Interleukin 28 is a potential therapeutic target for sepsis

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ABSTRACT

Identification of new therapeutic targets for the treatment of sepsis is imperative. We report here that cytokine IL-28 (IFN-λ) levels were elevated in clinical and experimental sepsis. Neutralization of IL-28 protected mice from lethal sepsis induced by cecal ligation and puncture (CLP), which was associated with improved bacterial clearance and enhanced neutrophil infiltration. Conversely, administration of recombinant IL-28 aggravated mortality, facilitated bacterial dissimilation and limited neutrophil recruitment, in the model of sepsis induced by CLP. This study defines IL-28 as a detrimental mediator during sepsis and identifies a potential therapeutic target for the immune therapy in sepsis.

1. Introduction

Each year, about 31.5 million individuals develop sepsis, and up to 5.3 million deaths due to sepsis occur worldwide [1]. The current treatment of sepsis relies on the administration of antibiotics and organ function support, and there is no specific therapeutic agent approved for the treatment of sepsis [2]. In sepsis, the host immune response triggered by an invading pathogen fails to return to homeostasis, resulting in aberrant inflammation and immune suppression, and septic patients fail to eradicate primary infections and are susceptible to secondary, mostly opportunistic, infections [3]. Identification of the immune factors involved in sepsis-induced aberrant inflammation and immune suppression will provide novel potential targets for individualized immune therapy in the patients with sepsis.

The classical IFN family cytokines, such as types I and II IFNs, have been shown to play an important role in regulating host immune responses during sepsis [4]. Type III IFNs, or IFN-λs, are a newly described member of the IFN family, and they consist of three members in humans, denoted IL-28A (IFN-λ2), IL-28B (IFN-λ3), and IL-29 (IFN-λ1), and two members in mice (IL-28A and IL-28B) [4]. IFN-λs signal through a heterodimeric receptor consisting of the IL-28 receptor (IL-28R) and IL-10RB, which is expressed predominantly on mucosal surfaces and on neutrophils [5,6]. IL-28 cytokine family members could be produced by various cells upon viral infection or Toll like receptor ligation [7]. Although the antiviral and anti-tumor activity has been extensively studied during the past decade, the role of IL-28 in the immunopathology of sepsis is still poorly understood.

To address this issue, we examined the potential role of IL-28 in the progression of sepsis. Blood specimens from patients with sepsis demonstrated increased release of IL-28A. We used the cecal ligation and puncture (CLP) model of sepsis to test effects of IL-28 on sepsis. Neutralization of IL-28 reduced CLP-induced sepsis mortality, while IL-28 administration in sepsis increased mortality. This study identifies cytokine IL-28 as a new immunotherapeutic agent for the treatment of sepsis.

2. Materials and methods

2.1. Study subjects and data collection

Patients who met the clinical criteria for sepsis-3 were screened for eligibility within the first 24 h after they were admitted to the Intensive Care Unit of The Second Affiliated Hospital of Chongqing Medical University between September 2015 and December 2017 [8]. A total of 46 septic patients were enrolled. Patient data such as Acute Physiology and Chronic Health Evaluation II (APACHE II) score, Sequential Organ Failure Assessment (SOFA) score, the counts of white blood cells (WBC) and the levels of C-reaction proteins (CRP), microbial culture results, the length of ICU stay and hospital stay, and the outcome of ICU stay were recorded. Patients with malignancy, organ transplantation, HIV-infected patients, and patients receiving immunosuppressive agents in the past 8 weeks were excluded from the study. 26 healthy donors with

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Table 1

Baseline characteristics of patients with sepsis and healthy controls.

