International Immunopharmacology 75 (2019) 105792

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Platelet-derived growth factor B attenuates lethal sepsis through inhibition of inflammatory responses

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ARTICLE INFO

Keywords:
Platelet-derived growth factor B
Sepsis
Inflammatory cytokines
Chemokines

ABSTRACT

Sepsis is a systemic inflammatory response during infection and remains a major clinical problem with high morbidity and mortality. Platelet-derived growth factor B (PDGF-B) is a member belongs to PDGF family and has been recently reported higher expressed in survivors of severe sepsis patients. However, the exact role and underlying mechanisms of PDGF-BB in sepsis remains unclear. In this study, we found that PDGF-BB levels were significantly elevated in patients with sepsis, and higher PDGF-BB levels were negatively correlated with the levels of proinflammatory cytokines (TNF-α, IL-6, IL-1β, IL-8), and chemokines (CXCL-1 and CCL2). PDGF-BB was also found increased in experimental sepsis in mice. Blockade of PDGF-BB using Tyrphostin AG 1296 aggravated, whereas recombinant PDGF-BB treatment improved survival and tissues injury in both two murine models of CLP-induced sepsis and LPS- induced endotoxemia. PDGF-BB blockade increased, whereas PDGF-BB administration decreased the inflammatory responses, as reflected by proinflammatory cytokines (TNF-α, IL-6, IL-1β, IL-8), and chemokines (CXCL-1 and CCL2). PDGF-BB also showed inhibitory effect on immune cell activation and cytokines production *in vivo* and *in vitro*. Therefore, our findings suggest that PDGF-BB plays a protective role in sepsis by decreasing the production of pro-inflammatory cytokines and chemokines. PDGF-BB thus may be a potential therapeutic strategy for treating sepsis.

1. Introduction

Sepsis is a life-threatening complication that caused by an overwhelming immune response to infection [1]. This inflammation then triggers a cascade of changes that cause tissue damage, organ failure, and ultimately, death. It is now known that pro-inflammatory cytokines and chemokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1β, IL-8, CXCL-1 and CCL2 are considered important to the pathogenesis of sepsis [2]. Currently, experimental or clinical approaches interventions using LPS sequestrants, HMGB-1 inhibitors, TLR4 antagonists or C5aR antagonists showed potential advantages in treatment in sepsis. However, the majority of them did not provide better therapeutic outcomes in clinical settings [3]. Therefore, understanding the pathogenic of sepsis and exploring new mediators as interventions targets have become a significant focus.

Platelet-derived growth factor B (PDGF-B) is a member belongs to PDGF family, which has four isoforms, PDGF-A, B, C and D [4,5]. PDGFs must form homo- or heterodimers to exhibit activity, including PDGF-AA, BB, AB, CC, or DD dimers [4]. PDGF can interact with either one of two PDGF receptors, namely the PDGF receptor (PDGFR) α and β [5]. PDGF-BB binds to PDFGFRβ and results in receptor dimerization, and activation of tyrosine kinase activity, leading to activation of protein kinase C, and intracellular calcium signaling pathways [6] PDGF family members play a crucial role in wound healing [7], embryologic development [8], tumor angio- and lymphangiogenesis [9,10], as well as cell proliferation, survival and migration [5,11]. Dysfunction of PDGF signaling has been observed in a wide array of pathological conditions, such as cancer, fibrosis, neurological conditions and atherosclerosis [4]. However, the role of PDGFs in the pathogenic of sepsis remains largely unknown. Although the previous report has demonstrated that PDGF-BB was higher in survivors of severe sepsis patients, as well as in patients with sepsis-associated encephalopathy (SAE) [12,13], the exact role and mechanism of PDGF-BB in regulating sepsis remains unclear.

In the present study, we first determined the production of PDGF-BB in clinical and experimental sepsis models. In addition, we evaluated the function of PDGF-BB and its associated mechanism in two animal sepsis models, CLP-induced polymicrobial sepsis and lipopolysaccharide (LPS)-induced endotoxemia. Our findings suggest that PDGF-BB plays a protective role in sepsis by decreasing the production of pro-inflammatory cytokines and chemokines. PDGF-BB thus may be a potential therapeutic strategy for treating sepsis.

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https://doi.org/10.1016/j.intimp.2019.105792
Received 22 November 2018; Received in revised form 25 July 2019; Accepted 26 July 2019
Available online 03 August 2019
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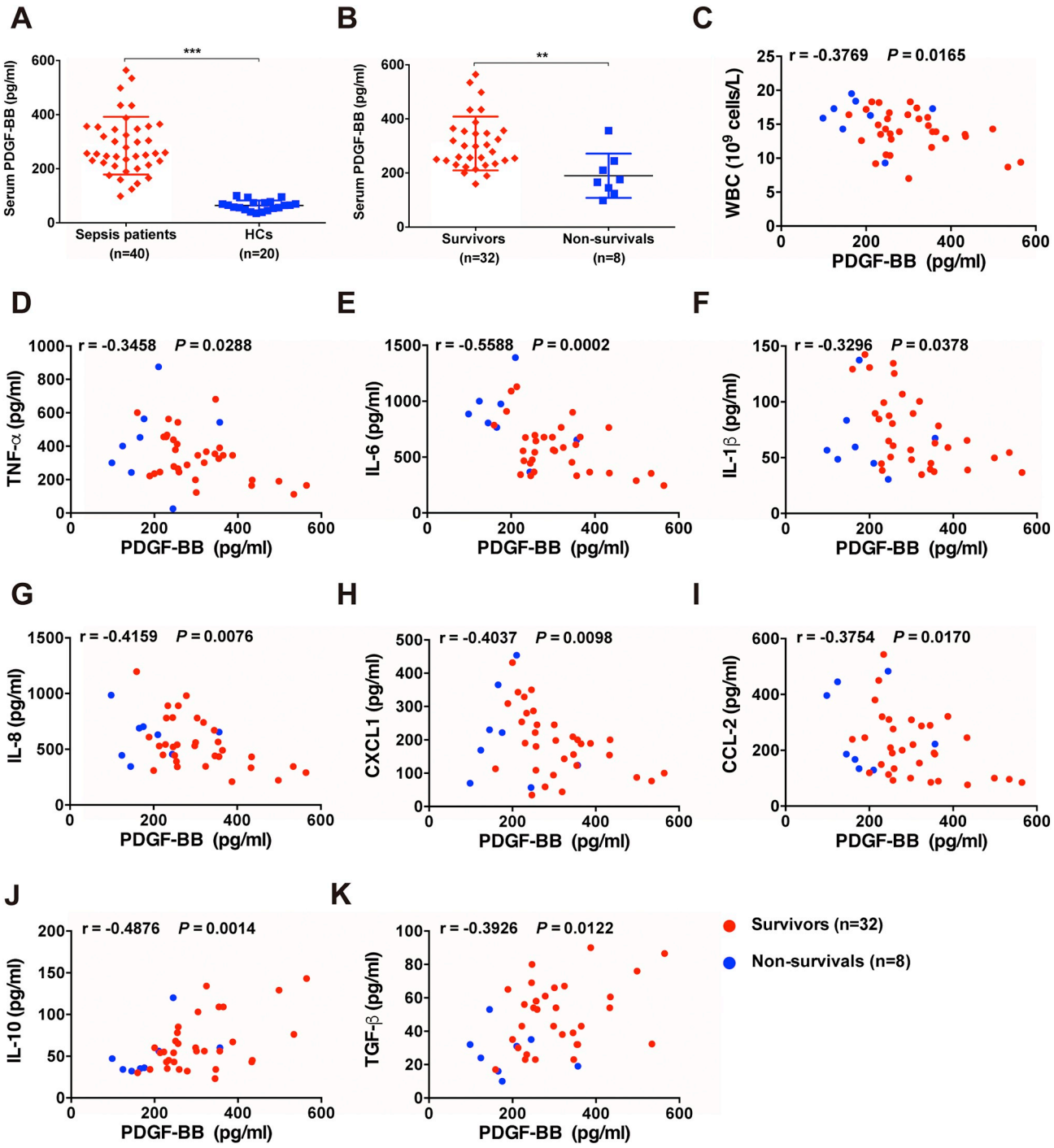

