

## Review Article

# The role of regulatory T cells in immune dysfunction during sepsis

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**BACKGROUND:** Although regulatory T cells (Tregs) are key to the maintenance of immunologic homeostasis and tolerance, little is known about Treg-mediated immunosuppression in the stage of sepsis. This article aimed to review the current literature on the role of Tregs in the pathophysiology of septic response, attempting to investigate the role of Tregs in immune dysfunction during sepsis.

**DATA SOURCES:** A literature search was conducted in January 2014 using the China National Knowledge Infrastructure and PubMed. Articles on the role of Tregs in immune dysfunction during sepsis were identified.

**RESULTS:** The identified articles indicated that Treg levels can be used for the assessment of the course of sepsis. The inhibition of Treg activity can promote the recovery of immune function.

**CONCLUSION:** Since the mechanism of Tregs is complex during the sepsis, more studies are needed.

**KEY WORDS:** Regulatory T cells; Sepsis; Immune dysfunction

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## INTRODUCTION

Sepsis is defined as a complex syndrome, which results from a serious infection followed by amplified and dysregulated inflammatory response. The complex immune response associated with sepsis results in a high rate of morbidity and mortality, despite substantial basic science and clinical advances. The mortality rate of sepsis in ICU is 33%, whereas that of severe sepsis is as high as 50%. A multicenter epidemiology survey<sup>[1]</sup> shows that in China the occurrence rate of severe sepsis was 8.68% in ICU and the mortality rate was as high as 44.7%. The treatment of sepsis has become a worldwide problem in the modern critical care medicine.

A recent report<sup>[2]</sup> has shown that regulatory T cells (Tregs) play an important role in suppression of immune response, as demonstrated by the increase in number and functional enhancement following the onset of severe sepsis or septic shock. This might be associated with the increased morbidity and mortality in septic

patients. Although Tregs are key to the maintenance of immunologic homeostasis and tolerance, little is known about Treg-mediated immunosuppression in the stage of sepsis. Thus, an increasing interest has emerged regarding the investigation of the biology of Tregs as well as their potential mechanisms underlying immune dysfunction after acute insults, infection, and sepsis or septic shock. This review aimed to investigate the role of Tregs in immune dysfunction during sepsis.

## Biological characteristics of Tregs and their regulation mechanism

In general, there are two kinds of Tregs that impact on peripheral immunity. The first is natural CD4<sup>+</sup>CD<sup>+</sup>Foxp3<sup>+</sup> T cells that are derived from the thymus, which have been extensively studied for their roles in autoimmunity and tolerance. The second group, such as Treg1 and helper T cells, comprises a group of Treg cells that develop from the activation, which

contains interleukin-1 and transforming growth factor- $\beta$ , and differentiation of mature CD4<sup>+</sup>CD25<sup>-</sup> T cells in the periphery; these Tregs are called adaptive or induced Tregs.

Natural Tregs are now classified as CD4<sup>+</sup>CD25<sup>+</sup> Tregs, which are derived from the thymus and have been extensively studied. In humans or an animal model, the latter cells comprise 5%–10% of CD4<sup>+</sup> T cells in the peripheral circulation and lymphoid compartment,<sup>[3]</sup> but are relatively scarce in tissues and bone marrow.<sup>[4]</sup>

The crucial role of T cell system in the pathogenesis of immunopathological disorders has been fully accepted. The anergic and suppressive function are the most important functional properties for Tregs. In addition to T cell receptor (TCR) signals, costimulatory molecules and cytokines, which include CD28 and IL-2 respectively, contribute to the development of Tregs. Tregs can inhibit the activation and proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the TCR mediated signal activated.<sup>[5]</sup> In addition, studies have shown that Tregs could inhibit neutrophil, B lymphocytes, monocytes/macrophages, and dendritic cells.