Characteristics	Sepsis patients ($n = 46$)	Healthy controls ($n = 26$)
Male sex	22	11
Age, years	55 (45–71)	49 (40–65)
WBC, 10^9/L	9 (6–19)	6 (4–9)
CRP, mg/L	120 (30–216)	NA
Infection site, no. of patients		
Respiratory	17	NA
Abdominal	16	NA
Vascular	3	NA
Urinary	5	NA
Other	5	NA
Bacteremia	30	NA
Isolates, no. of patients		
Gram positive	12	NA
Gram negative	22	NA
Fungus	1	NA
Miscellaneous	6	NA
Unknown	5	NA
APACHE II score	16.2 (13.1–22.5)	NA
SOFA score	8.2 (5.1–15.5)	NA
ICU stay, days	11 (3–19)	NA
Died/survived	6/40	NA

NOTE. Data are expressed as median (interquartile range) unless otherwise indicated. APACHE II: acute physiology and chronic health evaluation II; SOFA: sequential organ failure assessment; ICU: intensive care unit; WBC: white blood cells; CRP: C-reaction protein; NA: not applicable.

no medical problems in the medical examination center of The First Affiliated Hospital of Chongqing Medical University were also included as controls. This protocol was approved by the Clinical Research Ethics Committee of Chongqing Medical University, and informed consent was obtained from all participants according to the Declaration of Helsinki.

2.2. Sepsis model

Cecal ligation puncture (CLP) was used as a model of sepsis as described in our previous studies [9,10]. Briefly, C57BL/6 mice were anesthetized intraperitoneally (i.p.) with a mixture of xylazine (4.5 mg/kg) and ketamine (90 mg/kg), and the cecum was exposed, ligatured at its external third, and punctured through with a 21-gauge needle. The cecum was then returned to the peritoneal cavity, and incisions were closed. Sham-operated (control) animals underwent identical laparatomy, 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. Survival was monitored twice daily for 14 d. All experiments involving animals adhered to guidelines and received the approval of the Institutional Review Committee for Animal Care and Use at Chongqing Medical University.

