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ORIGINAL ARTICLE

Interleukin-1β Protection Against Experimental Sepsis in Mice

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Abstract— The inflammatory response involving interleukin-1β (IL-1β) has been thought to play an important role in the development of late-phase sepsis. However, in this study, we wanted to explore the possibility of using IL-1β to improve the prognosis of sepsis by triggering local differentiation of bone marrow cells (BMCs) into regulatory dendritic cells (DCs) *in vivo*, thereby reversing the immune paralysis in late-phase sepsis. Sepsis mouse models were induced by cecal ligation and puncture (CLP) and lethal *Escherichia coli* O18 infection. Mice were injected intraperitoneally with IL-1β after CLP and after the lethal infection. Septic BMCs and liver immune cells were isolated at 0, 3, 6, 9, and 14 days post-CLP. BMCs and liver cells isolated from septic mice treated with IL-1β were adoptively transferred into CLP mice. GFP^+-C57BL/6 parabiosis models were established. Serum IL-1β levels were determined by enzyme-linked immunosorbent assay (ELISA) kit, and the number, ratio, and phenotype of immune cells were observed by flow cytometry. IL-1β treatment improved the survival of sepsis and increased the numbers of BMCs and liver immune cells in septic mice. Moreover, IL-1β stimulation increased the number and the percentage of $CD11c^-CD45RB^{high}$ DCs in septic BM and liver. Adoptive transfer of septic BMCs, liver immune cells, and $CD11c^-CD45RB^{high}$ DCs treated with IL-1β into CLP mice attenuated sepsis. IL-1β triggered the redistribution of $CD11c^-CD45RB^{high}$ DCs as well as BMCs in parabiosis models. IL-1β protects against sepsis

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by stimulating local proliferation and differentiation of BMCs into CD11c^{-}CD45RB^{high} DCs at immune organs and non-immune organs.

KEY WORDS: interleukin-1β; bone marrow cells; CD11c^{-}CD45RB^{high} dendritic cell; parabiosis; sepsis.

INTRODUCTION

The concept of SEPSIS1.0 was proposed in the early 1990s. Despite the subsequent proposal of SEPSIS2.0 years later, sepsis was still defined as the systemic inflammatory response (SIRS) to infection [1]. SEPSIS1.0 and SEPSIS2.0 in terms of SIRS have been used as a clinical standard for judging whether or not patients suffer from sepsis [2]. Furthermore, SIRS is considered to be the chief culprit in the induction of multiple organ dysfunction syndrome (MODS) in sepsis [3]. Recently, however, the concept of SIRS has been eliminated from SEPSIS3.0 [4]. This updated definition implies that SIRS is no longer necessary for the diagnosis of sepsis, opposing the previous clinical diagnostic criteria [5]. In the early phase following injury, the inflammatory response is initiated by acute-phase reactants or pro-inflammatory factors [6]. As an important inflammatory factor, tumor necrosis factor (TNF) causes fever, hemodynamic abnormalities, anorexia, joint pain, and neutrophil aggregation, which are regarded as typical symptoms related to sepsis [7]. Therefore, it is critical to explore the potential role of inflammatory factors in the pathophysiology of sepsis. Moreover, removing the concept of SIRS from sepsis will inevitably result in continuous issues regarding the basic research and clinical treatment of sepsis. For instance, "cytokine storm" is considered to be the major driver of morbidity and mortality for severe COVID-19 patients who suffer from septic shock. Current therapeutic options include steroids, intravenous immunoglobulin, selective cytokine blockade (*e.g.*, anakinra or tocilizumab), and JAK inhibition. It is clear that even now, the concept of SIRS still has a great influence in the study of sepsis.

Similar to TNF, interleukin-1β (IL-1β), produced primarily by monocytes and macrophages in response to endotoxin, is another inflammatory factor of SIRS and plays an irreplaceable role in sepsis. Other laboratories have previously revealed that prophylactic administration of IL-1 can protect mice from lethal Gram-negative infection [8, 9]. A recent research has shown that IL-1 receptor antagonist treatment aggravates staphylococcal septic arthritis and sepsis in mice [10]. Additionally, IL-1β contributes to macrophage recruitment and *Streptococcus pneumoniae* clearance [11]. All these findings suggest that IL-1β may improve the prognosis of sepsis by initiating innate immunity in sepsis. However, the immune-regulatory mechanism of IL-1 remains largely unknown. Previous studies have reported that IL-1β accelerates hematopoietic recovery in the bone marrow (BM) [12]. Recent work in our laboratory has demonstrated that high mobility group protein 1 (HMGB1) improves the prognosis of sepsis by boosting differentiation of bone marrow cells (BMCs) into regulatory dendritic cells (DCs) [13]. Thus, we hypothesize that IL-1β can enhance innate immunity by stimulating local proliferation and differentiation of BMCs into CD11c^{-}CD45RB^{high} DCs in both immune and non-immune organs, thereby leading to sufficient bacterial eradication and the alleviation of sepsis.

RESULTS

IL-1β Reduced the Mortality of Septic Mice Induced by CLP

The protective effects of various doses of IL-1β against a lethal challenge of cecal ligation and puncture (CLP) administered immediately post-CLP are summarized in Table 1. Usually, the onset of sepsis occurs at around 12 h after CLP. The main part of lethality proceeds within the first 48 h, whereas the frequency of death events decreases with time, which correlates with the different concentrations of IL-1β treatment, as does the overall outcome. Septic mice treated with IL-1β at 50 μg/kg of body weight exhibited 80% survival, in contrast to 10% of controls treated with saline ($P < 0.05$). Mice treated with either 5–12.5 μg/kg IL-1β or 100–125 μg/kg IL-1β demonstrated similar results but did not have a significant survival advantage over the controls. Mortality due to sepsis did not decrease when the injected dose of IL-1β was less than 5 μg/kg, whereas the mortality of septic mice increased remarkably when the injected dose of IL-1β was higher than 500 μg/kg (Table 1).

The protective effects of IL-1β were significant when IL-1β (50 μg/kg) was administered either 1 h prior to or immediately after CLP ($P < 0.01$ or $P < 0.05$) (Table 2). However, there was no improvement in survival when the cytokines were administered between 1 and 6 h after CLP.