Fig. 1. PDGF-BB was elevated in patients with sepsis. (A and B) Serum PDGF-BB levels were quantified in 40 patients with sepsis and 20 healthy controls (HCs). Sepsis patients were divided into survivors (n = 32) and nonsurvivors (n = 8). (C-K) Correlation between PDGF-BB levels and Blood leukocyte counts, TNF-α, IL-6, IL-1β, IL-8, CXCL-1 CCL2, IL-10 and TGF-β were determined by nonparametric spearman correlation test. Red dots represent survivors (n = 32), blue dots represent nonsurvivors (n = 8). Data shown are mean ± SD. Dots represent individual subjects. $^{**}P < 0.01$, $^{***}P < 0.001$, by the Mann-Whitney *U* test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

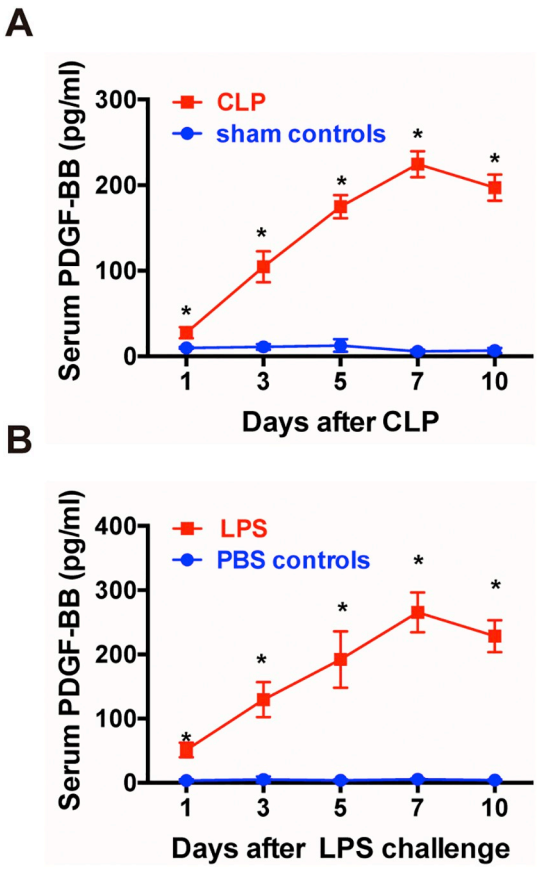

Fig. 2. PDGF-BB was increased in experimental sepsis in mice. PDGF-BB levels in serum of mice after CLP-induced sepsis (A) as well as LPS-induced endotoxemia (B) were measured (n = 3 per time points). Data shown are mean ± SD. *P < 0.01, by the multiple test using Holm-Sidak method, compared with sham controls (A), and PBS controls (B).

2. Materials and methods

2.1. Patients

From September 2016 to February 2017, 40 patients with sepsis from Department of Intensive Care Unit, The Affiliated Huai'an NO.1 People's Hospital of Nanjing Medical University were recruited in this study. All patients meet the clinical criteria of the third International Consensus Definitions for Sepsis and Septic Shock. Clinical data, including Acute Physiology and Chronic Health Evaluation II (APACHE II) score, Sequential Organ Failure Assessment (SOFA) score, the counts of white blood cell count, CRP level, Creatinine level, microbial culture results, or mortality during the 28-day study period, were recorded. Blood samples were obtained from patients within 24 h of the diagnosis in ICU. Blood samples from 20 healthy controls (HCs) with no medical problems were obtained from the blood bank of The Affiliated Huai'an NO.1 People's Hospital of Nanjing Medical University. Details of demographic and clinical characteristics of the patients and healthy controls (HCs) are provided in Table S1. The study was approved by the Clinical Research Ethics Committee of The Affiliated Huai'an NO.1 People's Hospital of Nanjing Medical University (KY-P-2015-007-01), and informed consent was obtained from each participant according to the committee's regulations.

2.2. Mice and sepsis models

Male and female C57BL/6 mice (6–8 week-old) were purchased from Charles River Laboratories, Beijing, China) and housed under specific pathogen-free conditions. All animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86–23 revised 1985). Animal study protocols were approved by the Research Ethics Committee of The Affiliated Huai'an NO.1 People's Hospital of Nanjing Medical University. CLP model was performed as previously described [14]. Briefly, mice were anesthetized with isoflurane; the cecum was exposed, ligated, and punctured twice with a 21-gauge needle. The cecum was returned to the peritoneal cavity, and incisions were closed. Sham-operated animals underwent identical laparotomy, and the cecum was exposed but not ligated or punctured and was then replaced in the peritoneal cavity. Mice received saline (5 mL per 100 g body weight) subcutaneously for resuscitation. For the LPS-induced endotoxemia model, mice were injected intraperitoneally with LPS from Salmonella abortus equi (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 100 μg per mice as described previously [15]. LDH levels were measured to evaluate the degree of tissue damage in LPS-induced endotoxemia (Fig. S1A).

2.3. In vivo blockade of PDGF-BB

To block PDGF-BB during experimental sepsis, tyrphostin AG 1296 (50 mg/kg), or vehicle (10% 1-methyl-2-pyrrolidinone and 90% polyethylene glycol 300) was intravenously injected into the mice each day for 7 days after CLP or LPS challenge.

2.4. In vivo administration of recombinant murine PDGF-BB

PDGF-BB recombinant mouse protein (Cat: PMG0044, Thermo Fisher, USA) was injected each day for 7 days at a dose of 1 μg/injection or 10 μg/injection after CLP or LPS challenge. PBS was delivered as vehicle control.

