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Original Article

New paradigm of immune checkpoint immunotherapy in sepsis

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Abstract: Sepsis is a severe life-threatening infection with organ dysfunction, initiating a complex interplay of host pro-inflammatory and anti-inflammatory processes. In particular, the number of T-cells decreases as thymic function decreases. Sepsis could accurately be regarded as a race to the death between pathogens and the host immune system. It is the proper balance between often competing pro- and anti-inflammatory pathways that determines the fate of the individual. The current study discusses the roles of costimulatory molecules, regulatory T-cells, and neuroendocrine factors in the immunosenescence of sepsis, aiming to build a novel understanding of this disorder by exploring potential targets for immune therapy. The final goal is to improve sepsis outcomes.

Keywords: Sepsis, immunosuppression, co-stimulatory molecules

Introduction

Sepsis, a syndrome of dysregulated host response to infections leading to life-threatening organ dysfunction, is a common problem [1]. Clinical manifestations of sepsis were already known to Hippocrates (460-377 BC), introducing the term 'wound putrefaction'. For thousands of years, sepsis has been a major contributor to the worldwide burden of disease. Since the early 1980s, many therapeutic agents, for treatment of sepsis, have been evaluated in randomized controlled clinical trials. With few exceptions, results from these trials have been disappointing. No specific therapeutic agents are currently approved for treatment of sepsis [2]. Incidence of sepsis has continually increased due to the aging population. The elderly have impaired immunity as a result of immunosenescence.

In the past, sepsis was commonly thought to be caused by overactivation of the innate immune system and the ensuing pro-inflammatory cascade, in response to severe microbial infection or extensive tissue damage (such as caused by burns or multiple injuries). In recent years, the dynamic nature of the disease been fully recognized. The cytokine profile changes with time, underlying infections can move from being localized to disseminated, and the immunological profile can change from being pro-inflammatory to more immunosuppressed [3, 4]. It is now clear that the host response is disturbed in a much more complex way, involving both sustained excessive inflammation and immune suppression, as well as a failure to return to normal homeostasis. Sepsis has been associated with immune suppression, characterized by lymphocyte exhaustion and the reprogramming of antigen-presenting cells.

Sepsis affects the immune system by directly altering the lifespan, production, and function of effector cells responsible for homeostasis [5]. Immunosenescence affects both innate and adaptive immunity. This is reflected in specific T-cell marks (such as an inverse CD4/CD8 ratio, loss of naïve T-cells, increased numbers of terminally differentiated T-cells, and a reduction in the function of NK cells [6]), increased Treg ratios [7], and upregulation of inhibitory immune checkpoint molecules (including PD-1, PD-L1, CTLA-4, TIM-3, LAG-3, 2B4) (see **Figure 1**) [8-11]. Intriguingly, a recent study demonstrated that the circadian clock controls immu-

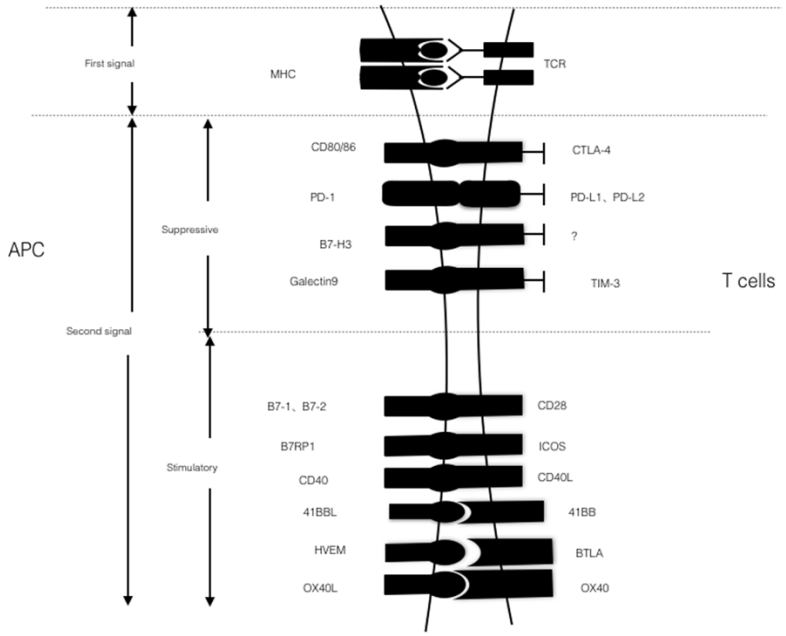

Figure 1. Multiple co-stimulatory and inhibitory interactions regulate T-cell response. Abbreviation: BTLA, B and T lymphocyte attenuator; GAL9, galectin 9; HVEM, herpesvirus entry mediator; ICOS, inducible T-cell co-stimulator; LAG3, lymphocyte activation gene 3; PD1, programmed cell death protein 1; PDL, PD1 ligand; TIM3, T-cell membrane protein 3.

ne checkpoint pathways in sepsis [12]. This review discusses the current understanding of the roles of co-inhibitory molecules during sepsis.