Table 1. Protective Effects of Various Doses of IL-1β Administered by IP Route After CLP Male C57BL/6

Dose of IL-1β (μg/kg)	14-day survival (%)
Untreated	1/10 (10)
5	2/10 (20)
10	3/10 (30)
12.5	5/10 (50)
25	6/10 (60)
50	8/10 (80)*
100	5/10 (50)
125	2/10 (20)
250	1/10 (10)
500	0/10 (0)

*$P < 0.05$ *versus* control

No difference was observed from the single dose of IL-1β (50 μg/kg) administered with a treatment interval from 12 to 48 h (Table 2).

IL-1β Prevented Against a Lethal Challenge Induced by *E. coli* Infection

The protective effects of various doses of IL-1β against a lethal infection of *Escherichia coli* bacteria administered immediately post-infection are summarized in Table 3. Septic mice treated with IL-1β at 25 μg/kg of body weight exhibited 80% survival, in contrast to 0% of controls treated with saline ($P < 0.01$). Mice treated with either 12.5 μg/kg IL-1β or 50 μg/kg IL-1β showed a significant survival advantage over the controls ($P < 0.05$). Mortality due to sepsis did not decrease when the injected dose of IL-1β was less than 2.5 μg/kg, whereas no survival of septic mice was observed when the dose of IL-1β injected was higher than 250 μg/kg (Table 3).

The protective effects of IL-1β were significant when IL-1β (25 μg/kg) was administered either 1 h prior to or

Table 2. Onset and Duration of Protection of IL-1β, 50 μg/kg by IP Route Against CLP in Male C57BL/6 Mice

Time prior to CLP	Survival (%)
Untreated	1/10 (10)
− 1 h	9/10 (90)**
0 h	7/10 (70)*
1 h	5/10 (50)
6 h	3/10 (30)
12 h	0/10 (0)
1 day	0/10 (0)
2 days	0/10 (0)

*$P < 0.05$ *versus* control; **$P < 0.01$ *versus* control

Table 3. Protective Effects of Various Doses of IL-1β Administered by IP Route After *E. coli* Infection of Male C57BL/6

Dose of IL-1β (μg/kg)	14-day survival (%)
Untreated	0/10 (0)
2.5	1/10 (10)
5	2/10 (20)
10	4/10 (40)
12.5	5/10 (50)*
25	8/10 (80)**
50	6/10 (60)*
100	2/10 (20)
125	1/10 (10)
250	0/10 (0)
500	0/10 (0)

*$P < 0.05$ *versus* control; **$P < 0.01$ *versus* control

immediately after infection ($P < 0.01$ or $P < 0.05$) (Table 4). However, there was no improvement in survival when the cytokines were administered 1 h after infection. No difference was observed from the single dose of IL-1β (25 μg/kg) administered with a treatment interval from 6 to 12 h (Table 4). These experiments demonstrated that a single intraperitoneal (i.p.) dose of IL-1β conferred significant protection against a lethal challenge induced by *E. coli* bacterial infection.

IL-1β Injection Inhibited the IL-1β Release in the Early Stage of Sepsis

The serum IL-1β level gradually decreased when intraperitoneal injection of IL-1β was administered immediately after CLP or a lethal *E. coli* infection (Fig. 1). However, the releases of IL-1β both in the CLP group and in the *E. coli* infection group were gradually increased. The IL-1β level in CLP experimental group was close to that in control group at 48 h (Fig. 1b), which was observed at 3 h in the lethal infection group (Fig. 1a). These indicated that the change of IL-1β level

Table 4. Onset and Duration of Protection of IL-1β, 25 μg/kg Administered by IP Route Against *E. coli* Infection in Male C57BL/6 Mice

Time prior to infection	14-day survival (%)
Untreated	0/10 (0)
− 1 h	8/10 (80)**
0 h	5/10 (50)*
1 h	4/10 (40)
6 h	0/10 (0)
12 h	0/10 (0)

*$P < 0.05$ *versus* control; **$P < 0.01$ *versus* control

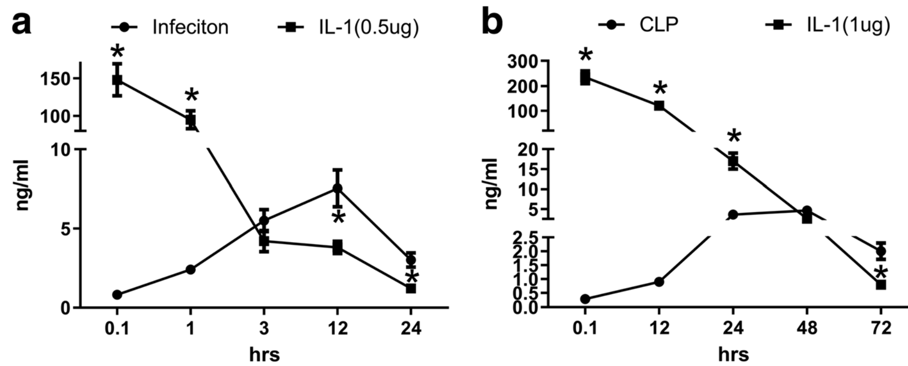

Fig. 1 . a The concentration of IL-1β was detected in the untreated group and IL-1β (25 μg/kg) treated group with intraperitoneal injection with IL-1β (0.5 μg/per mouse) post-*E. coli* bacteria infection at 0.1, 1, 3, 12, and 24 h ($n = 10$). $^{*}P < 0.05$ compared with untreated group by paired t test. b The concentration of IL-1β was detected in the untreated group and IL-1β (50 μg/kg) treated group with intraperitoneal injection with IL-1β (1 μg/per mouse) post-CLP at 0.1, 12, 24, 48, and 72 h ($n = 10$). $^{*}P < 0.05$ compared with untreated group by paired t test.

was rapid in the case of the lethal infection, regardless of the injection of IL-1β.

However, the IL-1β level in the infection control group gradually increased from 0 to 12 h and then showed a downward trend at 12–24 h. The point marked at each time interval showed significant difference in the levels of IL-1β between both the control and experimental groups.

IL-1β Improved Survival of Sepsis Induced by CLP

Then we investigated the effect of IL-1β on CLP-induced mortality. A single intraperitoneal dose of 12.5 or 25 μg/kg IL-1β immediately after CLP could not reduce sepsis mortality significantly (Fig. 2). In contrast, treatment with a single intraperitoneal dose of 50 μg/kg IL-1β significantly decreased mortality, and this effect was observed in a dose-dependent manner (Fig. 2a). Although survival rates were higher in high-grade sepsis with IL-1β treatment than in untreated sepsis, usually, most of the mice induced by high-grade sepsis die within 4 days after CLP without the late phase regarding mortality (6–14 days post-CLP).