2.5. Measurement of cytokine and chemokine levels using ELISA

Serum from sepsis patients and HCs were obtained by using a 2-step centrifugation protocol (3000*g* for 10 min and 12,000*g* for 5 min, all at 4 °C). All the serum samples were frozen immediately after collection and stored at −80 °C until analysis. Serum was diluted 2–10 times with assay buffer based on our preliminary test. Serum levels of PDGF-AA (Cat. No: DY221, Assay Range: 15.6–1000 pg/ml, Dilute: 1:10), PDGF-BB (Cat. No: DBB00, Sensitivity: 15 pg/ml, Dilute: 1:10), PDGF-CC (Cat. No: DBB00, Sensitivity: 24.7 pg/ml, Dilute: 1:10) and PDGF-DD (Cat. No: DDD00, Sensitivity: 3.51 pg/ml, Dilute: 1:10) were quantified by using a commercial ELISA kits purchased from R&D Systems (MN, USA) according to the manufacturer's protocol. Serum levels of TNF-α (Cat. No: 70-EK1822, Sensitivity: 0.42 pg/ml, Dilute: 1:10; Cat. No: 70-EK282HS-96, Sensitivity: 0.32 pg/ml, Dilute: 1:2), IL-6 (Cat. No: 70-

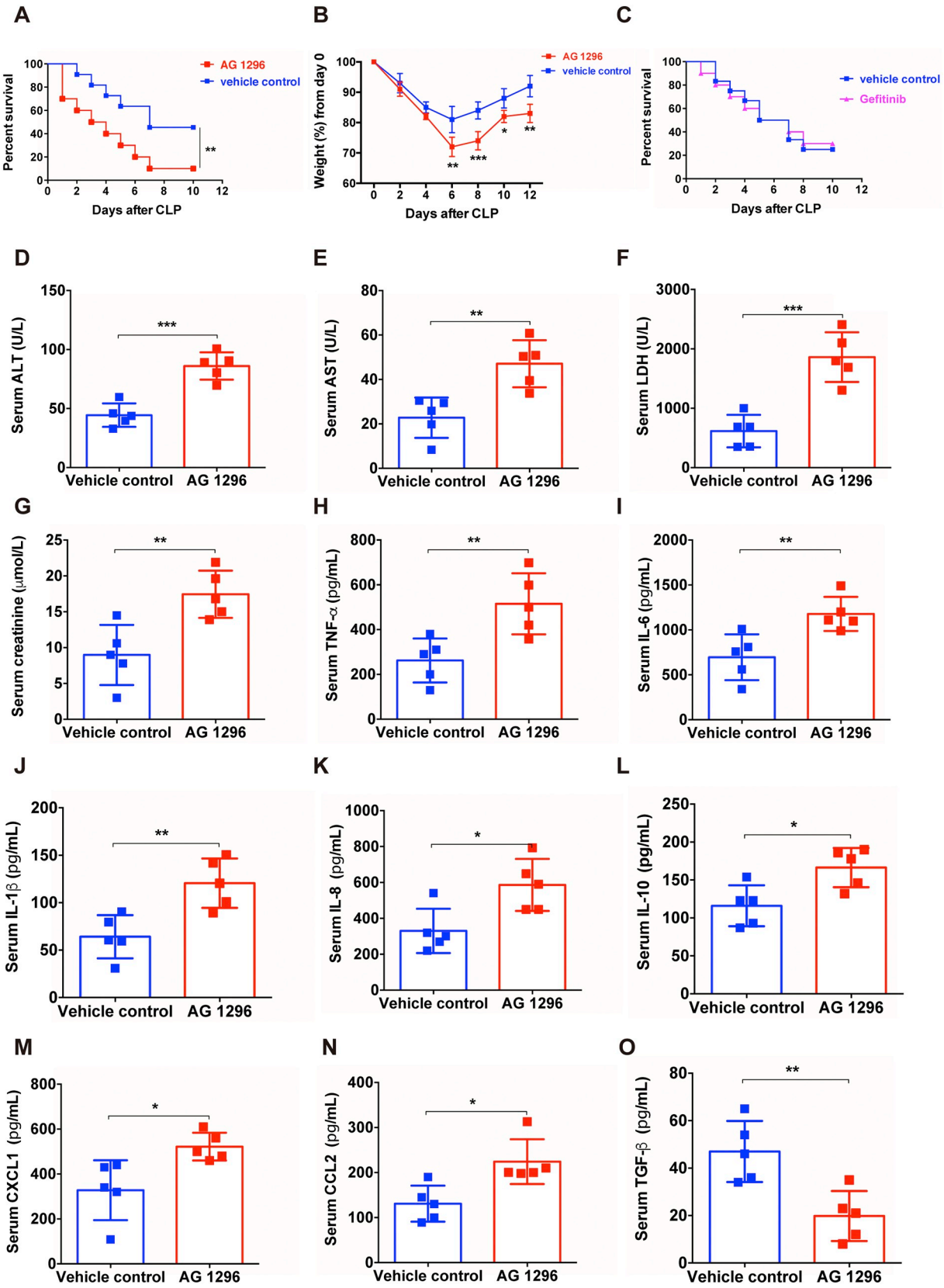

Fig. 3. PDGF-BB blockade aggravated sepsis through increasing pro-inflammatory cytokines and chemokines in CLP mice. (A) Survival and (B) overall weight loss between CLP mice treated with tyrphostin AG 1296 and Vehicle control (n = 10 per group). (C) Survival between CLP mice treated with Gefitinib and Vehicle control (n = 10 per group). (D–G) Levels of alanine aminotransferase (ALT), aspartate amino-transferase (AST), lactate dehydrogenase (LDH), creatinine, and (H–N) TNF-α, IL-6, IL-1β, IL-8, IL-10, CXCL-1, CCL2 and TGF-β at 48 h after CLP in septic mice treated with or without AG 1296 (n = 5 per group). Data shown are mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with vehicle control, by the student's *t*-test. Survival between two groups was performed by Kaplan-Meier analysis followed by log-rank tests. $^{**}P < 0.01$, when compared with vehicle control.

