

Alteration of macrophage immune phenotype in a murine sepsis model is associated with susceptibility to secondary fungal infection

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Abstract

Background: Secondary fungal infection is a major complication in patients with sepsis-associated immunosuppression. However, sepsis-induced immune alterations related to fungal susceptibility have not been well characterized.

Objective: To determine kinetic changes in the immune phenotype by determining the proportion of T cells, B cells and macrophages, and especially the expression of an immune exhaustion marker PD-1, in murine sepsis. In addition, sepsis-induced alterations of these immune cells were assessed in relation to susceptibility to secondary fungal infection.

Methods: Cecal ligation and puncture (CLP) was used as a mouse sepsis model, with *Candida albicans* as the secondary systemic fungal infection. Splenic T cells, B cells and macrophages were assessed by flow cytometry.

Results: Alterations in T cell and B cell numbers and the proportion of PD-1 expressing T cells and B cells in CLP mice were not clearly related to susceptibility to secondary *Candida* infection. By contrast, changes in levels of CD86⁺-activated macrophages, and the proportion of the PD-1⁺ population among the CD86⁺ macrophages in CLP mice were found to be related to secondary fungal infection susceptibility.

Conclusion: Macrophage activation and exhaustion might be a significant determinant in susceptibility to fungal infection, and outcomes of infection. This study provided more comprehensive knowledge pertinent to patient evaluation and therapeutics design in restoring host defenses against secondary fungal infection in those with sepsis.

Key words: macrophage, immune phenotype, sepsis, CD86, PD-1

Citation:

Vu, C. T. B., Thammahong, A., Leelahavanichkul, A., Ritprajak, P. (2022). Alteration of macrophage immune phenotype in a murine sepsis model is associated with susceptibility to secondary fungal infection. *Asian Pac J Allergy Immunol*, 40(2), 163-172. <https://doi.org/10.12932/ap-170519-0565>

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Introduction

Sepsis, a life-threatening condition caused by severe systemic infection, is one of the most common causes of death in critically ill patients.^{1,2} Immune responses in patients with sepsis consist of a cytokine-mediated hyper-inflammatory phase, which most patients survive, and a subsequent immunosuppressive phase (or immune paralysis) where immune cells are exhausted and dysfunctional. These two immune events in sepsis may occur simultaneously or sequentially.¹

Fungal infections have emerged as a problem over the last few decades in relation to an increased incidence of anti-fungal drug resistance. In addition, the immune status in patients is a crucial determinant for susceptibility to and outcome of fungal infections. Secondary fungal infections are a main cause of nosocomial infections worldwide, leading to high morbidity and mortality.^{3,4} Patients with sepsis-associated immunosuppression have increased susceptibility to secondary fungal infections, such as invasive candidiasis and aspergillosis.^{5,6} As an opportunistic pathogenic fungus, *Candida albicans* remains the leading cause of nosocomial bloodstream infections. The mortality rate of candidemia in intensive care patients is high, being approximately 50% without septic shock and 90% with septic shock.⁷

Programmed cell death-1 (PD-1 or CD279) is an immune checkpoint molecule, which triggers an inhibitory signal that modulates immune cell activation and function.⁸ Persistent upregulation of PD-1 on T cells in chronic viral and bacterial infections is associated with T-cell exhaustion.⁹ Substantial PD-1 expression on splenocytes and on circulating immune cells, including monocytes and T cells, has been observed in patients with severe sepsis.^{10,11} In addition, mice with cecal ligation and puncture (CLP)-induced sepsis displayed high PD-1 expression on splenic T cells, while blockade of the PD-1 pathway attenuated sepsis severity.^{12,13}

A previous study investigated the expression of PD-1 on T cells, B cells and CD11b⁺ cells in a CLP model, and examined the role of PD-1 in sepsis by pre-treatment with anti-PD-1 monoclonal antibody.¹³ However, the observation of PD-1 expression was limited to day 1 post-sepsis, and the authors only determined the role of PD-1 in the context of primary sepsis. In this current study, kinetic changes in the proportion of T cells, B cells and macrophages were examined, along with PD-1 expression by these cells, and their association with secondary fungal infection after sepsis, since this was a major complication in septic patients with immune paralysis.¹¹ *C. albicans* was used as the secondary fungal infection in the mouse model because it was a leading cause of opportunistic infection worldwide, and had been widely used as an experimental model of fungal infection. The findings provided more comprehensive knowledge of sepsis-induced immune alterations and their relation to secondary infection, which could be used for patient evaluation and the selection of therapeutic approaches.

Methods

Animals and the animal model

All animal procedures were conducted according to guidelines of the National Institutes of Health (NIH), USA, and were approved by the Institutional Animal Care and Use Committee (IACUAC) of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (protocol number 031/2561). Six to eight-week-old female C57BL/6 mice were purchased from the National Laboratory Animal Center of Mahidol University, Bangkok, Thailand, and were housed in the Animal Facility Center of the Medical School, Chulalongkorn University.

CLP was performed under isoflurane anesthesia as described previously.¹⁴ Briefly, one centimeter from the cecal tip, the cecum was ligated and punctured with a 21-gauge needle. For the control mice, the cecum was exteriorized, but neither ligated nor punctured. Primaxin IV (Merck, Kenilworth, NJ, USA) at 10 mg/kg in 100 μ L normal saline (NSS) was subcutaneously administered immediately after CLP, and once daily thereafter. For post-operative analgesia, 10 mg/kg tramadol (Madhya Pradesh, India) in 100 μ L NSS was subcutaneously administered immediately after the operation, and at 6 h and 24 h later.

The CLP and control mice were secondary infected with *C. albicans* SC5314 (or ATCC MYA-2876). The yeast was cultured on yeast extract peptone dextrose (YPD) agar (HiMedia, Mumbai, India) at 30°C for 48 h, and was then harvested and washed with phosphate-buffered saline (PBS). Yeast cells at 2×10^5 cells in 100 μ L NSS were intravenously administered via a tail vein at day 1, day 5 and day 12 post-CLP, under isoflurane anesthesia.

Blood analysis of mice

Renal and liver injury was assessed by the level of serum creatinine and alanine transaminase (ALT), using QuantiChrom Creatinine and EnzyChrom Alanine Transaminase assay kits (BioAssay Systems, Hayward, CA, USA), respectively. The detection of serum cytokines was performed with enzyme-linked immunosorbent assay (ELISA) kits (Biolegend, San Diego, CA, USA). To determine blood fungal burden, different volumes of blood were plated onto Sabouraud dextrose agar (SDA) containing 0.1% chloramphenicol (Thermo Scientific, Basingstoke, UK), and were then incubated at 35°C, for 72 h before fungal colony counting.