The numbers of BMCs and liver immune cells increased gradually from 3 days post-CLP to 9 days post-CLP (Fig. 2b, c). At 14 days post-CLP, the numbers of BMCs and liver immune cells in each group approached the normal level, and there was no difference between groups. Although larger numbers of BMCs and liver cells were observed in mice in late sepsis than in the sham-operated group, there were some moribund mice with lower numbers of BMCs or liver cells than those in active mice.

IL-1β Treatment Increased the Numbers and Percentages of Regulatory DCs (CD11c^{-}CD45RB^{high} DCs) in the BM and Liver

We tested the effects of IL-1β on the numbers and percentages of CD11c^{-}CD45RB^{high} cells in the BM and liver of the CLP group at different time points (Fig. 3). The number of CD11c^{-}CD45RB^{high} cells was elevated in late sepsis (from 3 to 9 days post-CLP), with an increasing percentage of CD11c^{-}CD45RB^{high} DCs (Fig. 3c–f). Subsequently, during the stage from 9 to 14 days post-CLP, the percentages of regulatory DCs gradually approached the level of sham-treated groups. In addition, we found that both the phenotype and the function of CD11c^{-}CD45RB^{high} DCs in the BM and liver were similar to those in the spleen, with low expression of CD40, CD80, CD86, and I-A/E and high secretion of IL-10 (see Fig. S2 in the supplemental material). Therefore, we supposed that CD11c^{-}CD45RB^{high} DCs triggered by IL-1β potentially played a pivotal role in controlling the pathophysiology of sepsis. To confirm this, we designed subsequent adoptive cell transfer experiments using BMCs, liver cells, and CD11c^{-}CD45RB^{high} DCs isolated from CLP mice with IL-1β treatment.

Beneficial Immunomodulation of Septic BMCs and Liver Immune Cells Treated by IL-1β *In Vivo*

A single intraperitoneal injection of BMCs or CD11c^{-}CD45RB^{high} DCs in BM 9 days post-CLP, isolated from CLP mice with IL-1β (50 μg/kg) stimulation, could reduce the mortality of septic mice in a dose-dependent manner (Fig. 4a, c). Surprisingly, a

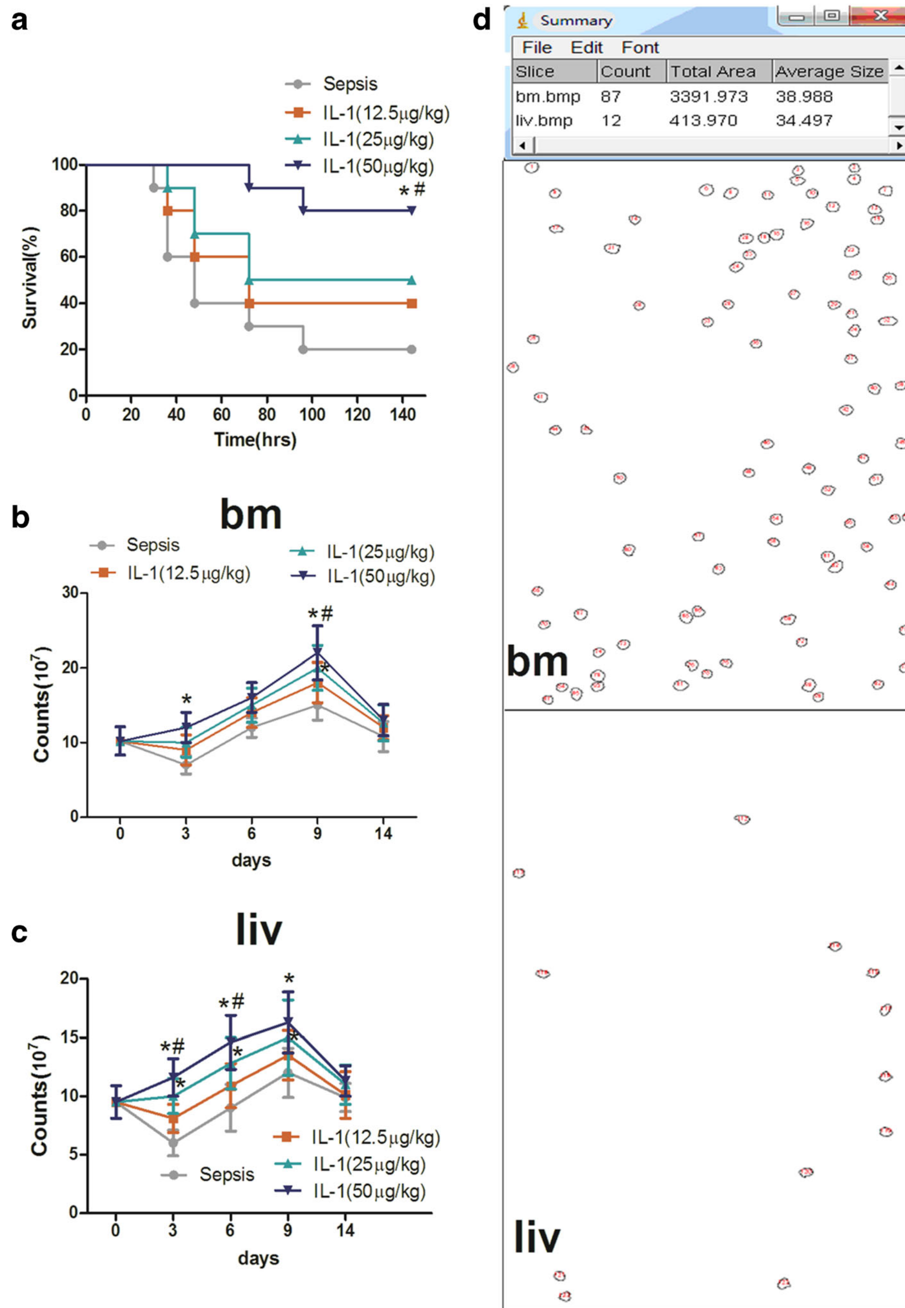

Fig. 2 . **a** Septic mice received intraperitoneal injection of IL-1β (12.5 μg/kg, 25 μg/kg, and 50 μg/kg) immediately after CLP ($n = 10$). *$P < 0.05$ compared with the CLP group by the log-rank test; #$P < 0.05$ compared with CLP mice treated with IL-1β (12.5 μg/kg) by the log-rank test. **b**, **c** The number of BMCs and liver immune cells ($n = 7$). *$P < 0.05$ compared with the CLP group; #$P < 0.05$ compared with mice treated with IL-1β (12.5 μg/kg) by one-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* test for multiple comparisons. **d** Selected digital images were saved as 8-bit images in an uncompressed tagged image file format (TIFF) for further analysis. The number of BMCs and liver immune cells of a sham CLP mouse was counted by ImageJ Macro Script Programming, and the data were representative of five consecutive experiments. All these images were in the same treatment conditions.