PD-1/PD-L1

Since the first observation of spontaneous autoimmune diseases in PD-1 (programmed cell death 1) knockout mice, PD-1 has been postulated to play essential roles in the regulation of autoimmunity. However, the precise mechanisms are largely unknown. PD-1 is known to play critical roles in cancer immunology. Blocking antibodies against this receptor have been shown to provide benefits in clinical trials, with the first of this class recently approved by the FDA for treatment of patients with refractory malignancies. PD-1 has two ligands, PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; CD273). PD-L2 has a three-fold higher affinity for PD-1, compared to PD-L1. The PD-1 ligand PD-L1 is expressed in epithelial and endothelial cells, monocytes, macrophages, and DCs [13]. The binding of PD-1 to its ligands, PD-L1 or PD-L2, is vital for physiological regulation of the immune system. However, PD-L2 is expressed by significantly fewer cell types than PD-L1. While not commonly present on resting cells, PD-L2 is inducibly expressed in dendritic cells and macrophages [14].

Since PD-1 is upregulated in both $CD4^+$ and $CD8^+$ cells during viral infections and cancer states, it has often been associated with the phenomenon of "T-cell exhaustion". This has been thought to stem from prolonged periods of exposure to self-antigens [15, 16]. Engagement of PD-1 by its ligands creates the second signal that inhibits T-cell function. To be specific, ligation of PD-L1 or PD-L2 on an antigen presenting cell with PD-1 on T-cells results in the inhibition of PI3K-AKT, LAT-Zap70, and C3G-Rap1 signaling. This inhibition leads to decreased cytokine production, T-cell proliferation, and T-cell adhesion, respectively. Likewise, PD-1 pathways can limit inflammation-associated cytokine production, including IL-2, which is a key cytokine released by T-cells that is involved in T-cell activation and proliferation [17]. These investigators found that not only expression of PD-1, but also expression of its 2 ligands, PD-L1 and PD-L2, associates with poor outcomes in sepsis and shock [18]. Both anti-PD-1 and anti-PD-L1 therapies have demonstrated promising results in human trials involving cancer and sepsis [19]. Therefore, sepsis biologists have postulated that anti-PD-1 and anti-PD-L1 therapy could also have similar beneficial results, reducing sepsis-induced immune dysfunction that drives ongoing infectious complications. Patients with septic shock demonstrate increased levels of PD-1 and PD-L1 in their monocyte and T-lymphocyte cell types [20, 21]. Studies by Huang et al. showed that mice, in which the gene for PD-1 was deleted, exhibited a marked reduction in mortality in response to cecal ligation and puncture (CLP)-induced polymicrobial septic challenges [22]. Similar to the survival analysis undertaken for PD-1-/- mice, survival analysis of PD-L1 gene-deficient mice, after sepsis, demonstrated comparable results [23].

A recent study by *Wenjun D* found that *Bmal1*, a core circadian clock gene, plays a role in preventing the development of a sepsis phenotype through counter-regulating PD-L1 expression and T-cell exhaustion. [12]. They also found that loss of *Bmal1* increased PD-L1 expression in macrophages. Importantly, anti-PD-L1 neutralizing antibodies improved septic survival, reduced lymphocyte apoptosis, and improved bacterial clearance in Bmal1 Mye-/- mice. This finding indicates that targeting the circadian clock and immunometabolism pathways has potential for treatment of infectious diseases that lead to lethal sepsis.

Notably, recent studies have suggested that treatment with checkpoint blockade antibodies targeting the PD-1-PD-L1 axis might be effective in the treatment of sepsis. Further research should address how PD-1 and/or PD-L1 blockades improve the immune response during sepsis, examining whether these relate to the induction of immunosuppressive mechanisms described by Roquilly et al. [24]. Considering the beneficial impact on adaptive immunity and tumor eradication strategies, it makes sense that PD-1 and PD-1L could concomitantly serve as biomarkers of sepsis-initiated immune suppression, as well as prospective therapeutic targets in reversing adaptive immune dysfunction and improving long-term survival.