Flow cytometry of splenocytes

Fluorochrome-conjugated antibodies were obtained from Biolegend (San Diego, CA, USA): anti-mouse F4/80 (BM8), anti-mouse CD86 (GL1), anti-mouse MHC class II (M5/114.15.2), anti-mouse PD1 (29F.1A12), anti-mouse CD4 (RM4-5), anti-mouse CD8 (53-6.7), and anti-mouse B220 (RA3-6B2). Splenocytes were prepared, stained with the fluorochrome-conjugated antibodies and processed for flow cytometry on a CytoFLEX system according to the manufacturer's protocols, and data were analyzed with Kaluza software (Beckman Coulter, Brea, CA, USA).

Total numbers of splenocytes were counted. The number of each type of immune cell was then calculated by multiplying its percentage from the flow cytometry analysis with the total numbers of splenocytes.

Statistical analysis

SPSS Statistics for Windows version 23.0 (IBM Corp., Armonk, NY, USA) and Prism 7.0 (GraphPad, San Diego, CA, USA) were used for statistical analysis. Data were presented as mean \pm standard error (SE). Student's *t*-test and One-way ANOVA with Bonferroni's post-hoc analysis were used to compare the differences between two groups and more than two groups, respectively. The log-rank test was used for survival analysis. Significant differences were accepted at $p < 0.05$.

Results

Innate and adaptive immune response phase in the CLP mouse model

To determine the phase of the immune response following sepsis, serum parameters were determined on day 1, 5 and 12 post-CLP. On day 1 post-CLP, serum creatinine and ALT were highly increased in CLP mice, indicating acute renal and liver injury, respectively (Figure 1A-B). Consistent with these tissue injuries, innate pro-inflammatory cytokines, TNF- α and IL-6, were markedly upregulated in serum of CLP mice, which defined a hyper-inflammatory state (Figure 1C-D).

Increased serum IL-10 production was also observed on day 1 post-CLP (Figure 1E). Serum creatinine, ALT, TNF- α , IL-6, and IL-10 declined to baseline levels (equal to those of the control) from day 5 to 12 post-CLP (Figure 1A-E). Conversely, IFN- γ was notably increased at day 5, and partially decreased at day 12 post-CLP (Figure 1F). The high upregulation of organ injury markers and pro-inflammatory cytokines at an early phase of sepsis (day 1 post-CLP) might indicate a hyper-inflammatory state. By contrast, the decline in these markers and pro-inflammatory cytokines, in concert with the upregulation of the protective cytokine IFN- γ ,¹⁵ suggested a protective or recovery state of sepsis.

Alteration of T cell, B cell and macrophage numbers during sepsis

The numbers of immune cells during sepsis were determined, and compared with the immune phases that were identified in Figure 1. CD4 T cells, CD8 T cells, and B cells were identified as CD3⁺CD4⁺, CD3⁺CD8⁺, and B220⁺ cells, respectively (Figure 2A). In CLP mice, total splenocyte numbers were reduced at day 1, restored by day 5, and significantly increased on day 12 post-CLP (Figure 2B). At day 1 post-CLP, CD4 T cell, CD8 T cell, and B cell numbers in CLP splenocytes were decreased in accordance with total splenocyte numbers (Figure 2C-H). Loss of CD4 T CD8 T and B cells in CLP splenocytes was further observed at day 5 and day 12 post-CLP (Figure 2C-H), contrary to increased total splenocyte numbers (Figure 2A).