single intraperitoneal injection of liver immune cells or $CD11c^{-}CD45RB^{high}$ DCs in liver 9 days post-CLP, isolated from CLP mice with IL-1β (50 μg/kg) stimulation, could also reduce the mortality of septic mice

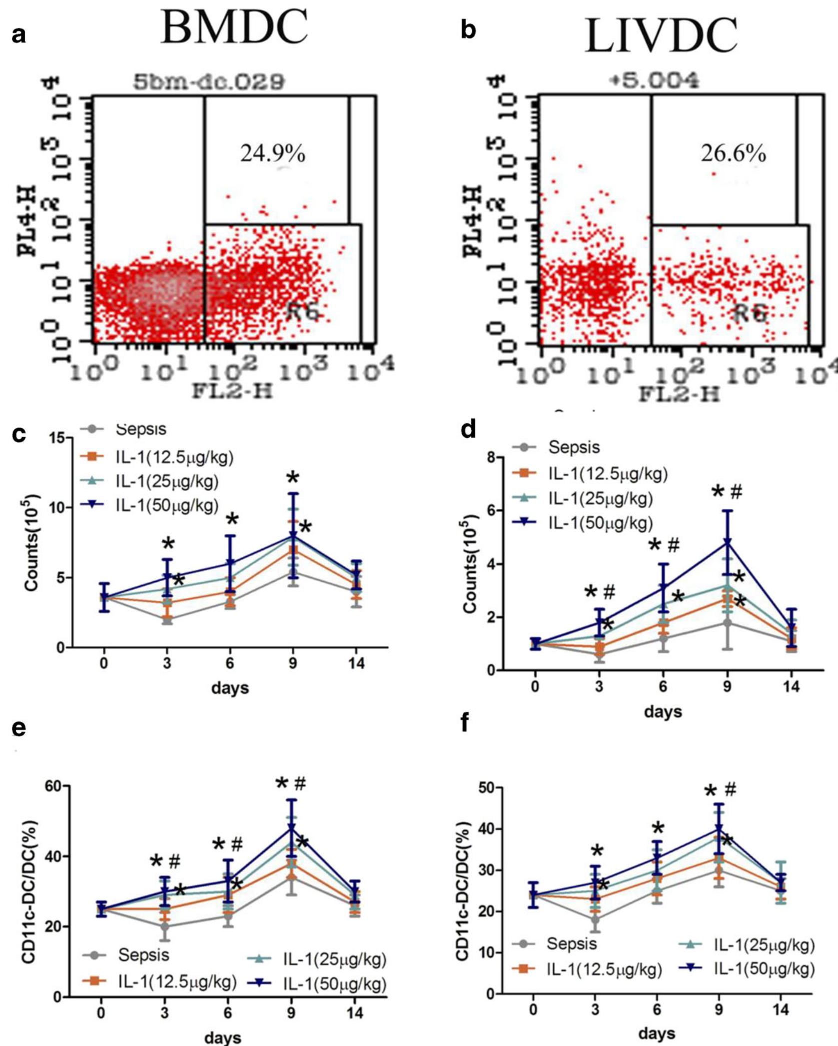

Fig. 3. **a**, **b** BM regulatory dendritic cells (BMDCs) and liver regulatory dendritic cells (LIVDCs), using mouse Dendritic Cells Enrichment Set-DM, were negatively selected from BMCs and liver immune cells in sham-operated mice and, subsequently, stained with phycoerythrin-conjugated mAbs to mouse CD45RB (FL2-H) and allophycocyanin-conjugated mAbs to mouse CD11c (FL4-H). Data were representative of six consecutive experiments. **c**–**f** The number and percentage of $CD11c^{-}CD45RB^{high}$ DCs in BM and liver ($n = 6$). $^{*}P < 0.05$ compared with the CLP group by one-way ANOVA test. $^{\#}P < 0.05$ compared with mice treated with IL-1β (12.5 μg/kg) by one-way ANOVA followed by Bonferroni *post hoc* test for multiple comparisons.

in a dose-dependent manner (Fig. 4b, d). In another experiment, these four types of cells isolated from sham-operated group were intraperitoneally injected into CLP mice, and no significant improvement could be observed in mortality (data not shown). Based on these data, the numbers of BMCs and liver immune cells were increased, and the immune function of these cells in CLP mice with IL-1β treatment was enhanced remarkably.

IL-1β Enhanced the Migration of $CD11c^{-}CD45RB^{high}$ DCs in a Parabiosis Model

To examine how IL-1β triggers the immune redistribution of BMCs, a green fluorescent protein (GFP) transgenic mouse was surgically joined to a C57BL/6 mouse (Fig. 5(A)). The parabiotic mice were separated at 1 week after intraperitoneal injection of IL-1β or anti-IL-1β antibody (Fig. 5(B)). Flow cytometric analysis revealed large numbers of GFP^{+} cells engrafted into the C57BL/6