CTLA-4

CTLA-4 (Cytotoxic T-lymphocytee associated antigen 4), also known as CD152, is a CD28 homolog with much higher binding affinity for B7 [25, 26]. However, unlike CD28, binding of CTLA-4 to B7 does not produce a stimulatory signal. The relative amount of CD28:B7 binding versus CTLA-4:B7 binding determines whether a T-cell will undergo activation or anergy [27]. CTLA-4 is subject to regulation, particularly by localization within the cell. CTLA-4 is also involved in other aspects of immune control. Unlike effector T-cells, Tregs constitutively express CTLA-4. This has been thought to be important concerning their suppressive function [28, 29]. Mechanisms of action of CTLA-4 in inhibiting T lymphocyte proliferation and activation involve a reduction in IL-2 production and IL-2 receptor expression, as well as arresting T-cells at the G1 phase of the cell cycle [27, 30]. A CTLA-4-specific antibody, ipilimumab, has been clinically used in anticancer immunotherapy. However, it induces severe autoimmune side effects. Ipilimumab has been FDA approved for treatment of metastatic melanoma and may obtain clinical approval for additional oncological indications, most likely in combination with other ICIs targeting PD-1 or its ligand PD-L1 [31].

Using a murine model of CLP-induced sepsis, Inoue et al. demonstrated that CTLA-4 expression was progressively increased in both $CD4^+$ and $CD8^+$ T-cells and regulatory T-cells, starting at 24 hours after induction of sepsis, along with T-cell apoptosis and depletion [32]. In the same study, treatment with anti-CTLA-4 inhibited T-cell apoptosis by more than 50%, significantly improving survival. While anti-CTLA-4 at a low dose (33 μg per mouse) also improved survival in a two-hit model of sepsis, comprised of slowly progressive CLP-induced sepsis followed by infection with a fungus, Candida albicans. Another study by Chang et al. using a two-model of sepsis, including a primary Candida albicans fungal sepsis and a two-hit model (CLP-induced sepsis followed by Candida albicans), demonstrated that anti-CTLA-4 increases T lymphocyte IFN-γ production, significantly improving survival [33]. Dong-Na G et al. demonstrated that $CD4^+CTLA\text{-}4^+$ was positively correlated with SOFA scores and APACHE II scores of sepsis patients [34]. Blocking of CTLA-4-mediated negative regulatory pathways can increase pathogen clearance and reverse T-cell dysfunction in septic animals, as well as improve survival in animal models of bacterial and fungal sepsis. Patients with acute liver failure (ALF) have defects in innate immune response to microbes (immune paresis) and are susceptible to sepsis. Khamri et al. found that the proportion of CTLA4 expressing-$CD4^+$ T-cells remained significantly higher, compared to healthy controls, throughout the course of admission.

BTLA

BTLA was discovered in a search for T-cell-helper (Th) type 1 (Th1)-specific genes [35, 36]. BTLA expression in murine splenic B-cells is constitutive, with low expression in splenic T-cells. It (B and T lymphocyte attenuator) is a type I transmembrane receptor belonging to the Ig-superfamily. It bears similarities to PD-1.

Its extracellular domain has an IgV-like fold and its cytoplasmic domain harbors an ITIM and an ITSM motif, two classical inhibitory motifs. BTLA and CD160 share a common ligand, HVEM (herpesvirus entry mediator), which also binds LIGHT, another costimulatory molecule that positively regulates T-cell response [37]. However, HVEM can function bidirectionally, as a positive or negative signal transducer, depending upon the receptor it binds [38]. BTLA expression is downregulated by virus specific CD8 T-cells but not by tumor-specific cells. Therefore, suitable immunotherapeutic approaches, like vaccination with CpG, have been suggested [39]. In mice, BTLA deficiency is associated with hyper-reactive B- and T-cells and enhanced susceptibility to autoimmunity [40]. Several reports have found that BTLA blockers can enhance human T-cell response when used alone or in combination with antibodies against PD-1 [41-43]. However, recent studies have indicated that the roles of BTLA in tumor-resident T-cells are complex, as engagement by its ligand HVEM inhibits proliferation and cytokine production but promotes survival of tumor-infiltrating lymphocytes (TILs) [44].

Shubin and others have shown that B and T lymphocyte attenuator (BTLA) expression contributes to apoptotic cell loss of primary and secondary lymphoid organs in experimental septic mice, suggesting that BTLA-induced apoptotic lymphocyte loss might be a mechanism for increased risk of nosocomial infections in critically ill patients [45, 46]. Shubin and colleagues reported that higher mean BTLA expression in critically ill patients may have value in identifying patients with infections. Further studies have provided evidence that BTLA activation contributes to T-lymphocyte apoptosis during sepsis [45]. In addition, Sean F. M et al. found that soluble BTLA is increased in human and murine models of critical illness [47]. It has biological significance in altering cellular proliferation and can predict the development of sepsis in critically ill patients. These data illustrate the value of BTLA as a potential therapeutic target and a possible biomarker, identifying critically ill patients that are most susceptible to developing subsequent or secondary infections.