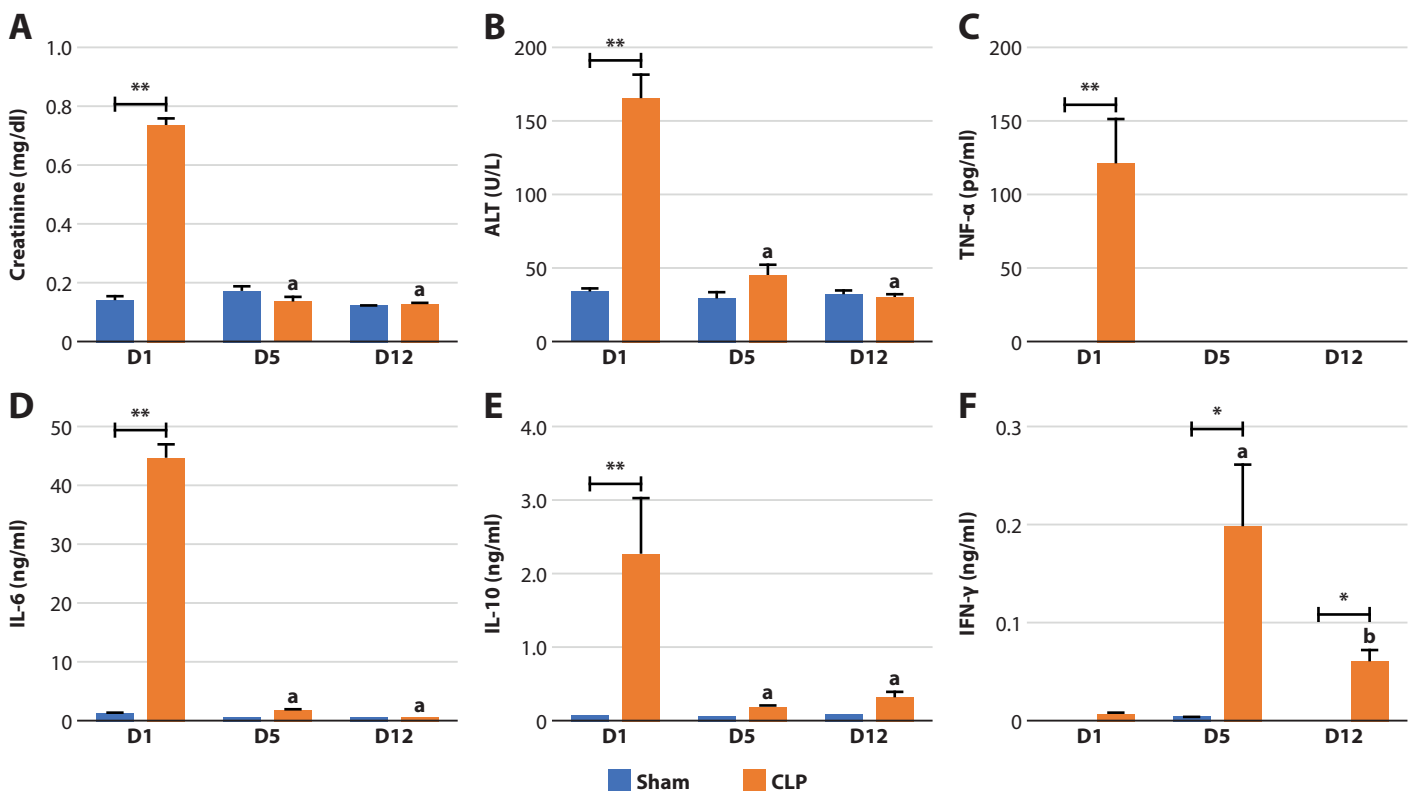


Figure 1. Kinetic changes of renal failure, liver injury and pro-inflammatory cytokines in CLP mouse model.

(A) Serum creatinine, (B) serum alanine transaminase (ALT), and (C-F) serum cytokines of CLP or sham control mice were determined at day 1 (D1), day 5 (D5) and day 12 (D12) post-CLP. $n = 4$ /group/time point. Data represent two independent experiments. * $p < 0.05$; ** $p < 0.01$; ^a $p < 0.05$ vs. D1; ^b $p < 0.05$ vs. D5.

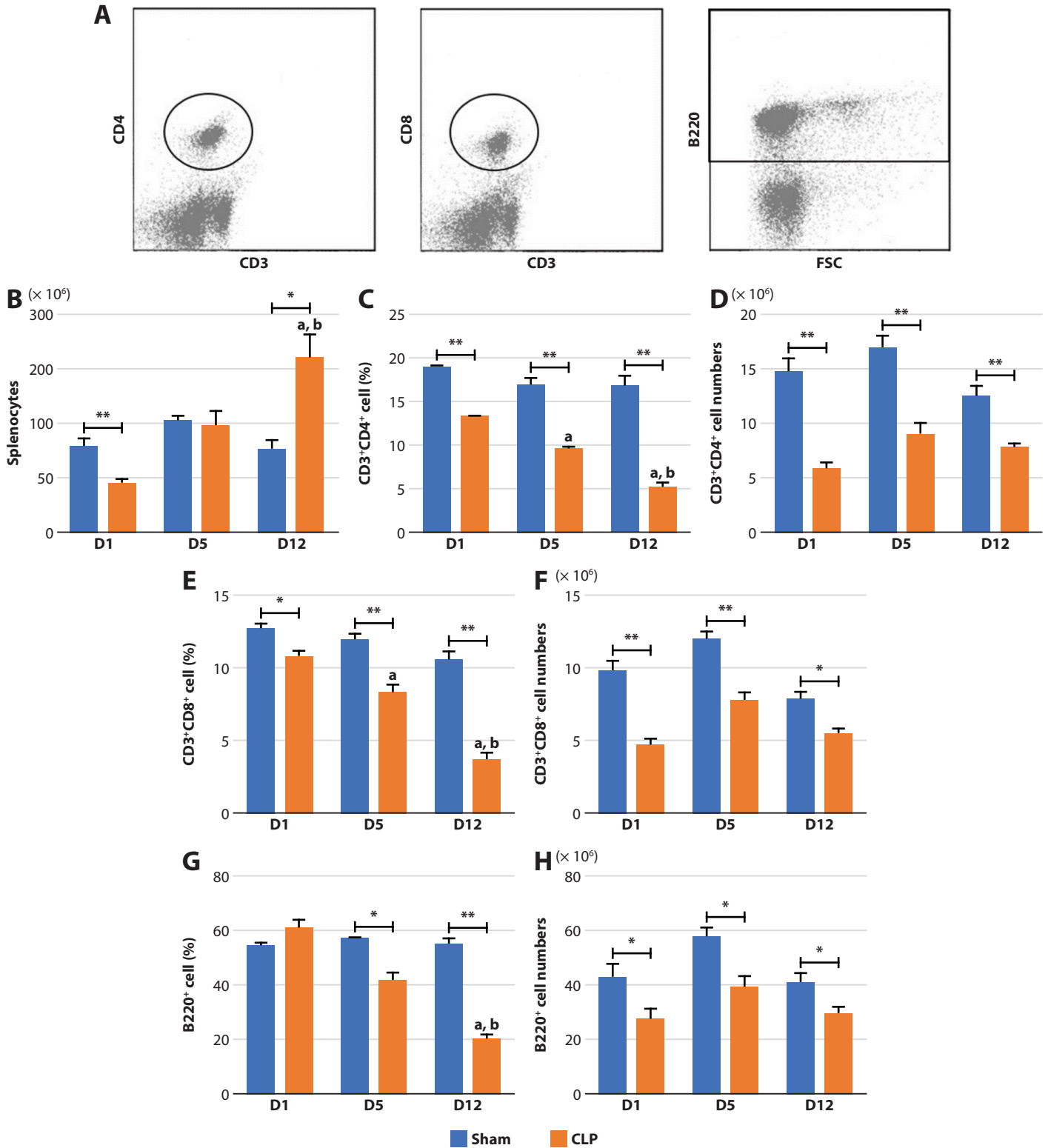


Figure 2. Alteration of T cell and B cell numbers during sepsis.

(A) Dot plot analysis of CD4 T cells (CD3⁺CD4⁺ cells), CD8 T cells (CD3⁺CD8⁺ cells) and B cells (B220⁺ cells), (B) Total splenocyte numbers, (C) percentage of splenic CD4 T cells, (D) numbers of splenic CD4 T cells, (E) percentage of splenic CD8 T cells, (F) numbers of splenic CD8 T cell, (G) percentage of splenic B cells, and (H) numbers of splenic B cell of CLP and sham control mice were assessed at day 1 (D1), day 5 (D5) and day 12 (D12) post-CLP. *n* = 5/group/time point. Data represent two independent experiments. **p* < 0.05; ***p* < 0.01; ^a*p* < 0.05 vs. D1; ^b*p* < 0.05 vs. D5.