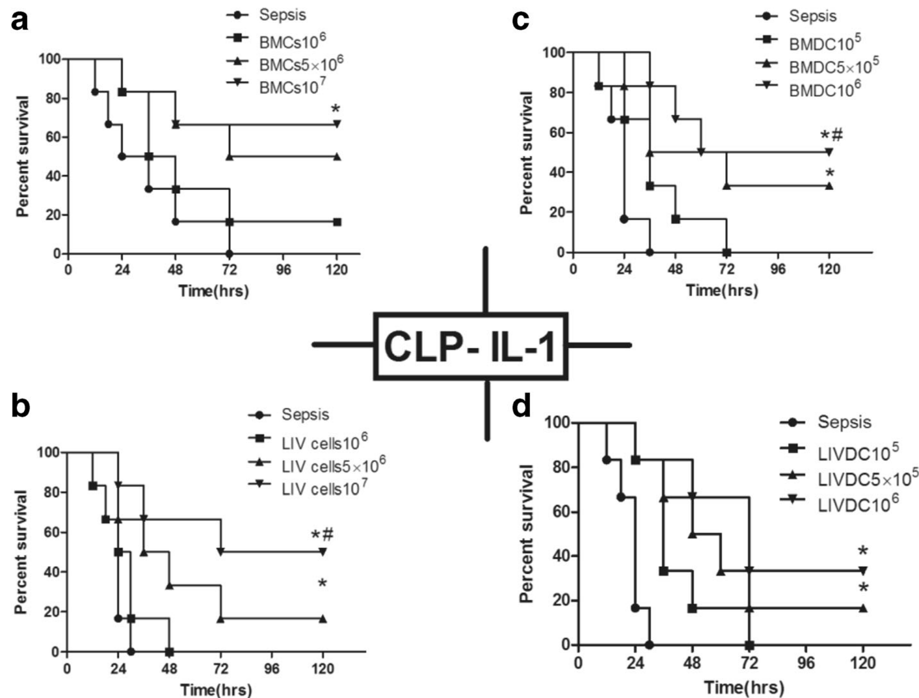

Fig. 4. **a**–**d** C57BL/6 mice were subjected to CLP and immediately thereafter were intraperitoneally injected with different cell concentrations of BMCs (10^6 to 10^7/mouse) (**a**), liver immune cells (10^6 to 10^7/mouse) (**b**), DCs in BM (10^5 to 10^6/mouse) (**c**), or DCs in liver (10^5 to 10^6/mouse) (**d**), which were harvested from CLP mice with IL-1β (50 μg/kg) treatment at 9 days post-CLP. $^*P < 0.05$ compared with the untreated mice; $^\#P < 0.05$ compared with mice given low-dose injections of DCs, BMCs, or LIV cells by the log-rank test ($n = 15$).

parabiont (Fig. 5(C)). After injecting IL-1β (50 μg/kg) intraperitoneally into GFP^+ mice, we found that the percentages of $GFP^+CD11c^-CD45RB^{high}$ DCs in BM and liver of C57BL/6 parabiont had significantly increased (Fig. 5(D)). On the other hand, the increasing percentages of $GFP^+CD11c^-CD45RB^{high}$ DCs in BM and liver of C57BL/6 parabiont could be limited when the anti-IL-1β antibody was injected 1 h post-IL-1β treatment on GFP^+ mice before parabiosis (Fig. 5(E)). C57BL/6 parabionts triggered by IL-1β were protected from sepsis after disconnection from GFP^+ mice (Fig. 5(F)), with more $GFP^+CD11c^-CD45RB^{high}$ DCs than untreated C57BL/6 parabionts or C57BL/6 parabionts induced by anti-IL-1β antibody after IL-1β treatment (Fig. 5(G)).

DISCUSSION

A recent study has shown that IL-1β could enhance cyprinid immunization [14]. Co-expression of IL-1β can enhance the potency of the vaccine, as demonstrated by a higher antibody level after the third immunizations. Indeed, a number of biologic effects of IL-1 are beneficial to the host in times of stress, contrary to its contribution to morbidity and mortality when produced in excessive quantities [15]. Instead of initiating excessive inflammatory response and increasing morbidity and mortality of sepsis, the secretion of IL-1β activates innate immune response to attenuate sepsis in our study. Either in the CLP group or the lethal *E. coli* infection group, artificially increasing the level of IL-1β showed a marked improvement in the prognosis of sepsis (Fig. 1). Hence, IL-1β should be utilized to treat patients with early sepsis or protect non-septic patients who are at risk of developing sepsis.

Previous work has been designed to evaluate the efficacy of single low-dose TNF for treatment of early sepsis. Administration of 10 to 50 μg/kg TNF would reduce mortality in septic model when pretreatment occurred 24 h before CLP [16]. Clearly, if TNF is used as a drug for the treatment of sepsis, there will be an extremely narrow dose range and a higher requirement for the time window. IL-1β has less toxicity when administered in a large dosage, but despite that, IL-1β and TNF have many similar pathophysiological effects [4, 10]. Effective dose range of IL-1β from 5 to 125 μg/kg in CLP-induced sepsis and 2.5 to 125 μg/kg in *E. coli* bacteria-induced sepsis has been investigated (Tables 1 and