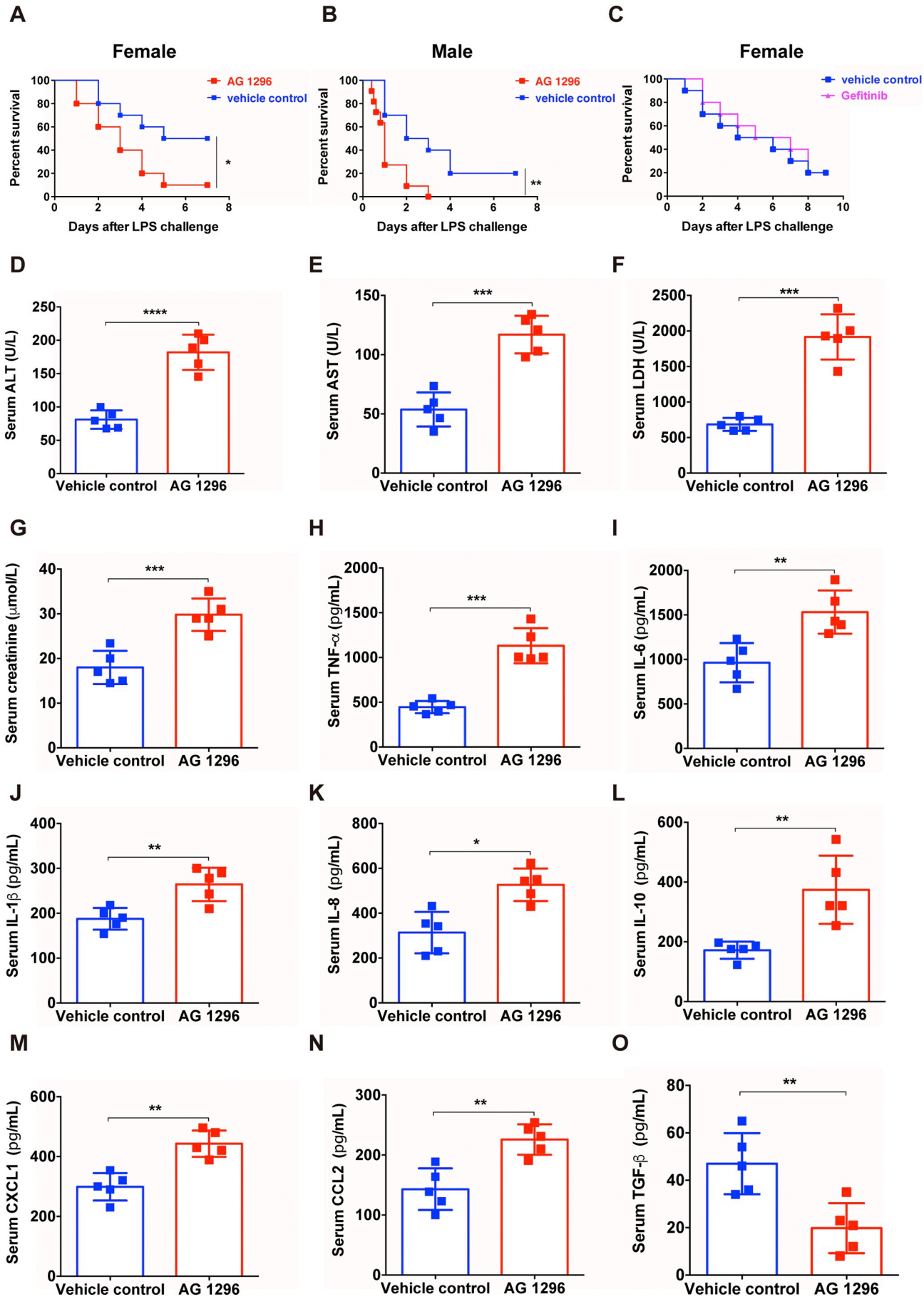

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Fig. 4. PDGF-BB blockade aggravated sepsis through increasing pro-inflammatory cytokines and chemokines in LPS-induced endotoxemia mice. Survival and between (A) female or (B) male mice treated with tyrphostin AG 1296 and vehicle control (n = 10 per group) after LPS challenge. (C) Survival between female mice treated with Gefitinib and vehicle control (n = 10 per group) after LPS challenge. (D–G) Levels of alanine aminotransferase (ALT), aspartate amino-transferase (AST), lactate dehydrogenase (LDH), creatinine, and (H–N) TNF-α, IL-6, IL-1β, IL-8, IL-10, CXCL-1, CCL2 and TGF-β at 48 h after LPS challenge in female mice treated with or without AG 1296 (n = 5 per group). Data shown are mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with vehicle control, by the student's *t*-test. Survival between two groups was performed by Kaplan–Meier analysis followed by log-rank tests. $^{*}P < 0.05$, $^{**}P < 0.01$, when compared with vehicle control.

EK1062, Sensitivity: 0.37 pg/ml, Dilute: 1:10; Cat. No: 70-EK206HS-96, Sensitivity: 0.43 pg/ml, Dilute: 1:2), IL-1β (Cat. No: 70-EK101B2, Sensitivity: 0.15 pg/ml, Dilute: 1:10; Cat. No: 70-EK201B2/2, Sensitivity: 1.45 pg/ml, Dilute: 1:2), IL-8 (Cat. No: 70-EK108HS-96, Sensitivity: 0.28 pg/ml, Dilute: 1:10; Cat. No: 70-EK2142/2-48, Sensitivity: 5.23 pg/ml, Dilute: 1:2), IL-10 (Cat. No: 70-EK1101, Sensitivity: 0.59 pg/ml, Dilute: 1:5; Cat. No: 70-EK210/3-96, Sensitivity: 4.8 pg/ml, Dilute: 1:2), CXCL-1 (Cat. No: 85-BMS2122, Sensitivity: 7.6 pg/ml, Dilute: 1:5; Cat. No: 70-EK2962/2, Sensitivity: 0.47 pg/ml, Dilute: 1:2) and CCL2 (Cat. No: 70-EK187-96, Sensitivity: 2.18 pg/ml, Dilute: 1:5; Cat. No: 70-EK287HS-96, Sensitivity: 2.08 pg/ml, Dilute: 1:2) from human and mice serum were measured using ELISA kits from MultiSciences (MultiSciences, Hangzhou, China) following manufacturer's protocol. TGF-β levels were measured using TGF beta-1 Human ELISA Kit (Cat. No: BMS249-4, Sensitivity: 8.6 pg/ml, Dilute: 1:5, Invitrogen, USA) or TGF beta-1 Mouse ELISA Kit (Cat. No: BMS608-4, Sensitivity: 7.8 pg/ml, Dilute: 1:2, Invitrogen, USA). Serum levels of mouse PDGF-AA (Cat. No: ELM-PDGFAA-1, Sensitivity: 3.2 pg/ml, Dilute: 1:10, Raybiotech, GA, USA), mouse PDGF-BB (Cat. No: MBB00, Sensitivity: 19.3 pg/ml, Dilute: 1:10, R&D Systems, MN, USA), mouse PDGF-CC (Cat. No: LS-F26133-1, Sensitivity: 0.63 pg/ml, Dilute: 1:10, LifeSpan Bioscience, WA, USA) were measured using commercial ELISA kits according to the manufacturer's protocol. For examining the effect of PDGF-BB on the IL-1β and IL-6 expression by DCs. BM-derived DCs were generated and expanded from B6 mice with GM-CSF (10 ng/ml) and IL-4 (10 ng/ml) for 7 days. The cells were harvested and stimulated with or without LPS (1 μg/ml) in the presence or absence of rmPDGF-BB (100 ng/ml) for 6 h. The supernatants were collected for ELISA. Serum levels of ALT, AST, LDH, and creatinine were measured using the Hitachi Model 7170 analyzer (Hitachi Ltd., Tokyo, Japan).

2.6. Flow cytometry

Splenocytes were obtained from mice on day 2 after LPS challenge. The cells were washed and 1×10^6 cells were surface stained with FITC anti-mouse CD3 (Cat: 100204), PE anti-mouse NK-1.1 (Cat: 108708), PE/Cy7 anti-mouse CD11c (Cat: 117318), APC anti-mouse F4/80 (Cat: 123116), Brilliant Violet 421 anti-mouse CD80 (Cat: 104726), APC/Cy7 anti-mouse CD86 (Cat: 105030), APC anti-mouse CD69 (Cat: 104514) for 30 min on ice. For intracellular cytokine staining, cells were stimulated with BD Leukocyte Activation Cocktail, with GolgiPlug (BD Biosciences, CA, USA) for 4 h, the cells were then fixed and permeabilized with Fixation/Permeabilization Buffer (BD Biosciences, San Jose, CA, USA) and stained with Brilliant Violet 421 anti-mouse IFN-γ (Cat: 505830), APC/Cy7 anti-mouse TNF-α (Cat: 506344), and APC anti-mouse IL-10 (Cat: 505010) for 30 min on ice. All antibodies were purchased from BioLegend (San Diego, CA, USA). Flow cytometry was performed using Gallios (Beckman Coulter, FL, USA), and analyzed using Flowjo software (FlowJo, Ashland, OR, USA).