2.3. Measurement of IL-28

IL-28 cytokines were quantified using specific ELISA kits, following the manufacturer's instructions. Mouse IL-28A/B kits were from R&D Systems, and human IL-28A (cross-reacting with IL-28B) kits were also from R&D Systems.
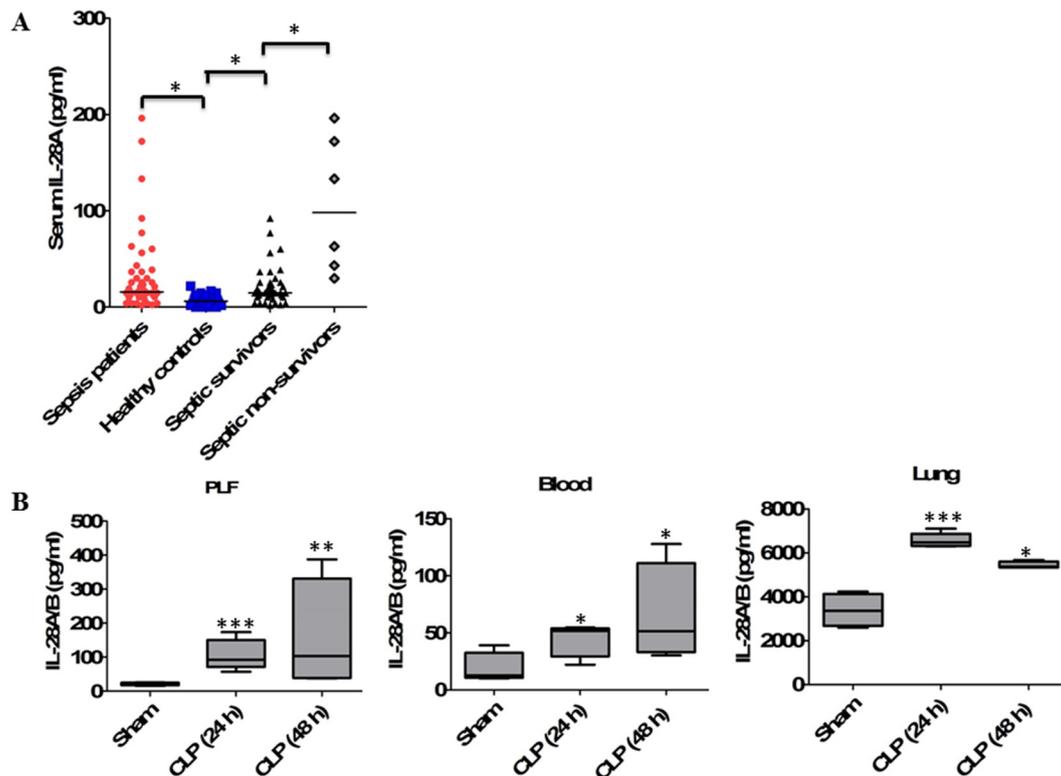
Fig. 1. IL-28 protein levels were elevated in clinical and experimental sepsis (A) IL-28A concentrations were measured by ELISA in serum samples collected from 46 patients with sepsis (40 survivors and 6 non-survivors) and from 26 healthy control subjects. Horizontal bars represent median values, and dots represent individual participants. *$p < .05$, compared between groups (denoted by horizontal bracket; Kruskal-Wallis test followed by Dunn's multiple comparisons post test). (B) Local and systemic IL-28 production in mice after cecal ligation puncture (CLP)–induced sepsis. C57BL/6 mice ($n = 6$ per group) were subjected to sham or CLP. Organs were removed at the indicated time points, blood was collected by cardiac puncture, and peritoneal lavage fluid (PLF) was obtained by washing the peritoneal cavity with 5 ml of sterile PBS. Samples were assayed for IL-28 content by ELISA. *$p < .05$, **$p < .01$,***$p < .001$, compared with sham control mice (Mann–Whitney U test).
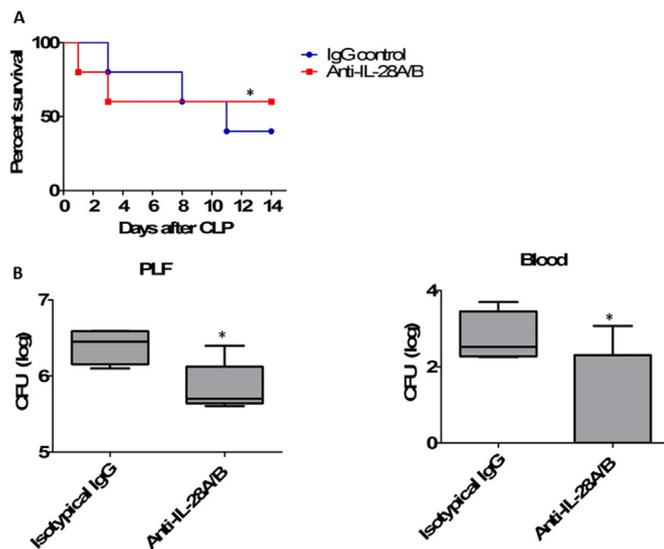
Fig. 2. Neutralization of IL-28 activity with monoclonal antibody attenuated CLP-induced sepsis. C57BL/6 mice were subjected to CLP, 10 μg of anti-mouse IL-28A/B neutralizing monoclonal antibody was administered intraperitoneally in 50 μl of PBS at 2 h after CLP, followed by a booster dose of 10 μg at 8 h later after CLP. As a control, rat IgG2b control antibody was used. (A) Survival of septic mice (n = 15 per group) following IL-28 neutralization after CLP with anti-IL-28 antibody. Comparison between groups was done by Kaplan–Meier analysis followed by log-rank tests. *p < .05 when compared with septic mice treated with isotypical IgG control. Results are representative of three independent experiments. (B) Dilutions of PLF and blood obtained from septic mice (n = 6) at 48 h after CLP were cultured on blood agar plates, and the number of bacterial colonies was counted as CFU. *p < .05 when compared with CLP-induced septic mice treated with isotypical IgG control (Mann–Whitney U test).

2.4. In vivo blockade of IL-28

To block IL-28 during experimental sepsis, we used rat anti-mouse IL-28A/B neutralizing monoclonal antibody (R&D systems, monoclonal rat IgG2b Clone # 244716). In vitro studies have shown that recombinant mouse IL-28B reduced the Encephalomyocarditis Virus (EMCV)-induced cytopathy in the HepG2 human hepatocellular carcinoma cell line, and inhibition of EMCV activity elicited by recombinant mouse IL-28B could be neutralized by this antibody. The 50% neutralizing dose (ND50) is typically 3–9 μg/mL. In mouse studies, 10 μg of anti-mouse IL-28A/B neutralizing monoclonal antibody was administered intraperitoneally in 50 μl of PBS 2 h after CLP, followed by a booster dose of 10 μg 8 h later after CLP. As a control, rat IgG2b control antibody was used.