As an innate immune cell, macrophages played multiple roles in sepsis, with extensive effects on inflammatory and homeostatic processes.^{16,17} For this reason, we determined macrophage numbers and activation. Macrophages were identified as F4/80⁺ cells, and their activation state was determined based on the expression of CD86 and MHC class II (Figure 3A). At day 1 post-CLP, the percentage of macrophages (F4/80⁺ cells) in spleen of CLP mice was comparable to that of the control (Figure 3B); however, macrophage numbers were significantly decreased (Figure 3C). Opposing the reduction in adaptive cell numbers, macrophages in CLP splenocytes were slightly increased at day 5 post-CLP, and substantial numbers of macrophages were detected at day 12 post-CLP

(Figure 3C), consistent with elevated splenocyte numbers (Figure 2B). The percentage of F4/80⁺ MHC class II⁺ cells was decreased on days 5 and 12 post-CLP (Figure 3D). Notably, the proportion of CD86⁺F480⁺ cells was reduced at day 5; however, it was greatly increased on day 12 post-CLP (Figure 3E).

Altogether, the numbers of splenic T cells and B cells were reduced through all phases of sepsis. Splenic macrophage numbers were decreased in the early phase of sepsis (day 1 post-CLP), but were increased in the late phase (day 12 post-CLP). Splenic macrophages might lose their function, with less activation markers at day 5 post-CLP; however, cell numbers and activation were restored at the late phase of sepsis.

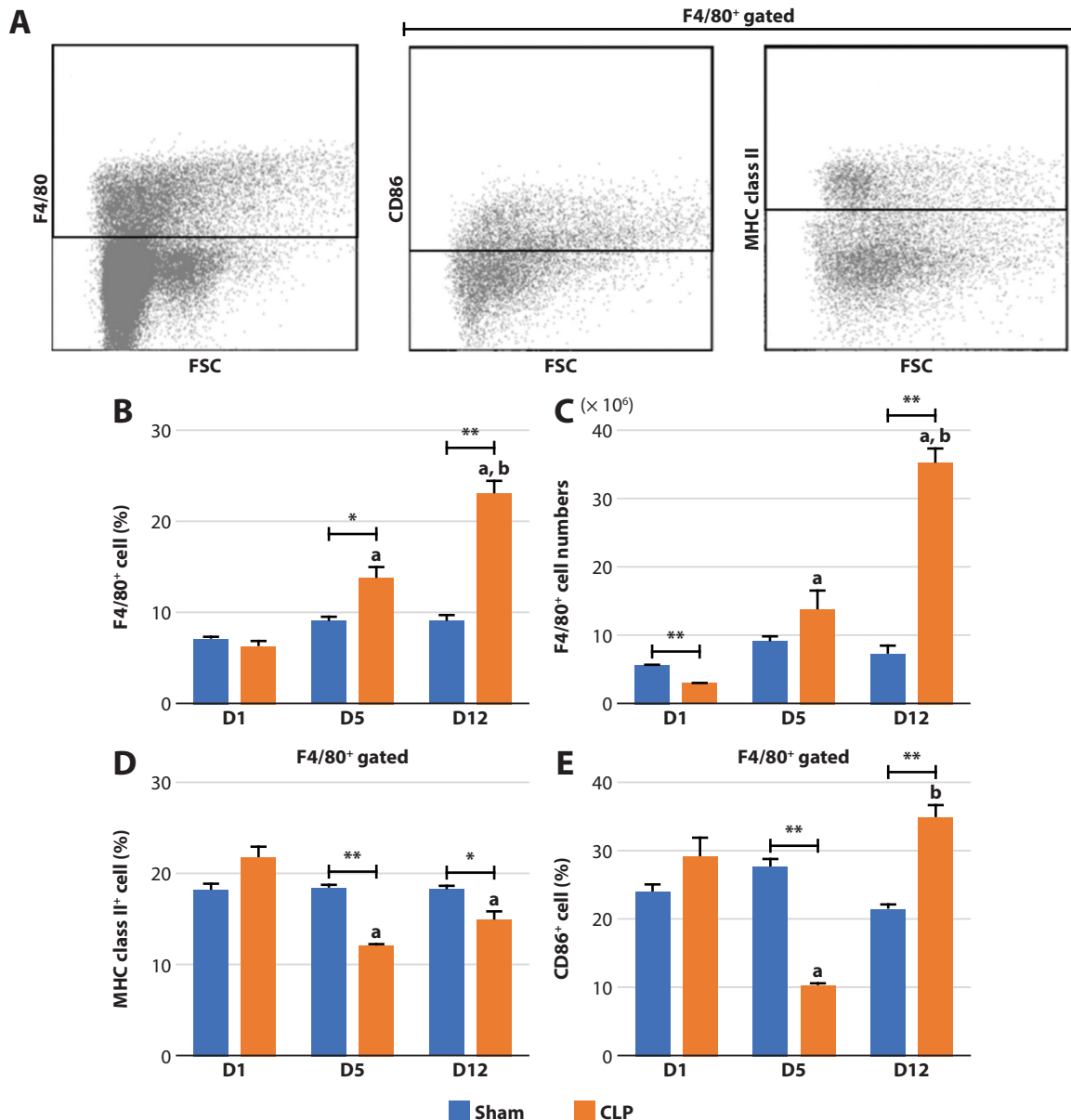


Figure 3. Changes in macrophage numbers and activation during sepsis.

(A) Dot plot analysis of macrophages. The bulk splenic macrophages were identified by F4/80 marker. The electronic gate was placed on F4/80⁺ population, and the macrophage activation markers, CD86 and MHC class II were analyzed. (B) Percentage of splenic macrophage, (C) numbers of splenic macrophage, (D) percentage of CD86⁺ macrophages (F4/80⁺CD86⁺ cells), and (E) percentage of MHC class II⁺ macrophages (F4/80⁺MHC class II⁺ cells) of CLP and sham control mice were determined at day 1 (D1), day 5 (D5) and day 12 (D12) post-CLP. $n = 5/\text{group}/\text{time point}$. Data represent two independent experiments. * $p < 0.05$; ** $p < 0.01$; ^a $p < 0.05$ vs. D1; ^b $p < 0.05$ vs. D5.