From a functional perspective, the suppression mechanisms for Tregs can be grouped into four basic "modes of action": suppression by inhibitory cytokines like interleukin-10 (IL-10), IL-35 and transforming growth factor- $\beta$  (TGF- $\beta$ ), suppression by cytotoxicity, suppression by metabolic disruption, and suppression by modulation of dendritic-cell (DC) maturation or function. Tregs can also influence immune responses by modulating the recruitment and function of other cell types.<sup>[6]</sup> For instance, Treg-cell-derived IL-9 can activate mast cells, which are essential to regulatory intermediaries in the establishment of peripheral allograft tolerance.

Forkhead/winged helix transcription factor p3 (Foxp3) belongs to the family of X chromosomes. The gene mutation affects the function of Tregs, leading to a series of autoimmune/inflammatory diseases. Mice (known as scurfy mice) and individuals that lack Foxp3 develop a profound autoimmune-like lymphoproliferative disease that graphically emphasizes the importance of Tregs in the maintenance of peripheral tolerance.<sup>[7]</sup> Humans that lack functional Foxp3 develop immunodysregulation, polyendocrinopathy and enteropathy X-linked syndrome (IPEX). IPEX is a severe autoimmune disease that develops early in infancy.<sup>[8]</sup> It is known that Foxp3 is essential for the differentiation and development of Tregs, meanwhile, these processes require Foxp3 gene stable<sup>[9]</sup> and high level expression.<sup>[10]</sup> Brunkow et al<sup>[11]</sup> found that CD4<sup>+</sup>CD25<sup>-</sup> T cells can express high level of

Foxp3 by the retroviral Foxp3 gene transfection carrier technology, and this phenotypic and functional activity was similar to Tregs. Foxp3 is considered as one of the most reliable markers of Tregs as a result of Foxp3 mRNA and its encoded protein is specifically expressed in Tregs, and directly influences the development, phenotype and activity of Tregs.

### Varieties of Tregs in sepsis and their clinical value

It is well-known that severe sepsis and septic shock undermine immune homeostasis by inducing an initial intense systemic inflammatory response that is rapidly followed by a negative feedback of anti-inflammatory process. Moreover, this inhibitory response may decrease host resistance to secondary nosocomial infections, thereby having a deleterious effect on patient outcome. More data have revealed the importance of immune function disorder in patients with sepsis and septic shock. It is considered that resistance to infection and its outcome is influenced by a complex interplay between the host, microbe, and environment.<sup>[12]</sup> Tregs play a pivotal role in the pathogenesis of septic complications with considerable effect on suppressing the adaptive immune response. Thus, it is important to understand the pathogenic mechanism of sepsis and to develop novel preventative and treatment strategies.

Although Tregs comprise only a small fraction of T lymphocytes in the immune system, these cells appear to possess potent regulatory properties on cellular activation, which make them an important participant in the inhibition of immune responsiveness during sepsis.

An animal study<sup>[13]</sup> revealed that the percentages of CD4<sup>+</sup>CD25<sup>+</sup> Tregs were significantly increased 24 hours after cecal ligation and puncture (CLP) in comparison with sham animals. A recent study<sup>[14]</sup> found that after the onset of septic shock, the number of Tregs and their suppressive function were increased progressively and significantly, indicating that they are active in the inhibition of immune responsiveness during sepsis. Another study<sup>[15]</sup> demonstrated that the increased proportion of Tregs was not due to a proliferation of Tregs but rather to a selective depletion of CD4<sup>+</sup>CD25<sup>-</sup> T cells. Hence we concluded that prolonged existence of CD4<sup>+</sup>CD25<sup>+</sup> T cells leads to severe immunoparalysis and is associated with a poor prognosis. Therefore, the sequential monitoring of Tregs in peripheral blood from septic patients might be helpful to appraise the patient's condition and to judge the prognosis.<sup>[13]</sup>

Tregs are involved in the induction of lymphocyte

anergy after sepsis, and the activity is cell-contact dependent and is mediated by increased cytokines. Anergy of T cells is a state of failure to proliferate or secrete cytokines and non-response to their specific antigen. A study<sup>[16]</sup> demonstrated that Tregs from septic mice showed more suppression of *in vitro* T cell proliferation and more suppression of TH1-type cytokine production than Tregs from sham-injured animals.