2.5. In vivo administration of IL-28

Recombinant murine IL-28A or IL-28B protein (2 μg, R&D systems) was injected immediately after CLP or S. aureus infection. PBS was delivered in a similar fashion as control vehicle.

2.6. Differential cell counts in peritoneum

Peritoneal lavage was performed with 4 mL of PBS containing 1 mM ethylenediaminetetraacetic acid. Peritoneal cell suspension was pelleted and resuspended. Cell viability was determined using Trypan blue exclusion assay, and cell numbers were counted with a hematology analyzer. Cytospin slides were prepared and stained with a Wright-Giemsa stain.

2.7. Determination of bacterial load

Serial dilutions of blood and peritoneal lavage fluid (PLF) were prepared in sterile PBS for plating on brain-heart-infusion agar plates. Colony-forming unit (CFU) counts were then determined after 24-hour culture.

2.8. Statistical analysis

Human data were expressed as scatter dot plots with medians. Mice data were expressed as box-and-whisker plots showing the smallest observation, lower quartile, median, upper quartile, and largest observation or as medians with interquartile ranges. Comparisons between groups were tested using the Mann-Whitney U test or Kruskal-Wallis test followed by Dunn's multiple comparisons post test as appropriate. For survival studies, Kaplan-Meier analyses followed by log-rank tests were performed. All analyses were done using GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA). p values < .05 were considered statistically significant.

3. Results

3.1. The levels of IL-28 were up-regulated in human and murine sepsis

To ascertain the relevance of IL-28 in human sepsis, we examined IL-28A (IFN-λ2) levels from the blood of septic patients and healthy controls (Table 1). Serum IL-28A levels were significantly elevated in patients with sepsis compared to healthy controls, and those who did not survive displayed significantly more serum IL-28A than did the survivors (Fig. 1A). We also analyzed the production of IL-28 in the murine model of CLP-induced sepsis, and found that IL-28 concentrations were significantly increased in the PLF, blood and lung at 24 or 48 h after CLP (Fig. 1B).

3.2. Antibody against IL-28 protected against lethal sepsis

Having established that IL-28 release was up-regulated in clinical and experimental sepsis, we next studied the role of IL-28 in CLP-induced sepsis. We firstly used an IL-28A/B blocking mouse monoclonal antibody (Clone # 244716) to neutralize the biologic activity of IL-28. Using CLP-induced sepsis model, we observed that the survival rate in mice treated with anti-IL-28A/B antibody was significantly higher than that in mice treated with immunoglobulin G (IgG) control (Fig. 2A). Furthermore, mice treated with anti-IL-28A/B antibody displayed a significant decrease in bacterial loads from peritoneum and blood at 48 h after CLP (Fig. 2B).

3.3. Treatment with anti-IL-28 antibody enhanced neutrophil infiltration into the peritoneum during sepsis

Because leukocytes are critical for host defense during sepsis, we investigated whether IL-28 modulates leukocyte influx in sepsis. There were significantly greater numbers of leukocytes in PLF from anti-IL-28A/B-treated mice compared with IgG-treated mice (Fig. 3A and B). Moreover, we found that the number of neutrophils but not macrophages and lymphocytes was significantly increased with IL-28A/B blockade (Fig. 3A and B).