2.7. Statistical analysis

Statistical analysis was performed using SPSS version 21.0 statistical software (SPSS, Chicago, IL, USA). The results are expressed as mean ± standard deviation (SD). Comparisons between groups were performed using the Mann-Whitney *U* test or student's *t*-test. Spearman correlation test was used to evaluate the associations between serum PDGF-BB levels and different variables. Survival was performed by Kaplan-Meier analyses followed by log-rank tests. P value < 0.05 was considered statistically significant.

3. Results

3.1. PDGF-BB was elevated in patients with sepsis

The previous report has demonstrated that PDGF-BB was elevated in survivors of severe sepsis patients [12]. However, the expression and clinical significance of PDGF-BB in sepsis remain unknown. We therefore first measured the serum PDGF-BB levels in 40 patients with sepsis and 20 healthy controls (HCs). As shown in Fig. 1A, patients with sepsis had significantly elevated serum PDGF-BB levels compared with HCs (285.4 ± 106.7 *vs.* 64.35 ± 18.52 pg/ml; $P < 0.001$). In addition, septic survivors showed higher PDGF-BB levels than those in septic non-survivals (309.2 ± 99.43 *vs.* 190.2 ± 81.72 pg/ml; $P < 0.001$; Fig. 1B), which was in accordance with a previous study [12]. We then evaluated the correlation between PDGF-BB levels with clinical characteristics of sepsis patients. We found that serum PDGF-BB levels were only negatively associated with WBC levels ($r = -0.3984$, $P = 0.01$; Fig. 1C). However, no obvious correlations were observed between serum PDGF-BB levels and other factors (data not shown). Pro-inflammatory cytokines TNF-α, IL-6, IL-1β, IL-8, anti-inflammatory cytokines IL-10 and TGF-β, and chemokines CXCL-1 and CCL2 are known to be key mediators in the pathogenesis of sepsis [2]. We found that serum PDGF-BB levels were negatively correlated with the levels of TNF-α, IL-6, IL-1β, IL-8 (Fig. 1D–G), CXCL-1 and CCL2 (Fig. 1H and I), whereas positively correlated with the levels of IL-10 and TGF-β (Fig. 1J and K), suggesting that PDGF-BB may play a protective role in sepsis.

3.2. PDGF-BB was increased in experimental sepsis in mice

To support our observation in clinical, we measured PDGF-BB expression in two sepsis mouse models, CLP-induced polymicrobial sepsis and LPS-induced endotoxemia. In accordance with what we found in sepsis patients, sepsis mice produced significantly higher levels of PDGF-BB, which increased from day 1 to day 7, and peak on day 7 (Fig. 2A and B), suggesting that PDGF-BB production is enhanced in experimental sepsis in mice.

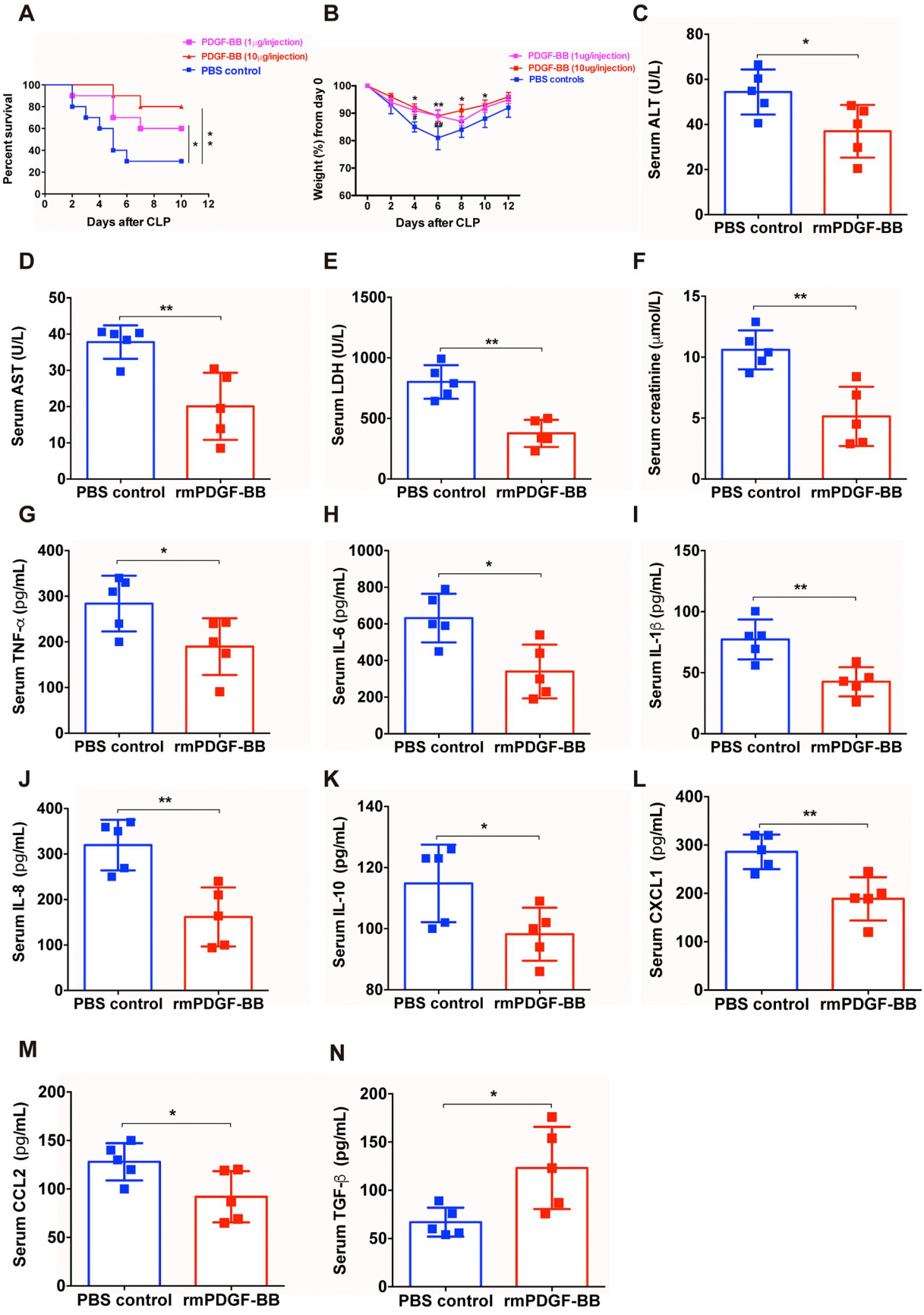

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Fig. 5. Administration of PDGF-BB protected against sepsis through inhibiting pro-inflammatory cytokines and chemokines in CLP mice. (A) Survival and (B) overall weight loss between CLP mice treated with rmPDGF-BB at a dose of 1 μg/injection or 10 μg/injection (n = 10 per group), PBS served as control. (C–F) Levels of alanine aminotransferase (ALT), aspartate amino- transferase (AST), lactate dehydrogenase (LDH), creatinine, and (G–N) TNF-α, IL-6, IL-1β, IL-8, IL-10, CXCL-1, CCL2 and TGF-β at 48 h after CLP in septic female mice treated with or without rmPDGF-BB (n = 5 per group). Data shown are mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with vehicle control, by the student's *t*-test. Survival between two groups was performed by Kaplan–Meier analysis followed by log-rank tests. $^{*}P < 0.05$, $^{**}P < 0.01$, when compared with PBS control.