PD-1 expressing immune phenotype during sepsis

PD-1 expression was a characteristic marker of immune cell exhaustion in several fatal diseases, including sepsis.¹ To verify an immune exhaustion state in the CLP mouse model, we investigated the PD-1 expressing immune phenotype in splenic CD4 T cells, CD8 T cells, B cells and macrophages by flow cytometry (Figure 4A). The proportions of PD-1⁺ cells in splenic CD4 T cells (CD3⁺CD4⁺ cells) and B cells (B220⁺ cells) from CLP mice were notably increased at day 5 post-CLP, and these increases were sustained until day 12 post-CLP, when compared to the control (Figure 4B, left and right panel). For the splenic CD8 T cell population (CD3⁺CD8⁺ cells), the high frequency of PD-1⁺ cells was only observed at day 12 post-CLP (Figure 4B, middle panel).

The proportion of PD-1⁺ cells in splenic macrophages (F4/80⁺ cells) of mice with sepsis was markedly increased at day 1 post-CLP, and decreased to baseline levels at day 5 and 12 post-CLP (Figure 4C, left panel). Similarly, increased PD-1⁺ cells were found in MHC class II⁺F480⁺ macrophages in CLP splenocytes at day 1 post-CLP, and these numbers were decreased on days 5 and 12 post-CLP (Figure 4C, middle panel). A high frequency of PD-1⁺ cells was also found in splenic CD86⁺F480⁺ macrophages of mice with sepsis on days 1 and 5 post-CLP (Figure 4C, right panel).

Collectively, our data indicated that macrophages and adaptive immune cells exhibited an exhausted phenotype in the early phase (day 1 post-CLP) and late phase (day 5–12 post-CLP) of sepsis, respectively.

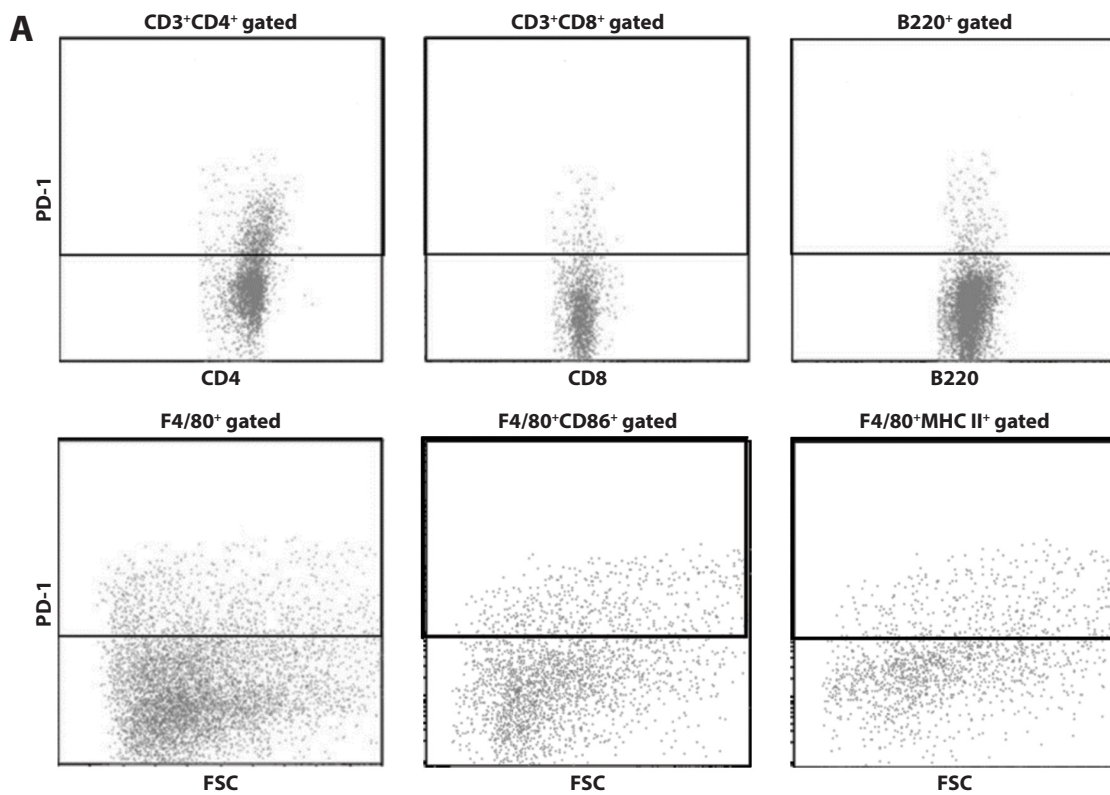


Figure 4. Kinetic expression of PD-1 molecules on immune cells during sepsis.

(A) Dot plot analysis of PD-1⁺ cells. Upper panel, splenic CD4 T cells (CD3⁺CD4⁺ cells), CD8 T cells (CD3⁺CD8⁺ cells) and B cells (B220⁺ cells) were identified by the electronic gates based on their lineage markers, and PD-1⁺ fractions were subsequently gated in each immune cell subset. Lower panel, splenic macrophages (F4/80⁺ cells), CD86⁺ macrophages (F4/80⁺CD86⁺ cells), and MHC class II⁺ macrophages (F4/80⁺MHC class II⁺ cells) were identified by the electronic gates, and PD-1⁺ fractions were subsequently gated. Percentage of PD-1⁺ cells in (B) CD3⁺CD4⁺ cells, (C) CD3⁺CD8⁺ cells, (D) B220⁺ cells, (E) F4/80⁺ cells, (F) F4/80⁺CD86⁺ cells, and (G) F4/80⁺MHC class II⁺ cells from spleens of CLP or sham control mice were examined at day 1 (D1), day 5 (D5) and day 12 (D12) post-CLP. *n* = 5/group/time point. Data represent two independent experiments. **p* < 0.05; ***p* < 0.01; ^a*p* < 0.05 vs. D1; ^b*p* < 0.05 vs. D5.