3.4. IL-28A delivery exacerbated lethality during sepsis

Because the administration of antibody against IL-28 was found to protect mice from lethal sepsis, we performed the reverse experiment and examined the effect of a bolus injection of recombinant mouse IL-28 given at the onset of CLP. IL-28A supplementation in the absence of sepsis had no effect on survival in healthy mice (Fig. 4A). In this model of CLP-induced sepsis, recombinant IL-28A significantly decreased
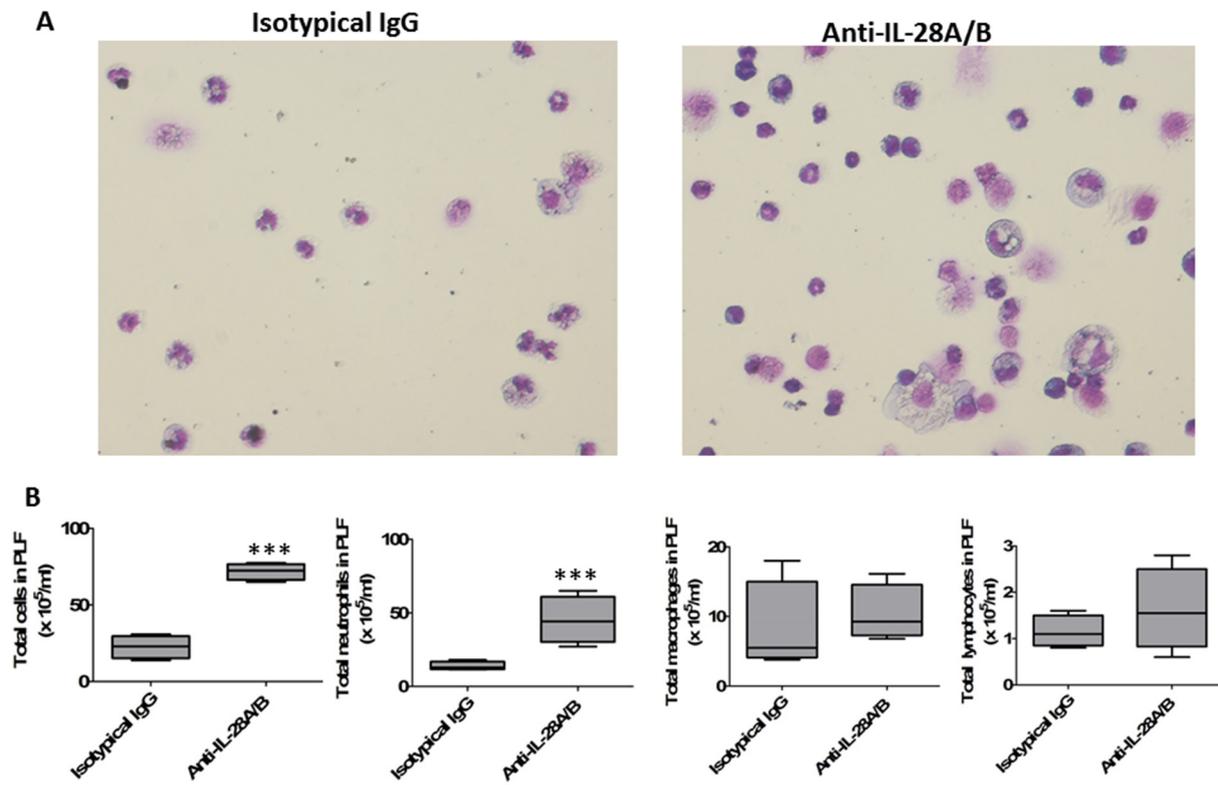
Fig. 3. Neutralization of IL-28 activity with monoclonal antibody enhanced neutrophil infiltration during CLP-induced sepsis. (A) Cytospin centrifugation was performed for Diff-Quik staining (x 40) to assess cell counts in PLF from septic mice (n = 6) with or without IL-28 neutralization at 24 h after CLP. (B) Number of cells in PLF from mice (n = 6) with or without IL-28 neutralization at 24 h after severe CLP. ***$p < .001$ when compared with septic mice treated with isotypical IgG control (Mann–Whitney U test).
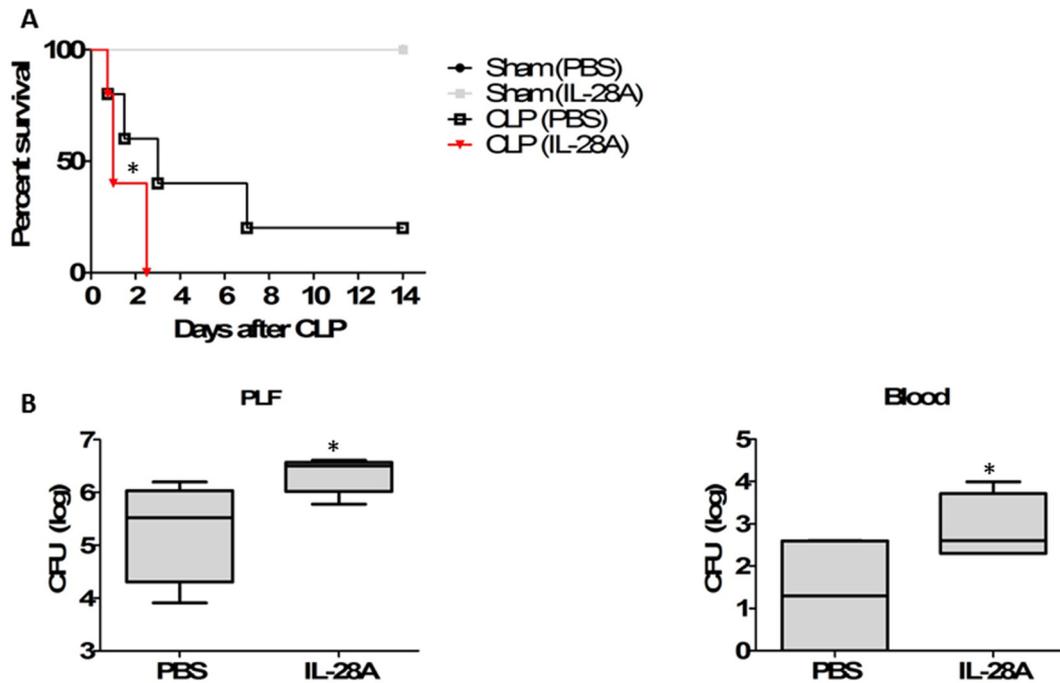