3.3. PDGF-BB blockade aggravated sepsis through increasing pro-inflammatory cytokines and chemokines in mice

To further explore the role of PDGF-BB in sepsis, we induced sepsis in C57BL/6 female mice by CLP, and treated with a specific PDGFR inhibitor Tyrphostin AG 1296 to block PDGF-BB signaling *in vivo* [16–18] after surgery (Fig. S1B). As shown in Fig. 3A, the survival rate in mice treated with AG 1296 was significantly lower compared with the vehicle-treated control mice. Overall weight loss was significantly increased in AG 1296-treated groups compared to vehicle-treated control groups (Fig. 3B). However, when using Gefitinib, an EGFR inhibitor that also inhibits receptor tyrosine kinase signaling, could not aggravate sepsis (Fig. 3C), suggesting that specifically blocking PDGFR signaling contributes to the development of sepsis.

We then evaluated the inflammatory cytokines expression after AG-1296 treatment. Serum levels of ALT, AST, LDH, and creatinine, which are the markers for tissue injury, were significantly increased in mice treated with AG 1296 after CLP (Fig. 3D–G), supporting that PDGF-BB blockade aggravated sepsis in mice. In addition, we found that treatment with AG 1296 resulting in a significant increase in levels of TNF-α, IL-6, IL-1β, IL-8, IL-10, and CXCL-1 and CCL2 (Fig. 3H–N), but markedly decreased TGF-β levels in CLP mice (Fig. 3O). The promoting effect of PDGF-BB blockade on sepsis was further confirmed in the LPS-induced endotoxemia mouse model (Fig. 4). The survival rate of the mice in the AG 1296 group was greatly lower compared to the vehicle group after LPS challenge (Fig. 4A). Since male mice are more sensitive to LPS than their female, we found that following LPS administration; males developed more severe endotoxemia after blockade of PDGF-BB (Fig. 4B). Treatment of Gefitinib also did not affect the development of sepsis in female mice (Fig. 4C). Additionally, serum levels of sepsis biomarkers and cytokines showed similar pattern in LPS-induced endotoxemia mouse model (Fig. 4D–O). Taken together, our results indicate that PDGF-BB blockade aggravates sepsis through increasing pro-inflammatory cytokines and chemokines.

3.4. Administration of PDGF-BB protected against sepsis through inhibiting pro-inflammatory cytokines and chemokines in mice

Since PDGF-BB blockade aggravated sepsis in mice, we next examined whether administration of recombinant mouse PDGF-BB (rmPDGF-BB) protein could protect mice against sepsis. As shown in Fig. 5A, rmPDGF-BB treatment (1 μg/injection) markedly reduced sepsis-associated mortality rate compared to PBS treated controls in CLP mice. In addition, mice treated with 10 μg rmPDGF-BB exhibited higher survival rates (Fig. 5A), suggesting that the protective effect is positively correlated to the treatment dose of PDGF-BB. Overall weight loss was significantly reduced in PDGF-BB-treated groups compared to PBS treated controls (Fig. 5B). Mice treated with rmPDGF-BB after CLP had significantly lower serum levels of ALT, AST, LDH, and creatinine on day 2 after CLP surgery (Fig. 5C–F). Moreover, the levels of TNF-α, IL-6, IL-1β, IL-8, IL-10, CXCL-1 and CCL2 were significantly down-regulated in septic mice treated with rmPDGF-BB (Fig. 5G–M), whereas the levels of TGF-β levels significantly up-regulated after rmPDGF-BB treatment (Fig. 5N). We also evaluated the inflammatory cytokines levels at day 7 after CLP surgery, our data showed that PDGF-BB still had inhibitory effect on inflammatory cytokines expression at the late stage of sepsis (Fig. S2). We confirmed the similar results of rmPDGF-BB effect in the LPS-induced endotoxemia mouse model (Fig. 6A–N). Taken together, our findings demonstrate that administration of PDGF-BB protected against sepsis through inhibiting pro-inflammatory cytokine and chemokine in mice.

3.5. PDGF-BB inhibited immune cell activation and cytokines production in vivo and in vitro

Previous studies reported that PDGF-BB could inhibit pro-inflammatory cytokine expression by activated T cells and NK cells [19–21]. To clarify the underlining mechanism of the inhibitory effect of PDGF-BB on inflammatory cytokines expression, we evaluated the immune responses during sepsis with PDGF-BB treatment. The results showed that PDGF-BB treatment did not affect the percentages of T cells, NK cells, DCs and macrophages in the spleen of sepsis mice treated with or without of PDGF-BB (Fig. 7A). However, on day 2 after LPS challenge, PDGF-BB significantly inhibited IFN-γ and TNF-α expression by T cells (Fig. 7B–C) and NK cells (Fig. 7E–F), but not IL-10 expression by T cells (Fig. 7D). In addition, the activation of T cells ($CD3^{+}CD69^{+}$) (Fig. 7G), as well as the activation of DCs and macrophages in terms of CD80 and CD86 levels was significantly decreased after PDGF-BB treatment, compared with PBS control groups (Fig. 7H–K). These data suggest that PDGF-BB may inhibit pro-inflammatory cytokines expression by inhibiting immune cell responses, including T cells, NK cells, DCs and macrophages *in vivo*. Additionally, we analyzed the effect of PDGF-BB on the pro-inflammatory cytokine expression by BMDCs with treatment of PDGF-BB *in vitro*. We found that addition of PDGF-BB could significantly suppress the proinflammatory cytokines IL-1β and IL-6 expression in the presence of LPS stimulation (Fig. 7L and M). Taken together, our results indicate that PDGF-BB suppresses immune cell activation and cytokines production *in vivo* and *in vitro*.

4. Discussion

Sepsis is one of the most common causes of death in hospitalized patients, with mortality rates between 30% and 70% [1]. Inflammation plays a significant role in the development pathogenesis of severe sepsis, and most current therapeutic strategies have targeted pro-inflammatory cytokines using monoclonal antibodies [2]. PDGFs have been served as a growth factor for more than three decades. Studies of PDGFs in human and animals have revealed roles for PDGFs signaling in health and diseases, including wound healing [7], embryologic development [8], tumor angio- and lymphangiogenesis [9,10], as well as cell proliferation, survival and migration [5,11]. However, there is very