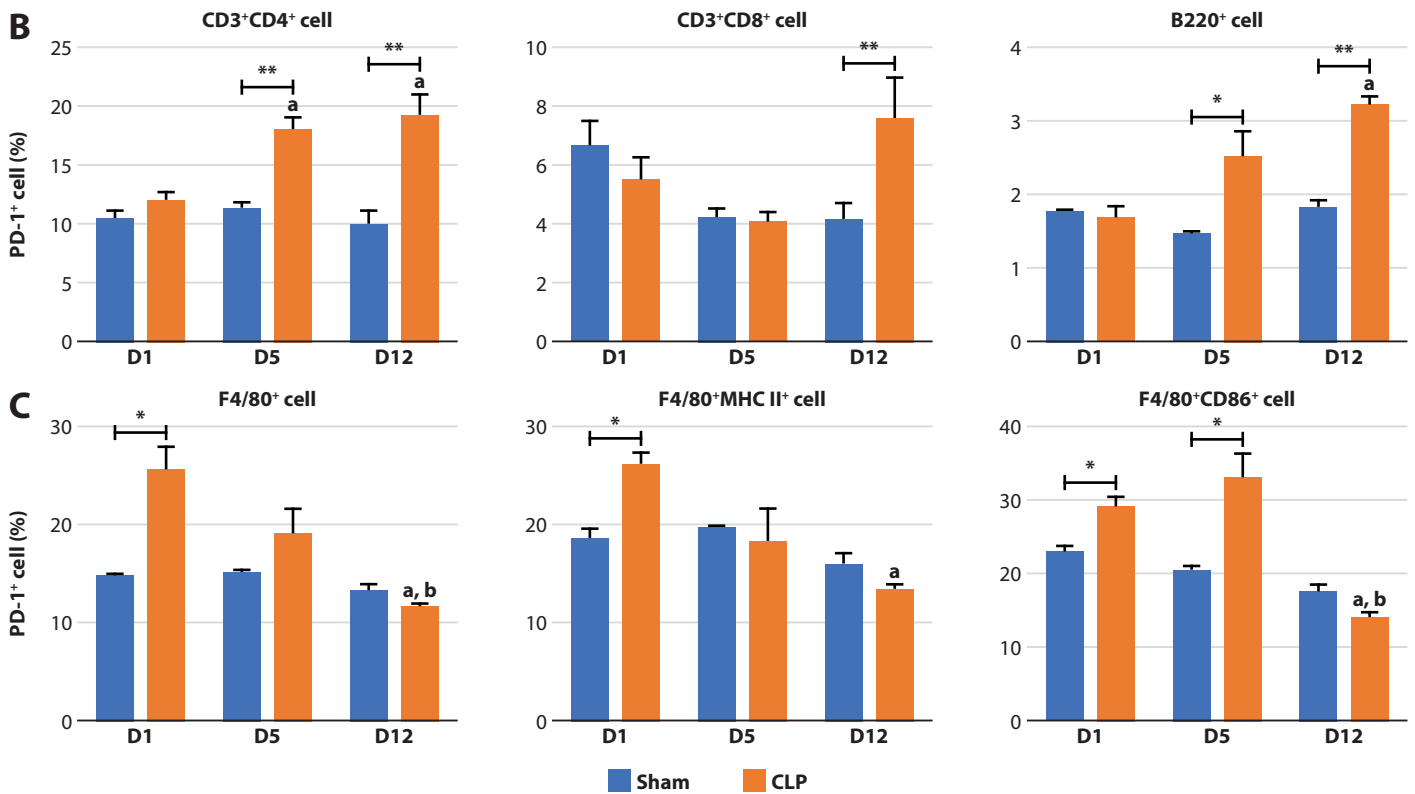
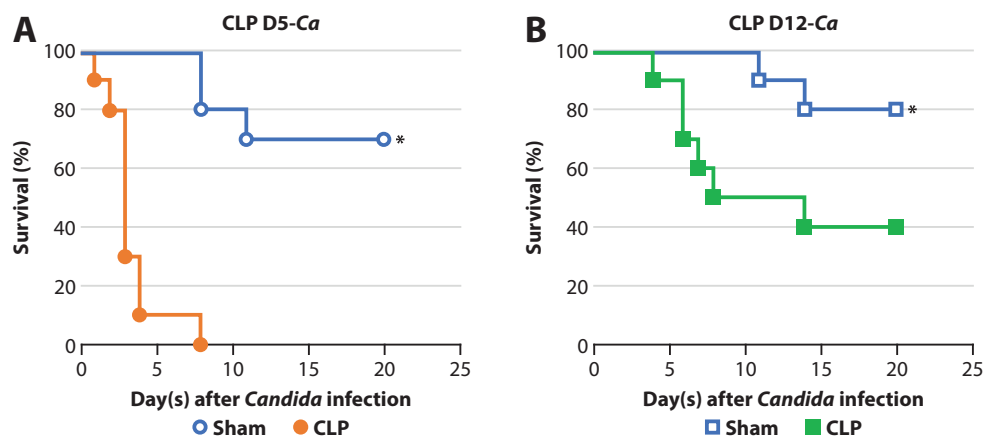


Figure 4. (Continued)

The influence of immune phenotype during sepsis on secondary fungal infection

To evaluate the clinical significance of the immune phenotype during sepsis, CLP and control mice were systemically infected with *C. albicans* on days 5 and 12 post-CLP. These time points were selected based on the changes in macrophage activation (Figure 3), and the alteration of PD-1 expression on macrophages, T cells and B cells (Figure 4). In the CLP group, fungal infection at day 5 post-CLP (CLP D5-Ca)

resulted in a mortality rate of 100% (Figure 5A), while infection at day 12 post-CLP (CLP D12-Ca) produced a 40% mortality rate (Figure 5B). Consistent with the mortality rate, fungal burden in the blood of the CLP D5-Ca group was high on day 3 post-fungal infection, when compared to those of the infected control mice (Figure 5C). By contrast, fungal burden of the CLP D12-Ca group was comparable to that of the infected control mice (Figure 5C).

Figure 5. Survival rate and fungal burden of sepsis mice with secondary *Candida* infection.

Survival analyses of CLP or sham control mice infected with *C. albicans* at (A) day 5 post-CLP (CLP D5-Ca), and (B) day 12 post-CLP (CLP D12-Ca). (C) Fungal burden in the blood of CLP or sham control mice infected with *C. albicans* at day 5 post-CLP (CLP D5-Ca), and day 12 post-CLP (CLP D12-Ca) were determined after intravenous *Candida* inoculation at the indicated time points. $n = 10$. * $p < 0.05$; † $p < 0.05$ vs. CLP D12-Ca.