Fig. 4. Treatment with recombinant IL-28A aggravated experimental sepsis. (A) Survival of septic mice (n = 15 per group) following IL-28A supplementation. Recombinant murine IL-28A (2 μg/injection) was injected at the time of CLP. PBS was delivered in a similar fashion as control vehicle. Comparison between groups was done by Kaplan–Meier analysis followed by log-rank tests. *$p < .05$ when compared with septic mice treated with PBS control. Results are representative of three independent experiments. (B) Dilutions of PLF and blood obtained from septic mice (n = 6) at 48 h after CLP were cultured on blood agar plates, and the number of bacterial colonies was counted as CFU. *$p < .05$ when compared with CLP-induced septic mice treated with isotypical IgG control (Mann–Whitney U test).
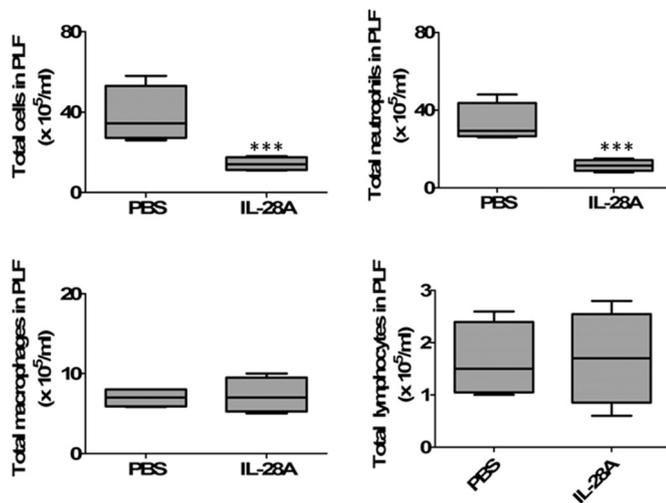
Fig. 5. Treatment with recombinant IL-28A restricted neutrophil infiltration during CLP-induced sepsis. Recombinant murine IL-28A (2 μg/injection) was injected at the time of CLP. PBS was delivered in a similar fashion as control vehicle. Number of cells in PLF from septic mice (n = 6) treated with or without recombinant IL-28A at 24 h after CLP. ***p < .001 when compared with septic mice treated with PBS control (Mann–Whitney *U* test).
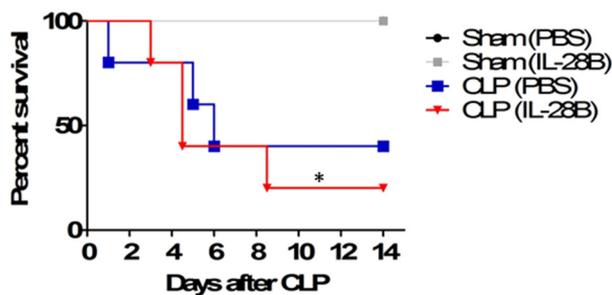