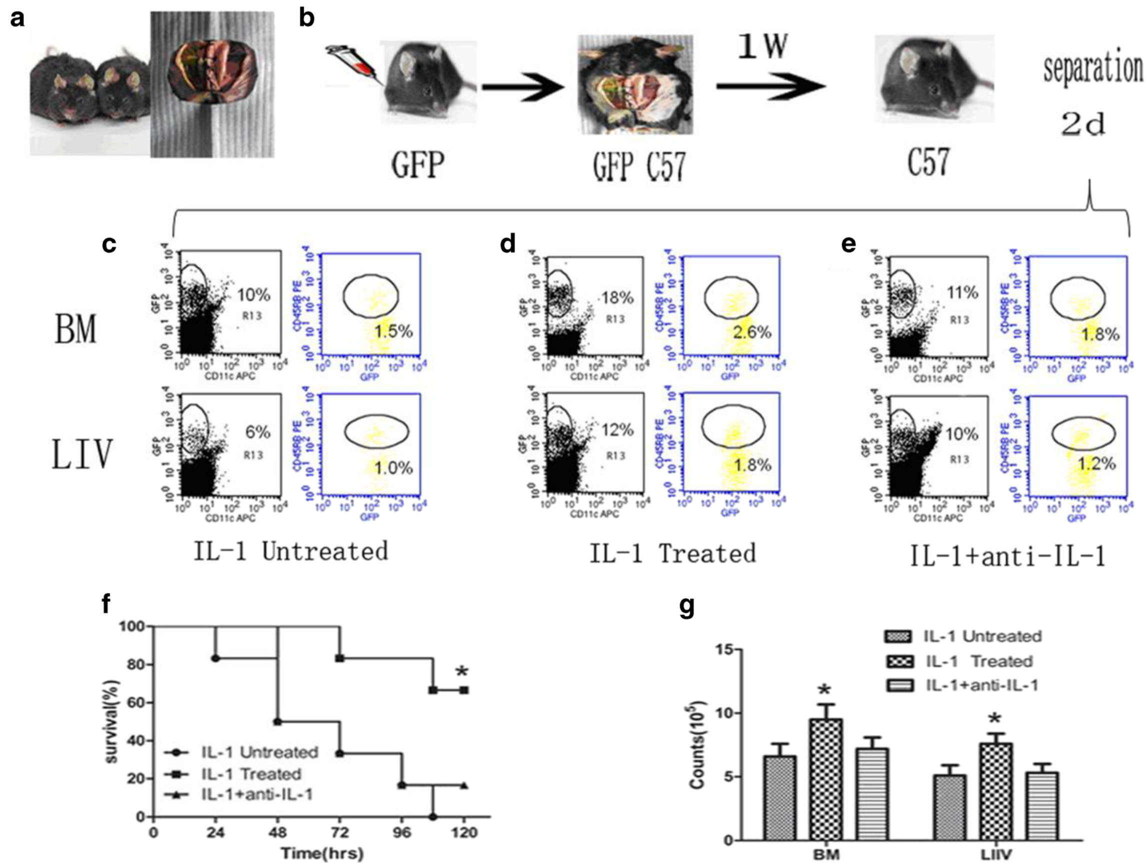

Fig. 5. (A) Photograph of the GFP$^+$-C57BL/6 mouse parabiosis model. (B) Diagram illustrating the experimental procedure designed to analyze the migration and local differentiation of circulating GFP$^+$ cells in a C57BL/6 parabiont and the survival rate of C57BL/6 parabionts after lethal *E. coli* infection. (C–E) In GFP$^+$-C57BL/6 mouse parabiosis models without IL-1β treatment (C), or with IL-1β treatment at 50 μg/kg (D), or with IL-1β treatment at 50 μg/kg and neutralization with anti-IL-1β antibody at 100 μg/kg (E), GFP cells were redistributed from GFP$^+$ mice into C57BL/6 mice through the symbiotic circulation system. After disconnection, BMCs and liver cells of C57BL/6 mice were isolated and detected by flow cytometry in order to analyze the migration of GFP$^+$CD11c$^-$ cells (left) and the percentages of CD11c$^-$CD45RB^{high} DCs (right) in BM and liver. (F) Lethal bacterial-induced mortality of C57BL/6 mice after disconnection from parabiosis with IL-1β or IL-1β + anti-IL-1β antibody injection into GFP mice (n = 6). The photograph and flow diagrams show the results of one representative experiment out of five performed under the same conditions (n = 6). *P < 0.05 compared with IL-1β untreated group by the log-rank test. (G) GFP$^+$ cell counts in C57BL/6 mice after parabiosis (n = 6). *P < 0.05 compared with IL-1β untreated group by one-way ANOVA followed by Bonferroni *post hoc* test.

3). Therefore, it is well tolerated through a large range of doses that confer a significant protective benefit when treating patients with sepsis in clinical practice. IL-1β also appears to protect against CLP when given 6 h after injury (Table 2). The results suggest that the early administration of low-dose IL-1β may be useful in the prevention and treatment of the lethality of sepsis.

Although IL-1β treatment has been shown to confer protection against a subsequent lethal bacterial infection and to reduce mortality in a CLP model, the mechanism of the protective action of it remains unknown [9, 10]. Previous studies have confirmed that LPS-induced tolerance or tachyphylaxis to the lethality of endogenous inflammation contributes to the protective role of IL-1β pretreatment. It has been recently shown that the protective effect of IL-1β stems from activating innate immunity [17]. After activation of the innate immune system, innate immune cells may undergo long-term functional reprogramming characterized by the ability to overcome either a stronger or attenuated inflammatory response upon reactivation. This phenomenon, which has been termed trained immunity, is a *de facto* innate immune memory [18, 19]. Also, the IL-1β family of cytokines and their receptors play a central role in the modulation of innate immunity and inflammation through trained

immunity [20]. Research by others shows injecting IL-1 at 30 min and 2 h before infection did not improve survival [8, 9]. Our study, however, indicated that IL-1 was protective when injected after CLP and a lethal challenge. The effective dose used in our trial was lower than the effective dose of IL-1 administrated according to trigger "trained immunity." Therefore, the mechanism of IL-1β protection explored in our experiment by injecting IL-1β post-CLP or post-lethal infection ought not to be considered as trained immunity. Because IL-1β treatment happens after the first stimulus (and yet is still able to provide protective effects for following lethal infections within 24 h), it is not a result of LPS tolerance or trained immunity. In the present study, we have found that IL-1β can decrease the mortality of sepsis and increase the number of BMCs and liver cells (Fig. 2). We argue that the protective role of IL-1β treatment post-CLP or post-*E. coli* infection on sepsis is probably derived from the mobilization and proliferation of BMCs accompanied by the increasing number of liver immune cells, which should be beneficial with respect to prevention of the potential lethality. BMCs are known to be responsible for peripheral immunity. We speculate that the protective effects of innate immunity are attributed to the redistribution of BMCs between traditional immune and non-immune organs, which is defined by our team as immune coordination or immune redistribution (ICO/IRD). Actually, both LPS tolerance and trained immunity should be regarded as the result of ICO/IRD.

The next key question we should examine is which cell subset differentiated from BMCs could be responsible for local defense. We focus on the immunological regulation of dendritic cells (DCs) between immune organs and non-immune organs. DCs play a critical role in innate immunity and respond to microbial challenges actively, in a magnitude comparable to that of monocytes/macrophages and their production of cytokines and induction of distinct phenotypic differentiation involved in host defense [21]. For instance, traditional DCs release large amounts of IL-12 with high-level expression of CD40, CD80, and I-A/E molecules, while $CD11c^{-}CD45RB^{high}$ DCs release large quantities of IL-10 with low-level expression of CD40, CD80, and I-A/E molecules (Fig. S in the supplemental material). Previous studies have shown that IL-1 is essential for the regulation of hematopoietic activity as well as clonal proliferation of primitive myeloid BMCs [11, 22], and $CD11c^{-}CD45RB^{high}$ DCs derived from myeloid BMCs alleviate sepsis accompanied by acute SIRS [23]. Other investigators have also recently demonstrated that mesenchymal stem cells alleviate bacteria-induced liver injury in mice by inducing regulatory DCs [24]. Therefore, we surmised that IL-1β could trigger local proliferation and differentiation of BMCs into $CD11c^{-}CD45RB^{high}$ DCs at traditional immune organs and non-immune organs. Based on our results, the administration of IL-1β post-CLP can increase the number of BMCs, liver cells, and $CD11c^{-}CD45RB^{high}$ DCs in the bone marrow and liver (Fig. 3). It is clear that IL-1β can trigger local proliferation and differentiation of BMCs into $CD11c^{-}CD45RB^{high}$ DCs at bone marrow and liver. This provides the possibility for the mobilization and migration of BMCs and $CD11c^{-}CD45RB^{high}$ DCs between immune and non-immune organs, subsequently promoting these organs to fight sepsis in coordination. $CD11c^{-}CD45RB^{high}$ DCs, as a regulatory factor of host inflammatory response, have certain prevention and treatment potential for the treatment of sepsis [23]. A single intraperitoneal injection of $CD11c^{-}CD45RB^{high}$ DCs, BMCs, and liver cells has improved the prognosis of sepsis significantly (Fig. 4). In addition, $CD11c^{-}CD45RB^{high}$ DCs would exert negative immunomodulatory effects by releasing IL-10 [23, 25]. We theorize that only when the body produces enough inflammatory factors, such as IL-1β, to eliminate bacteria, can the negative feedback regulation of inflammation-BMC-$CD11c^{-}CD45RB^{high}$ DCs produce a large number of anti-inflammatory factors, such as IL-10, to eliminate the side effects left by inflammatory factors. In short, sepsis has a good outcome when the inflammatory and anti-inflammatory reactions reach equilibrium.