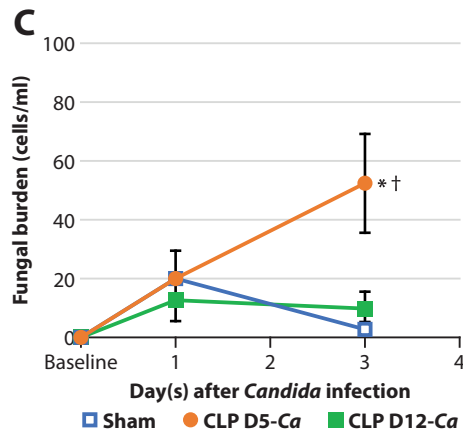


Figure 5. (Continued)

Serum parameters were evaluated to assess organ injury and immune status after secondary fungal infection. At day 3 post-fungal infection, only the CLP D5-Ca group showed a significant increase in serum creatinine and ALT, which indicated acute renal failure and liver injury, respectively (Figure 6A-B). The highest levels of serum TNF- γ and IL-6 were also detected in the CLP D5-Ca group (Figure 6C-D), which were related to the organ injury. Although IL-10 was enhanced in the serum of CLP D5-Ca mice (Figure 6E),

it did not reduce disease severity or the mortality rate (Figure 5A). IFN- γ had been shown to play a pivotal role in anti-fungal immunity.¹⁸ Therefore, we examined its level in serum following secondary candidiasis. Serum IFN- γ levels were significantly lower in both the CLP D5-Ca and CLP D12-Ca groups (Figure 6F), which possibly resulted from reduced T cell numbers and increased PD-1 expressing T cells (Figures 3 and 4). Notably, IFN- γ levels in the CLP D12-Ca group did not differ from those of the CLP D5-Ca group.

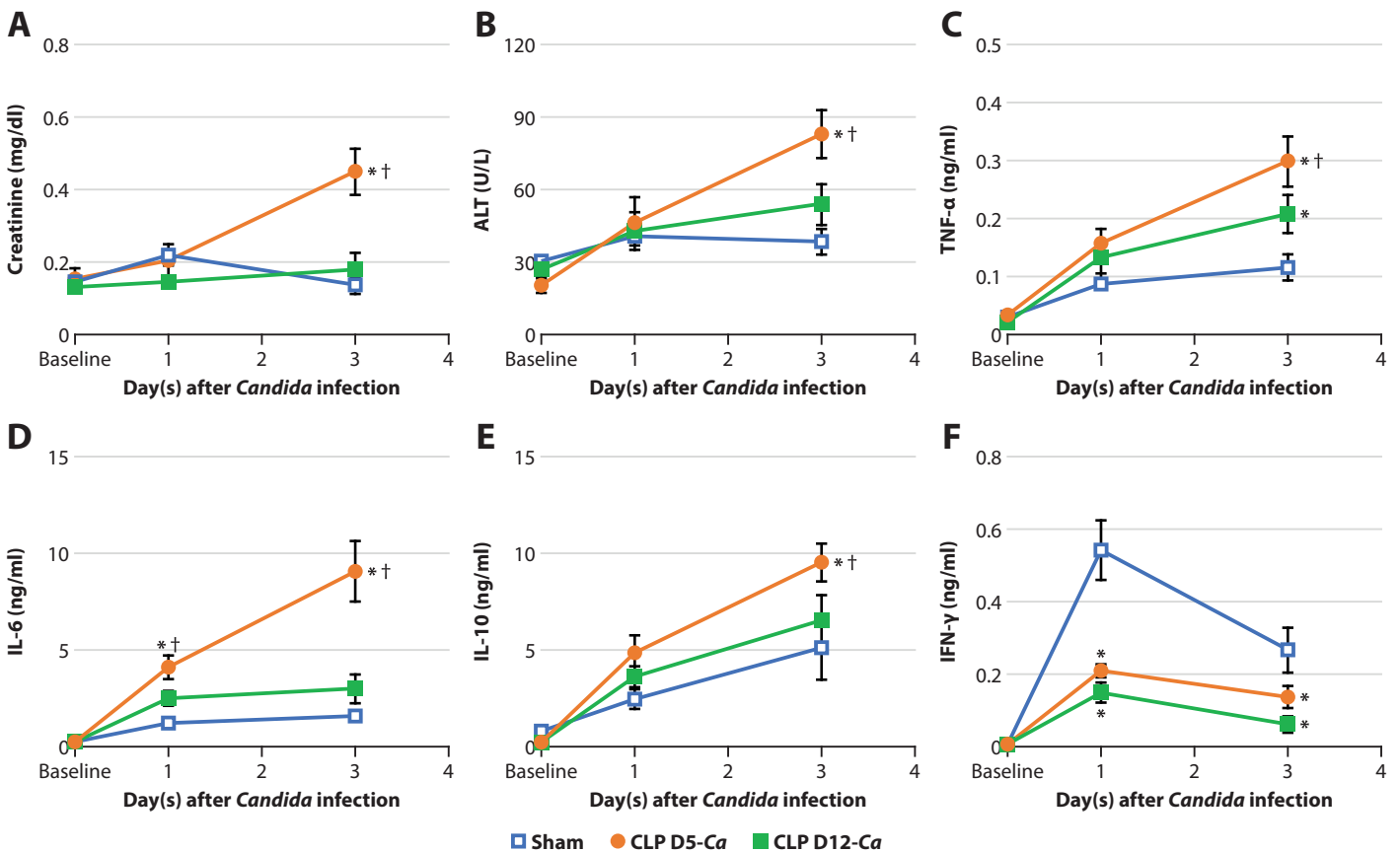


Figure 6. Severity of sepsis mice with secondary *Candida* infection.

(A) Serum creatinine, (B) serum alanine transaminase (ALT), and (C-F) serum cytokines of CLP or sham control mice infected with *C. albicans* at day 5 post-CLP (CLP D5-Ca) were assessed after intravenous *Candida* inoculation at the indicated time points. $n = 10$. * $p < 0.05$; † $p < 0.05$ vs. CLP D12-Ca.

Our data suggested that mice with sepsis were highly susceptible to secondary *Candida* infection at day 5 post-CLP, and disease severity might be due to acute organ injury and the hyper-inflammatory reaction. Mice with sepsis developed resistance against secondary *Candida* infection, with lower inflammatory responses, after day 12 post-CLP.

Discussion

Secondary fungal infection frequently occurs as a complication of bacterial sepsis due to concomitant immunosuppression.¹ Our CLP-induced sepsis model exhibited hyper-inflammation (cytokine storm) as early as day 1 post-CLP, and immune exhaustion thereafter, as demonstrated by an increased susceptibility to secondary *Candida* infection at 5 days post-CLP. CLP mice eventually restored their immunity to overwhelm the fungal infection after 12 days post-CLP.