Fig. 6. Treatment with recombinant IL-28B aggravated mortality during CLP-induced sepsis. Survival of septic mice (n = 15 per group) following IL-28B supplementation. Recombinant murine IL-28B (2 μg/injection) was injected at the time of CLP. PBS was delivered in a similar fashion as control vehicle. Comparison between groups was done by Kaplan–Meier analysis followed by log-rank tests. *p < .05 when compared with septic mice treated with PBS control. Results are representative of three independent experiments.

survival of septic mice compared to PBS control (Fig. 4A). Treatment with recombinant IL-28A also impaired bacterial clearance in the peritoneum and blood (Fig. 4B). Furthermore, there were significantly lower numbers of leukocytes in PLF from IL-28A-treated septic mice compared with PBS-treated septic mice, and the number of neutrophils but not macrophages and neutrophils was reduced with IL-28A treatment in septic mice (Fig. 5).

3.5. Treatment with IL-28B exacerbated lethality during sepsis

To further confirm the detrimental effects of IL-28 on sepsis, recombinant IL-28B (IL-28A and -B are 96% identical [11]) was instilled into septic mice. Addition of recombinant IL-28B (2 μg) to septic mice also increased CLP-induced lethality (Fig. 6).

4. Discussion

In this study, we identified IL-28 to be centrally involved in the pathogenesis of sepsis. We made the following key observations: (1) patients with sepsis have increased circulating IL-28 levels, and IL-28 release was enhanced in the experimental model of sepsis; (2) Neutralization of the IL-28 activity with antibody against IL-28

produced substantial survival benefit in experimental sepsis; (3) IL-28 supplementation in the presence of sepsis worsened outcome of experimental sepsis.

Since its discovery in 2003, many groups have identified contributions of IL-28 to host immune responses. IL-28 production has been found to be increased in serum from patients with chronic hepatitis C virus (HCV) infection or dengue virus infection [12,13]. Increased serum levels of IL-28 were also found in patients with Hashimoto's Thyroiditis [14]. This is the first study to analyze IL-28 levels in blood samples from patients with sepsis. Serum IL-28 levels were elevated in patients with sepsis compared to healthy donors. Notably, those who did not survive had significantly more serum IL-28 than did the survivors. However, we are unable to determine whether this is a biomarker for illness severity of human sepsis, and we acknowledge this can only be definitively established in humans with a large cohort of clinical study.

Our data suggest a novel role of IL-28 in the progression of experimental sepsis. Neutralization of the IL-28 activity with antibody against IL-28 could attenuate CLP-induced sepsis. The survival benefit obtained with neutralizing antibody against IL-28 was associated with lower local and circulating bacterial counts. This finding is in agreement with the recent observation that activation of IL-28 signaling in response to influenza virus infection increased susceptibility to pulmonary infection by methicillin-resistant *Staphylococcus aureus* (MRSA) [15]. The improved bacterial clearance obtained with IL-28 neutralization was associated with enhanced neutrophil infiltration during CLP. A previous study has demonstrated that treatment with recombinant IL-28A restricted neutrophil infiltration into joint in the mouse model of collagen-induced arthritis [6]. Here we also confirmed that IL-28 supplementation in septic mice suppressed neutrophil influx during CLP. Taken together, these results suggest that IL-28 might impair bacterial clearance during sepsis by restricting neutrophil influx to the site of infection.

Collectively, our data suggest that IL-28 plays a detrimental role in the pathogenesis of sepsis. Elevated IL-28 production during sepsis may promote bacterial dissimilation by limiting neutrophil filtration, leading to a failure to contain the infection, thereby resulting in the aggravation of sepsis. Therefore, blocking IL-28 may offer a new strategy for the future of immune therapy in sepsis.

Conflicts of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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