The parabiosis model has played an important role in studying innate immune regulation [26]. Nakatsuji et al. successfully performed parabiosis experiments, tracing the movement of natural adiponectin in different tissues [27]. To further observe how IL-1β regulates innate immunity by promoting migration and differentiation of BMCs into $CD11c^{-}CD45RB^{high}$ DCs in BM and liver, we performed GFP^{+}-C57BL/6 mouse parabiosis experiments. GFP^{+} mice were treated with IL-1β or anti-IL-1β antibody to illustrate how IL-1β promotes the migration and differentiation of GFP^{+}BMCs into $GFP^{+}CD11c^{-}CD45RB^{high}$ DCs in the BM and livers of C57BL/6 parabiont (Fig. 5(A, B)). C57BL/6 parabiont isolated from GFP^{+}-C57BL/6 parabiosis with IL-1β treatment showed significantly more $GFP^{+}CD11c^{-}$DCs/$CD11c^{-}CD45RB^{high}$ DCs in the BM and liver than those without IL-1β stimulation. Furthermore, antibodies against IL-1β successfully inhibited the migration and differentiation of $GFP^{+}CD11c^{-}$DCs and $CD11c^{-}CD45RB^{high}$ DCs (Fig. 5(C–E)). Therefore, IL-1β might be required for controlling the pathophysiology of sepsis *via* accumulation of $CD11c^{-}CD45RB^{high}$ DCs in systemic and local regions. The redistribution of BMCs triggered by IL-1β post-sepsis observed through the

parabiosis model allows for clear identification of the innate immune homeostasis and underlying mechanism in terms of BMCs flowing from the BM into circulation, infiltrating into peripheral organs, differentiating into the subsets of immune cells, and then returning to the blood.

In summary, the presence of IL-1β post-sepsis stimulates proliferation, migration, and differentiation of BMCs into $CD11c^{-}CD45RB^{high}$ DCs at traditional immune organs and non-immune organs, activating innate immunity characterized by the synergistic effect of these organs and attenuating sepsis.

MATERIALS AND METHODS

Reagents and Kits

IL-1β (product no. SRP8033) and collagenase D from *Clostridium histolyticum* were purchased from Sigma, St. Louis, MO. Ficoll-Paque was purchased from Axis-Shield Co., Oslo, Norway. RPMI 1640 medium, fetal calf serum (FCS), glutamine, penicillin, streptomycin, and HEPES were purchased from Beyotime Biotech Co. Ltd., Beijing, China. Polymyxin B was added to the cell culture medium at 10 μg/ml to neutralize the activity of endotoxin. 7-Aminoactinomycin D (7-AAD) was purchased from BD Biosciences, Mountain View, CA, USA. A biotinylated mouse dendritic cell enrichment cocktail, containing monoclonal antibodies (MAbs) that recognized antigens expressed on peripheral erythrocytes and leukocytes that were not DCs, was obtained from BD Biosciences.

Cell Culture

$CD11c^{-}CD45RB^{high}$ DCs isolated from BM and liver were cultured in 200 μl modified RPMI 1640 medium containing 10% FCS in 96-well round-bottom plates at 37 °C and then placed in a 5% CO_2 humidified incubator at 37 °C overnight for the next step. Subsequently, the incubated cells were collected by gentle pipetting, washed with a 10× excess volume of 1× BD IMag buffer, and centrifuged at 300×*g* for 10 min. The resulting supernatants were collected and stored until analysis at − 70 °C for the determination of IL-12 and IL-10 levels with enzyme-linked immunosorbent assay (ELISA) kits.

Cell Counting System

Cells in biological microscope images were counted utilizing ImageJ Macro Script Programming, a macro for the ImageJ platform (version 1.50 provided in the public domain by the National Institutes of Health, Bethesda, MD, USA), and IJ1 programming language on Fiji image-processing software (fiji.sc; in the public domain). After entering the tagged image file format (TIFF) file, the most relevant image processing steps were performed.

Mice/Animals

Approved by the Scientific Investigation Committee of the General Hospital of the Chinese People's Liberation Army (Beijing, China), we studied male C57BL/6 wild-type (WT) mice with 8- to 10-week-old and GFP-expressing mice on the C57BL/6 background. All animals were housed in cages separately in a temperature-controlled room with 12 h of light and 12 h of darkness in order to be acclimatized for at least 3 days prior to use. All experimental procedures were undertaken in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health [28].

CLP Model and Bacterial Sepsis Model

Polymicrobial sepsis was induced by cecal ligation and puncture (CLP) according to the method described by Chaudry et al. and Rittirsch et al. [29, 30]. A laparotomy was performed, and the cecum was exposed and ligated below the ileocecal junction, without causing bowel obstruction. The cecum was punctured once with an 18-gauge needle and then gently squeezed to ensure the leakage of the cecum contents through the puncture. The cecum was returned to the peritoneal cavity, and the body wall and skin incision were closed with a 6-0 silk suture. In CLP mice, large cecal ligation, which comprises 70% of the cecum, results in a survival rate of 10–20% (referred to as high-grade sepsis). In the bacterial sepsis models, bacteria were suspended in sterile 0.9% saline at a final concentration of 1×10^{10} CFU/ml. Mice were challenged with 0.1 ml of this suspension (1×10^{9} CFU) of *E. coli* O18. Sham-operated animals were subjected to laparotomy and intestinal manipulation but were neither ligated nor punctured. All animals received 1 ml saline subcutaneously immediately post-CLP and post-bacteria infection. The mice were observed for morbidity each 6 h after the CLP and bacteria infection, and any moribund mice were sacrificed immediately for the isolation of BMCs and liver immune cells. Mice were randomly assigned to four groups: the sepsis group, on which CLP was performed under anesthesia with an intraperitoneal injection of pentobarbital sodium (untreated group), and the low-, moderate-, and high-dose IL-1β groups, receiving 12.5,

25, and 50 μg/kg, respectively, by intraperitoneal injection immediately after CLP.