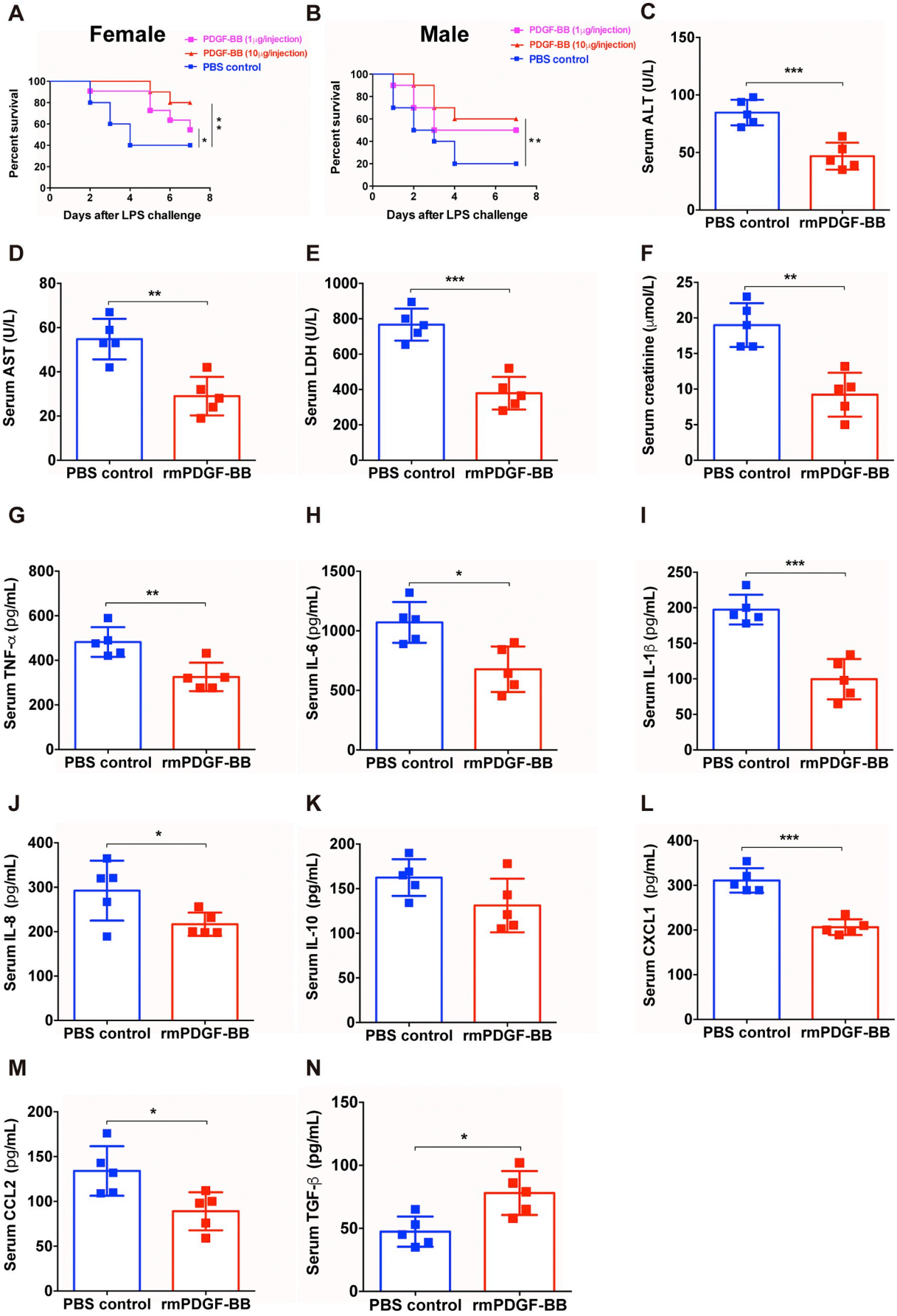

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Fig. 6. Administration of PDGF-BB protected against sepsis through inhibiting pro-inflammatory cytokines and chemokines in LPS-induced endotoxemia mice. Survival and between (A) female or (B) male mice treated with rmPDGF-BB at a dose of 1 μg/injection or 10 μg/injection (n = 10 per group) after LPS challenge, PBS served as control. (C–F) Levels of alanine aminotransferase (ALT), aspartate amino-transferase (AST), lactate dehydrogenase (LDH), creatinine, and (G-N) TNF-α, IL-6, IL-1β, IL-8, IL-10, CXCL-1, CCL2 and TGF-β at 48 h after LPS challenge in female mice treated with or without rmPDGF-BB (n = 5 per group). Data shown are mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with vehicle control, by the student's *t*-test. Survival between two groups was performed by Kaplan-Meier analysis followed by log-rank tests. $^{*}P < 0.05$, $^{**}P < 0.01$, when compared with PBS control.

limited evidence on the expression or function of PDGFs in sepsis.

In the present report, we identified that PDGF-BB to be significantly involved in the pathogenesis of clinical and experimental sepsis. Septic patients had significantly increased serum PDGF-BB levels, which were negatively correlated with inflammatory cytokines TNF-α, IL-6, IL-1β, IL-8 and chemokines CXCL-1 and CCL2, but were positively correlated with IL-10 and TGF-β. PDGF-BB expression was also increased in experimental sepsis in mice. PDGF-BB blockade aggravated sepsis, whereas administration of rmPDGF-BB protected against sepsis through modulating immune cell activation and pro-inflammatory cytokines and chemokines production. Taken together, our findings suggest that PDGF-BB exerts a protective effect against sepsis, and maybe act as a potential therapeutic strategy for sepsis treatment. In addition to PDGF-BB, we also took a look at the other PDGF dimers, including PDGF-AA, CC and DD in both sepsis patients as well as mice models. We found that PDGF-AA and CC were significantly elevated in sepsis patients (Fig. S3). However, the level of PDFD-DD was not changed in sepsis patients. In sepsis mice, PDGF-AA increased first but then fell rapidly, whereas PDGF-CC remained elevated even day 10. We did not examine PDGF-DD in mouse model due to no available ELISA kit. Although PDGF-AA and BB showed 56% homology, they bind to different receptors. PDGF-AA mainly activates PDGFRα while PDGF-BB activates PDGFRβ. PDGF-AA is mainly synthesized and secreted by epithelial cells. The decreased PDGF-AA levels indicating that there may have some factors that could inhibit PDGF-AA expression during sepsis. PDGF-CC was found over-expressed in macrophages in murine renal fibrosis [22]. Macrophages play essential roles throughout all phases of sepsis and affect both immune homeostasis and inflammatory processes [23]. Therefore, whether PDGF-CC was produced by macrophages needs further investigations. Taken together, our findings indicate that in addition to PDGF-BB, other PDGF dimers maybe also involved in the development of sepsis, further studies therefore are needed to explore their functions in sepsis.

PDGF-BB is predominantly released in α-granules of platelets but can also be expressed by macrophages, fibroblasts and endothelial cells [10]. So far, the major role of PDGF-BB has been connected with organ fibrosis [24], osteogenesis [25], vascular diseases [26], as well as cancer diseases [10,27]. PDGF-BB has been considered as an important oncogene in cancer development by PDGF/PDGFR signaling pathways. However, litter is known about its role in inflammatory disease. Recently, Malgorzata et al. documented high PDGF-BB in the blood of patients with inflammatory bowel disease [28], which is a chronic inflammatory disease. PDGF-BB has been found elevated in survivors of severe sepsis patients, and serve as a good predictor for survival [12]. In addition, Tomasi et al. reported that SAE patients presented higher levels of PDGF-AB/BB when compared to delirium patients [13]. PDGF-BB in sepsis was found to be released by endothelial cells, and could be induced by drotrecogin alfa and activated protein C [12]. Our present work also supported the high PDGF-BB expression in sepsis patients, especially in septic survivors. In addition, previous study and our data both showed that PDGF-BB levels did not correlate with the severity of sepsis, as reflected by APACHE II and SOFA scores. However, we here found that elevated PDGF-BB levels were negatively correlated with the inflammatory cytokines TNF-α, IL-6, IL-1β, IL-8, and chemokines CXCL-1 and CCL2, but positively correlated with IL-10 and TGF-β, suggesting the anti-inflammatory potential of PDGF-BB in sepsis. To support our findings, a previous study demonstrated that PDGF-BB was a potent inhibitor of IL-1β-mediated activation of NF-κB and apoptosis in chondrocytes [29]. PDGF-BB could induce TGF-β expression in vascular smooth muscle cells and was positively correlated with TGF-β levels in systemic lupus erythematosus [30,31].