We found that as the cecal ligation and puncture (CLP) model was used to induce polymicrobial sepsis in mice, the proliferative activity of CD4<sup>+</sup>CD25<sup>-</sup> T cells was inhibited when co-cultured with CD4<sup>+</sup>CD25<sup>+</sup> Tregs. In addition, the apoptosis of CD25<sup>-</sup> T cells was enhanced. Tregs acted on monocytes by inhibiting LPS-induced survival through a pro-apoptotic mechanism involving the Fas/FasL pathway or secretion of IL-10, which affects the apoptosis of monocyte and neutrophil.<sup>[17]</sup>

Apart from a higher percentage of circulating Tregs in the blood samples from patients with septic shock, the expressions of Treg phenotypes such as CTLA-4 and Foxp3 and gene/protein expression of cytokines (IL-10 and TGF- $\beta$ ) were all elevated after burn. And there were obvious differences in various burn sizes. They were also higher in septic patients than those without sepsis. Among the septic patients, the expressions of Treg phenotypes and the levels of cytokines were markedly lower in the survival group than those in patients with fatal outcome.<sup>[18]</sup>

Concomitantly, the mRNA expression of Foxp3 levels was found to show a similar pattern as the protein expression of Foxp3. It has been reported that the function of Tregs is governed by Foxp3, and without this expression Treg function is lost.<sup>[19]</sup> Whereas the transduction of Foxp3, the special transcription factor for the development and function of natural Tregs, induced the expression of CD25, CTLA-4, and GITR, and it exerted anergic and suppressive effects.

The aforementioned studies demonstrated that sepsis leads to a relative increase in the number of Tregs and increases their suppressive function. Consequently, a question emerging from these findings regarding the depth of Tregs investigation might indicate the outcome of patients with sepsis.

Hein et al<sup>[20]</sup> reported a negative correlation between severities of sepsis, assessed by simplified acute physiology score-II, sequential organ failure assessment or arterial lactate concentration and percentage of Tregs. Another study<sup>[21]</sup> revealed that sepsis was characterized by the increase in percentages of circulating CD4<sup>+</sup>CD25<sup>+</sup> Tregs and plasma level of soluble CD25, and the

elevation of these parameters might be a useful marker of infections in SIRS.

### The interventional strategy of Tregs for sepsis

Clinical and experimental evidences have indicated that sepsis causes a relative and absolute elevation in Treg number and enhancement of its suppressive function. For this reason, it is expected that a better understanding of Tregs and their physiological action will help develop new therapeutical approaches for modulating the immune response in conditions such as severe sepsis and septic shock. How to manage Tregs for the improvement of sepsis outcome has only received minor attention, limited to animal studies.

Heuer et al<sup>[22]</sup> reported that the adoptive transfer of *in vitro*-stimulated Tregs but not CD4<sup>+</sup>CD25<sup>-</sup> T cells significantly improved the survival in a mouse sepsis model before or after 6 hours CLP. It was the first time to demonstrate that Tregs improved the survival of mouse sepsis through host T cells. The reason may be that the adoptive Tregs significantly increased the secretion of TNF- $\alpha$  and improved peritoneal bacterial clearance. This result was supported by Murphy et al<sup>[23]</sup> who reported that immune function recovered by injecting exogenous Tregs into burned mice.

Heuer et al<sup>[22]</sup> reported treatment of mice before CLP models with antibody against CD25 could markedly down-regulate the percentage of Tregs, which restored proliferative capacity and Th1 cytokine release. Surprisingly, the depletion of CD25<sup>+</sup> cells before the occurrence of sepsis did not alter the mortality rate of sepsis. These findings were supported by other studies.<sup>[22,24]</sup> On the contrary, another experimental study reported that after the administration of anti-CD25 antibody 3 days before CLP, the depletion of Tregs decreased the mortality rate of sepsis after CLP. This finding suggested that the levels of TNF- $\alpha$  elevated by interacting with TNFR2 predominantly expressed by Tregs, together with the sepsis-induced expansion of Tregs, might contribute to post-septic immunosuppression and fatal consequences.