Hyper-inflammation developed in the early phase (1 day post-CLP) of the CLP mouse model, and this response was one underlying mechanism that perpetuated organ injuries (Figure 1).¹⁹ In the same phase, a reduction in total splenocytes, T cells, B cells, and macrophages was also observed (Figures 2, 3), and this loss of immune cells was probably the result of inflammation-induced apoptosis.^{19,20} Although an anti-inflammatory cytokine, IL-10, also increased at day 1 post-CLP, it was merely a negative feedback of inflammatory regulation to protect against self-damage.²¹

The adaptive immune cells were persistently decreased throughout all phases of sepsis (Figure 2), and cell loss in the later phase of sepsis (5 to 12 days post-CLP) might be, in part, due to immune exhaustion. PD-1 plays a pivotal role in immune suppression owing to its diverse immunoregulatory properties, and an increase and sustained expression of PD-1 is a hallmark of an exhausted immune phenotype in lethal infectious diseases, including sepsis.¹⁸ One well-known function of PD-1 is that it can direct exhausted immune cells to undergo apoptosis.¹³ Therefore, the loss of adaptive immune cells during the late phase of sepsis (day 5 to 12 post-CLP; Figure 2) was probably associated with the upregulation of PD-1 molecules (Figure 4). Although there was sustained exhaustion and reduction of T cells and B cells from day 5 to 12 post-CLP, mice with sepsis only displayed high susceptibility to secondary *Candida* infection at day 5, and not at day 12 post-CLP.

The number of activated macrophages (F4/80⁺CD86⁺ and F4/80⁺MHC class II⁺ cells) at day 5 post-CLP was notably decreased, and about half the F4/80⁺CD86⁺ population expressed PD-1 (Figures 3 and 4). Our results were consistent to previous observations of human and mouse sepsis, which found a decreased number of CD86⁺ and HLA-DR⁺ (or MHC class II⁺) cells in blood.^{10,22} In addition, substantial PD-1 expression was detected on peritoneal macrophages from mice with sepsis, and this was associated with macrophage dysfunction.²³ Therefore, the deficit of activated macrophages and their exhausted phenotype at day 5 post-CLP might contribute to the increased susceptibility to secondary *Candida* infection (Figure 5). By contrast, macrophages in mice with sepsis

at day 12 post-CLP were reconstituted, and the increased macrophage numbers were related to an increase in total splenocytes (Figures 1B and 3C). The macrophage reconstitution after sepsis was possibly from hematopoietic stem cell-derived macrophages or self-renewal tissue resident macrophages, as described previously.²⁴ A large number of CD86⁺F4/80⁺ activated macrophages were also observed at day 12 post-CLP, in accordance with decreased PD-1 expression on macrophages (Figure 3E and 4C), and these reinvigorated macrophages might account for the resistance to secondary *Candida* infection. In this regard, the activation and exhaustion of macrophages perhaps contributed to host defenses against secondary fungal infection during sepsis.

T helper 1 (Th1) cell responses are crucial for protection against *Candida* bloodstream infection, and IFN- γ is a key cytokine mediating anti-*Candida* immunity.¹⁸ Hence, systemic IFN- γ production in CLP mice, both pre- and post-fungal infection, was investigated. The level of serum IFN- γ from day 5 to 12 post-CLP was inconsistent with the reduced T cell numbers (Figure 1 and 2). It was likely that IFN- γ was not involved in fungal clearance, as consistently low IFN- γ production was observed in the CLP D5-*Ca* and CLP D12-*Ca* groups after *Candida* infection (Figure 6). Even so, IFN- γ has recently been used as an adjunctive therapy against fungal infection,^{25,26} though treatment with IFN- γ alone was not effective.²⁷ Although IL-17 has been suggested to play a key role in *Candida* eradication, its role is restricted to mucosal infections.²⁸ Furthermore, our recent investigation demonstrated that IFN- γ , but not IL-17, was a significant protective cytokine for host defense against secondary fungal infection in murine sepsis.²⁹

In summary, the reduced numbers of T and B cells during sepsis were possibly related to PD-1 expression; however, these changes were irrelevant to the susceptibility to secondary *Candida* infection. By contrast, for secondary fungal infection during sepsis, macrophage exhaustion and activation were possibly involved in susceptibility and resistance, respectively. In addition, IFN- γ might be a significant protective cytokine, enforcing protective immunity to eradicate *Candida* infection from the bloodstream. In the last decade, anti-PD-1 therapy has shown high efficacy in promoting immune responses against fungal infection and bacterial sepsis. Blockade of the PD-1 pathway has improved survival in primary and secondary *Candida* infections through enhanced IFN- γ production, and the restoration of antigen-presenting cell function.³⁰ In addition, combination immunotherapy of an anti-PD-1 monoclonal antibody (Nivolumab) and recombinant IFN- γ was highly effective in improving survival outcomes of patients with extensive abdominal mucormycosis that was unresponsive to conventional therapy.²⁶ A recent clinical trial has also demonstrated the high efficacy of anti-PD-1 therapy in patients with sepsis-associated immunosuppression, with no apparent adverse immune effects.³¹ The aforementioned evidence revealed that PD-1 was a promising target for immunotherapy of sepsis and fungal infections.

Conclusion

This study characterized the kinetic alterations of immune cell populations and immune phenotypes in CLP-induced sepsis, and their association with secondary fungal infection. The findings indicated that an alteration of innate immune phenotypes during sepsis might be reflected in the innate immune activation state and its function, which might be relevant in susceptibility to secondary fungal infections. From a clinical point of view, our results show the need for further studies in patients with sepsis-associated immunosuppression, to ascertain the implication of innate activation and exhaustion markers in secondary fungal infection. This fundamental knowledge would help in the design of therapeutic strategies aimed at restoring immune function in patients with sepsis, for fungal clearance.

Conflict of interests

This research was conducted in the absence of any financial interests.

Acknowledgements

This project was supported by Faculty Research Grant DRF 60003, the Faculty of Dentistry, Chulalongkorn University, the Thailand Government Fund (RSA-6080023), the Thailand Research Fund (RES_61_202_30_022), and the Ratchadaphiseksomphot Endowment Fund (76001-HR) at Chulalongkorn University. Research Units were supported by the Ratchadaphiseksomphot Endowment Fund. Chau T. B. V. was supported by The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship. We thank the Oral Biology Research Center, Faculty of Dentistry, Chulalongkorn University for the use of research equipment and flow cytometry services.

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