Parabiosis Model

For parabiosis, a lateral incision was made through the skin from the armpit to the iliac crest of each mouse, and then the subcutaneous fascia was dissected to create 0.5 cm of free skin. The armpit and iliac crest were attached using a 5–0 Vicryl suture, and the dorsal and ventral skins were closed by a continuous 5–0 suture line. The muscles of the anterior and posterior limbs were penetrated and attached using a 5–0 Vicryl suture to prevent flipping, and the retroauricular skins were attached by a continuous 5–0 suture line to prevent twisting. The sutures of muscles and retroauricular skins that were broken were sewed again. IL-1 was injected intraperitoneally into GFP mice immediately after they were joined in parabiosis with C57BL/6 mice. At 7 days post-parabiosis, the parabiotic mice were disconnected. C57BL/6 mice were separated from their parabiotic partners, stabilized for 2 days, and utilized for (i) exploring the protective effect of the redistribution of $CD11c^{-}CD45RB^{high}$ DCs triggered by IL-1 in sepsis and (ii) determining the number and percentage of $GFP^{+}CD11c^{-}CD45RB^{high}$ DCs that had been accumulated in C57BL/6 mice.

Harvest of BMCs, Live Cells, and Blood

BMCs were aspirated from the tibias and femurs of mice using a 25-gauge needle. The pellets of BMCs were resuspended in phosphate-buffered saline (PBS) containing 1% bovine serum albumin. After centrifugation, the sedimentary cells were collected. The isolated liver and spleen were placed in a 6-cm petri dish with sufficient collagenase D solution to thoroughly cover the bottom of the dish. The liver was then injected with a collagenase D solution using a 1-ml syringe and a 25-gauge needle, and the tissue was cut into small pieces using a pair of sharp scissors. The liver was incubated in a collagenase D solution for 30 min at 37 °C and was then gently passed through a cell strainer with the help of a plunger. Peripheral blood obtained in 2 to 3 ml of RPMI 1640 medium was treated with erythrocytolysin for cytokine detection.

Purification of DCs in BM and Liver

For the purification of DCs, we first separated mononuclear cells from BMCs or suspensions of tissue cells, such as liver cells, *via* Ficoll-Paque and Percoll medium. Secondly, BM and liver were selected from BMCs and liver cells by a Mouse Dendritic Cell Enrichment Set-DM (BD Biosciences). We used lineage cocktails of anti-mouse CD2 (LFA-2), anti-mouse CD3e (CD3 epsilon chain), anti-mouse CD45R/B220, anti-mouse CD49b (integrin α2 chain), anti-mouse CD147 (basigin), anti-mouse Ly-6G and Ly-6C (Gr-1), and anti-mouse TER-119/erythroid cells. Subsequently, T cells, B cells, NK cells, granulocytes, platelets, and red blood cells were deleted.

Gating Strategies for FACS of $CD11c^{-}CD45RB^{high}$ DCs in BM and LIVER

$CD11c^{-}CD45RB^{high}$ BM DCs and $CD11c^{-}CD45RB^{high}$ liver DCs were sorted as described in previous reports [22, 30] with some modifications. MAbs to R-phycoerythrin (PE)-conjugated CD45RB and allophycocyanin (APC)-conjugated CD11c were labeled on BM DCs and liver DCs for the detection and isolation of $CD11c^{-}CD45RB^{high}$ DCs. 7-AAD was used to delete dead cells. The $CD11c^{-}CD45RB^{high}$ BM DCs and $CD11c^{-}CD45RB^{high}$ liver DCs were isolated through a FACSAria II cell sorter. Fluorescence-activated cell sorting (FACS) was used to determine the purity of $CD11c^{-}CD45RB^{high}$ DCs to be > 95%. Those purified living cells were used for cell reinfusion.

Flow Cytometry for Detection of Phenotypes on $CD11c^{-}CD45RB^{high}$ DCs

MAbs to the following antigens were purchased from BD Pharmingen (San Diego, CA) for use in flow cytometry experiments: CD40, CD80, CD86, and I-A/I-E conjugated to fluorescein isothiocyanate (FITC), CD45RB conjugated to PE, and CD11c conjugated to APC. Isotypes corresponding to these MAbs, conjugated to the appropriate fluorochromes, were used as controls for non-specific binding of MAbs. Cells were washed in a FACS buffer comprising 5% FCS, 0.02% sodium azide, and 1 mM EDTA in PBS. FCS (15 μl) and 2 μl of purified anti-CD16/32 (FcεIII/II receptor) (2.4G2; BD Pharmingen, San Diego, CA) were added to each tube to inhibit non-specific binding. MAbs were added to the appropriate tubes and maintained at 4 °C for 30 min. Finally, cells were washed twice by centrifugation in FACS buffer at 350×*g* for 5 min and then fixed in 1% paraformaldehyde.

Adoptive Transfer of BMCs, Organ Cells, and $CD11c^{-}CD45RB^{high}$ DCs in BM and Organs

The potential of BMCs, organ cells, or $CD11c^{-}CD45RB^{high}$ DCs in BM and organs isolated from sham-operated mice or mice at 9 days post-CLP to improve the prognosis of sepsis was investigated. One

group was subjected to CLP, and the others were subjected to CLP with intraperitoneal injections of either BMCs (10^6, 5×10^6, or 10^7 per mouse), organ cells (10^6, 5×10^6, or 10^7 per mouse), CD11c$^-$CD45RB^{high} DCs (10^5, 5×10^5, or 10^6 per mouse) in BM, or CD11c$^-$CD45RB^{high} DCs (10^5, 5×10^5, or 10^6 per mouse) in organs. There were 10 mice in each subgroup. These cells were added to lactated Ringer's solution (1 ml/mouse) for intraperitoneal injection groups.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Experimental data were analyzed with Social Sciences (SPSS), version 18.0 (SPSS, Chicago, Inc., IL) and results were analyzed by *t* test, one-way variance (ANOVA) test with Bonferroni's *post hoc* test for pairwise comparisons, and log rank test. Fisher's exact test was used for chi-square analysis for comparison of survival between the groups. *P* values below 0.05 or less were considered significant.

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COMPLIANCE WITH ETHICAL STANDARDS

All experimental procedures were undertaken in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health [28].

Conflict of Interest. The authors declare that they have no conflicts of interest.

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