The present study also shows for the first time that PDGF-BB improved the survival rate of septic mice in two mouse models, and reduced the extent of tissues injury. During sepsis, large amounts of cytokines were releases, which produce pro-inflammatory or anti-inflammatory effects and regulate the body's inflammatory reactions and immune responses [2]. In our study, PDGF-BB intervention resulted in a reduction of the systemic inflammatory response, as shown by a significant decrease of the concentrations of TNF-α, IL-6, IL-1β, IL-8, IL-10, and chemokines CXCL-1 and CCL2, suggesting that PDGF-BB induced protection against experimental sepsis was associated with decreased inflammation response. Although IL-10 is an anti-inflammatory cytokines, our results showed that PDGF-BB did not inhibit IL-10 expression by T cells, suggesting that the anti-inflammatory effect of PDGF-BB maybe through inhibiting the expression of IL-10 by other cells, such as the DCs, macrophages and monocytes in mice [32]. Whereas in human subjects, the positive correlation between PDGF-BB and IL-10 and TGF-β suggesting that PDGF-BB could promote or increase IL-10 and TGF-β levels by DCs or other cells in human, which is not consistent with mice, and this need further investigation.

Since sepsis is associated with immune dysfunctions, and immune cells are the main source of inflammatory cytokines in sepsis [33]. We then evaluated the immune responses during sepsis with PDGF-BB treatment. Our data showed that PDGF-BB significantly inhibited the activation of T cells, DCs, and macrophages, suppressed IFN-γ and TNF-α expression by T cells and NK cells. Findings of this *in vivo* study are consistent with previous *in vitro* work showing that PDGF-BB can significantly inhibit IFN-γ production by activated T cells and NK cells [19–21]. In addition, PDGF-BB treatment resulted in a dramatic decrease in the proliferation of $CD4^{+}$ T from HCs [34]. However, there were also reports showed that PDGF-BB is a potent inducer of proinflammatory cytokines, as evidenced by increased IL-6, IL-8, CCL2, CCL5, and CCL7 production by orbital fibroblasts after PDGF-BB stimulation [35]. Therefore, the different role and underlying mechanism of PDGF-BB on cellular responses require further investigation.

In summary, the present study demonstrated that PDGF-BB release occurs in clinical and experimental sepsis and that PDGF-BB plays a protective role in sepsis by decreasing the production of pro-inflammatory cytokines and chemokines. PDGF-BB thus may be a potential therapeutic strategy for treating sepsis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105792.

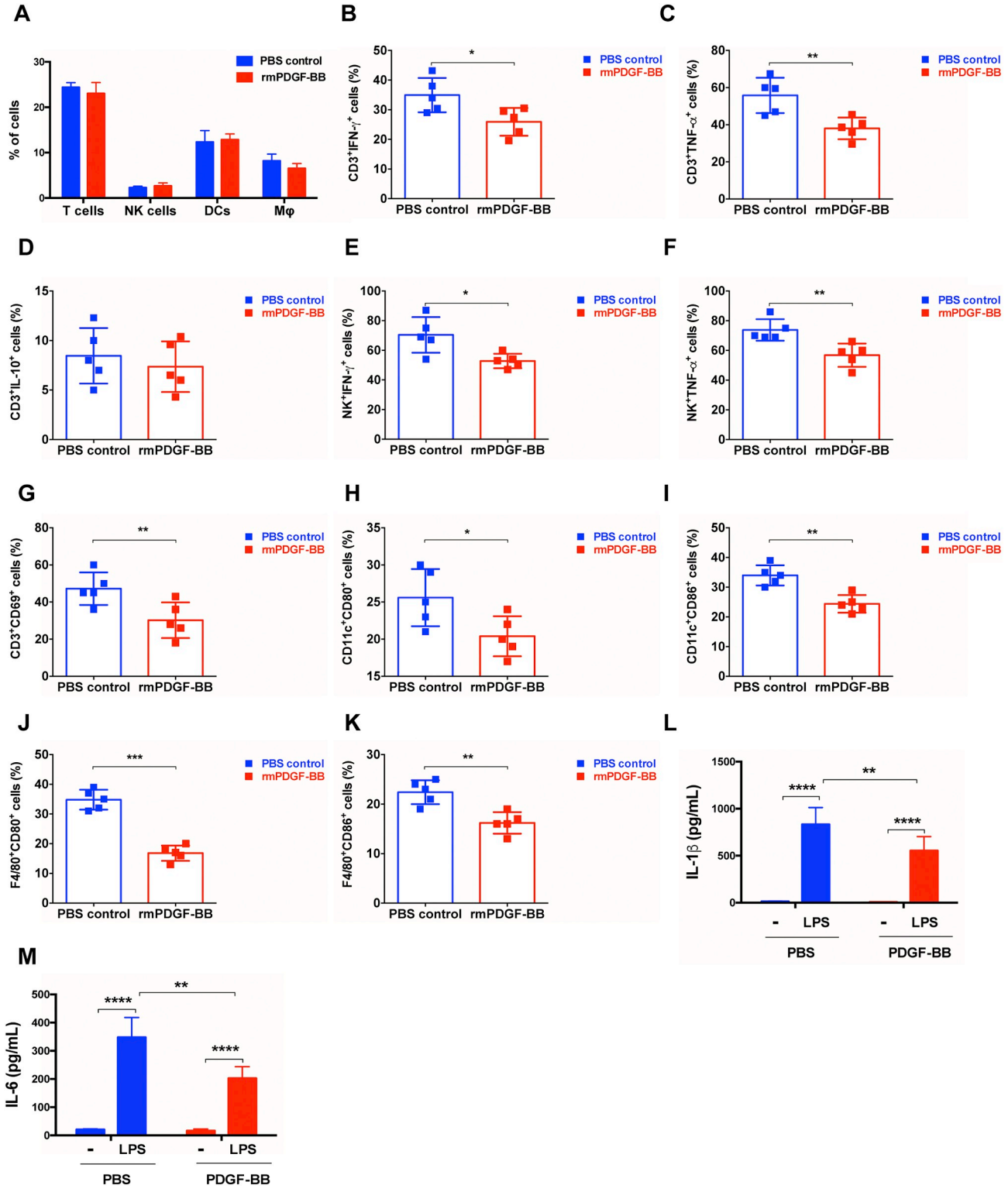

Fig. 7. PDGF-BB inhibited immune cell activation and cytokines production *in vivo* and *in vitro*. (A) The percentages of T cells ($CD3^+$), NK cells ($CD3^-NK1.1^+$), DCs ($CD11c^+$) and macrophages ($F4/80^+$) in the spleen of sepsis mice treated with or without of PDGF-BB. (B and E) IFN-γ and (C and F) TNF-α expression by T cells or NK cells, as well as (D) IL-10 expression by T cells were examined. (G) CD69 levels on T cells, CD80 levels on (H) DCs and (J) macrophages, CD86 levels on (I) DCs and (K) macrophages were evaluated in the spleen of sepsis mice treated with or without of PDGF-BB. (L and M) IL-1β and IL-6 expression by BMDCs treated with PDGF-BB in the presence of LPS stimulation. Data shown are mean ± SD. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with PBS control, by the student's *t*-test.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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