki, K. Abiko, T. Baba, J. Hamanishi, K. Yamaguchi, R. Murakami, et al., Snail promotes ovarian cancer progression by recruiting myeloid-derived suppressor cells via CXCR2 ligand upregulation, Nat. Commun. 9 (1) (2018) 1685.
[27] E. Tcyganov, J. Mastio, E. Chen, D.I. Gabrilovich, Plasticity of myeloid-derived suppressor cells in cancer, Curr. Opin. Immunol. 51 (2018) 76–82.
[28] L. Li, L. Wang, J. Li, Z. Fan, L. Yang, Z. Zhang, et al., Metformin-induced reduction of CD39 and CD73 blocks myeloid-derived suppressor cell activity in patients with ovarian cancer, Cancer Res. 78 (7) (2018) 1779–1791.
[29] H.J. Jeong, H.J. Lee, J.H. Ko, B.J. Cho, S.Y. Park, J.W. Park, et al., Myeloid-derived suppressor cells mediate inflammation resolution in humans and mice with autoimmune uveoretinitis, J. Immunol. 200 (4) (2018) 1306–1315.
[30] C.R. Lee, Y. Kwak, T. Yang, J.H. Han, S.H. Park, M.B. Ye, et al., Myeloid-derived suppressor cells are controlled by regulatory T cells via TGF-beta during murine colitis, Cell Rep. 17 (12) (2016) 3219–3232.
[31] C. Fu, L. Jiang, X. Xu, F. Zhu, S. Zhang, X. Wu, et al., STAT4 knockout protects LPS-induced lung injury by increasing of MDSC and promoting of macrophage differentiation, Respir. Physiol. Neurobiol. 223 (2016) 16–22.
[32] J. Dai, A. Kumbhare, D.A. Williams, D. Youssef, Z.Q. Yao, C.E. McCall, et al., Nfia deletion in myeloid cells blocks expansion of myeloid-derived suppressor cells during sepsis, Innate Immun. 24 (1) (2018) 54–65.
[33] S. Chang, Y.H. Kim, Y.J. Kim, Y.W. Kim, S. Moon, Y.Y. Lee, et al., Taurodeoxycholate increases the number of myeloid-derived suppressor cells that ameliorate sepsis in mice, Front. Immunol. 9 (2018) 1984.
[34] C. Onyilagha, S. Kuriakose, N. Ikeogu, P. Jia, J. Uzonna, Myeloid-derived suppressor cells contribute to susceptibility to *Trypanosoma congolense* infection by suppressing CD4+ T cell proliferation and IFN-γ production, J. Immunol. 201 (2) (2018) 507–515.
[35] Q. Yang, H. Qiu, H. Xie, Y. Qi, H. Cha, J. Qu, et al., A *Schistosoma japonicum* infection promotes the expansion of myeloid-derived suppressor cells by activating the JAK/STAT3 pathway, J. Immunol. 198 (12) (2017) 4716–4727.
[36] Y.Y. Luan, N. Dong, M. Xie, X.Z. Xiao, Y.M. Yao, The significance and regulatory mechanisms of innate immune cells in the development of sepsis, J. Interf. Cytokine Res. 34 (1) (2014) 2–15.