What's arousing our interests is the aforementioned studies which demonstrate that the depletion of CD4<sup>+</sup>CD25<sup>+</sup> T cells does not alter the mortality rate of sepsis.<sup>[25]</sup> However, dispute could not be considered to be so simplistic, since CD4<sup>+</sup>CD25<sup>+</sup> Tregs are not the only cells that express the CD25 receptor. For this reason, such a depletion approach might remove both the detrimental effects of CD4<sup>+</sup>CD25<sup>+</sup> Tregs and CD25<sup>+</sup> non-Tregs. For example, most activated CD4<sup>+</sup>CD25<sup>-</sup>

T cells are beneficial to a functional innate/adaptive immune response to septic challenge. This indicates that the ablation of CD25<sup>+</sup> cells does not give rise to advantage or disadvantage in survival. In addition, the time of observation may affect the final result as well. The relatively elevated Tregs may not be enough early to play a role in sepsis, but a large volume of infusion of exogenous Tregs is probably related to immune effect.<sup>[26]</sup>

High mobility group box 1 protein (HMGB1) is a non-histone DNA-binding protein, which plays a critical role in regulating gene transcription.<sup>[27]</sup> HMGB1 has been identified as a late proinflammatory cytokine. A study<sup>[19]</sup> reported that HMGB1 stimulation can markedly down-regulate the expressions of CTLA-4 and Foxp3 from splenic Tregs in mice. HMGB1 appears to be involved in modulating cell-mediated immunity by influencing the proliferation of effector T cells, secretion of IL-2, and cell polarization.

Inoue et al<sup>[28]</sup> reported that mice underwent sham or CLP, and after surgery the spleens were harvested at various time points. Using anti-CTLA-4 antibody to block CTLA-4 apoptosis, they found the expression of CTLA-4 on CD4<sup>+</sup>, CD8<sup>+</sup>, and Tregs was increased during sepsis. Anti-CTLA-4 therapy decreased sepsis-induced apoptosis, but had little effect on pro- or anti-inflammatory cytokines. There was a dose dependent effect of anti-CTLA-4 on survival. At a high dose, anti-CTLA-4 lowered the survival, but at a lower dose, the survival was significantly improved.

GITR was expressed in both CD4<sup>+</sup>CD25<sup>+</sup> Tregs and CD4<sup>+</sup>CD25<sup>-</sup> effector T cells. This indicated that CD4<sup>+</sup>CD25<sup>-</sup> T cells but not CD4<sup>+</sup>CD25<sup>+</sup> Tregs were necessary for the survival benefit offered by anti-GITR treatment. Scumpia et al<sup>[29]</sup> reported that in a septic mice model, treatment with GITR agonistic antibody corrected adaptive immune dysfunction. Surprisingly, the depletion of CD4<sup>+</sup> T cells, but not CD25<sup>+</sup> Tregs, eliminated the survival benefit of anti-GITR treatment. This study indicated that the depletion of CD4<sup>+</sup> or CD4<sup>+</sup>CD25<sup>+</sup> Tregs did not alter the mortality rate of sepsis.

Another experiment duplicated sepsis murine models and *ex vivo* down-regulated Foxp3 expression using siRNA transfection. The relative increase in circulating Tregs might play a role in lymphocyte anergy after septic shock and represent a standard surrogate marker of declining proliferative capacity after sepsis.<sup>[30]</sup>

## Prospect

Further research would enable us to know more about changes and clinical significance of Tregs during

sepsis, but problems need to be studied including the relationship among Treg changes, sepsis-induced immune disorders, the exact pathophysiology of Tregs in sepsis, and the effects of Tregs on the outcome of sepsis at different stages. With the understanding of the relationship between Tregs and sepsis immune disorder and between the immune status and regulation mechanism, we will find a new way for the prevention and treatment of sepsis.

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**Contributors:** Cao C proposed the study and wrote the first draft. All authors read and approved the final manuscript.

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