TIM-3

Tim-3 (Tcellimmunoglobulinandmucin-domain-containing-3) is a co-inhibitory receptor expressed in IFN-γ-producing T-cells, FoxP3$^+$ Treg cells, macrophages, and dendritic cells. It has been shown to suppress their response upon interaction with their ligands. It has gained prominence as a potential candidate for cancer immunotherapy, as blockade of Tim-3 with other checkpoint inhibitors enhanced anti-tumor immunity and suppresses tumor growth in several preclinical tumor models. Tim-3 was first identified 12 years ago as a molecule selectively expressed on IFN-g-producing CD4$^+$ T helper 1 (Th1) and CD8$^+$T cytotoxic 1 (Tc1) T-cells [48]. In addition, Tim-3 may be a key immune checkpoint in tumor-induced immune suppression, as Tim-3 marked the most suppressed or dysfunctional population of CD8$^+$ T-cells in preclinical models of both solid and hematologic malignancy [49, 50]. In these models, all CD8$^+$ Tim-3+ T-cells co-expressed PD-1. These dual-expressing cells exhibited greater defects in both cell-cycle progression and effector cytokine production than cells expressing PD-1 alone. Together, these data indicate that Tim-3 pathways may cooperate with PD-1 pathways to promote the development of a severe dysfunctional phenotype in CD8$^+$ T-cells in cancer.

Tim-3 in monocytes has been reported to promote immune homeostasis during sepsis [51]. Moreover, F. Ren et al. identified that soluble Tim-3 was reduced in sepsis and severe sepsis patients but was elevated in septic shock patients [52]. Recently, Zhengping W et al. identified that Tim-3 was highly upregulated in liver CD8$^+$ T-cells in a mouse cecal ligation and puncture model, as well as in peripheral blood CD8$^+$ T-cells of human patients with sepsis [53]. Using a CLP mouse septic model, they found that liver CD8$^+$ T-cells expressed high Tim-3 in a bi-phasic fashion and apoptosis of liver CD8$^+$ T-cells was mediated by Tim-3. They also claimed that administration of α-lactose, a molecule with a similar structure to galactin-9, reduced Tim-3 expression and liver injuries in sepsis. These findings provide a basis for targeting Tim-3 in the management of sepsis, protecting septic patients from liver damage. In addition, Xiandong L et al. identified the capacity of Tim-3+Tregs to protect against lung struc-

tural injuries, inflammatory infiltrates, and subsequent fibrosis after acute lung injuries.

Selective expression of TIM-3 in tumor tissues, along with its roles in multiple mechanisms of immunosuppression, highlights its value as a target for cancer immunotherapy. Sepsis and cancer share many immunological defects. Therefore, recent immunomodulatory findings in cancer provide hope and insight into potential immunostimulatory therapies for sepsis.

LAG-3

LAG-3 (Lymphocyte activation gene-3) was discovered 28 years ago as a molecule that is upregulated in activated $CD4^+$ and $CD8^+$ T-cells. It is a subset of natural killer (NK) cells [54]. In addition to its expression in T-cells, one study suggested expression in activated B-cells. However, that data has not been widely replicated [55]. In addition, LAG-3 mRNAs can be found in the thymic medulla, splenic red pulp, and base of the cerebellum [56]. After a T-cell is activated to kill its target T-cell, LAG-3 expression is increased to turn off immune response. Thus, T-cells do not go on to attack healthy cells. Inhibition of immune response is accomplished through the binding of LAG-3 to an antigen-presenting complex called MHC II, which together signals T-cells to stop activation and multiplication.

Initial examinations of Tim-3 function have suggested that Tim-3 is a negative regulator of type 1 immunity. Anti-Tim-3 antibody has been shown to exacerbate experimental autoimmune encephalomyelitis (EAE) [48]. Tim-3 pathways are perfectly poised as a target for anticancer immunotherapy, due to their expression on both dysfunctional CD8þ T-cells and Tregs, two key immune cell populations that constitute immunosuppression in tumor tissues. In Wilms tumor-3 (WT3) sarcoma and transgenic adenocarcinoma of the mouse prostate C-1 (TRAMP-C1) cancer models, Tim-3 blockade alone was effective, in a dose-dependent manner. In preclinical models of colon carcinoma (CT26 and MC38), Tim-3 blockade alone exhibited similar efficacy to PD-1 pathway blockade [57]. Targeting Tim-3 pathways may produce promising results, according to preclinical cancer models.

The use of anti-LAG-3 Abs in a melanoma tumor model led to increased $CD8^+$ IFNγ producing cells and decreased tumor growth, compared to non-treated mice. The combination of anti-LAG-3 and anti-PD-1, in a variety of tumor models, has led to synergistic antitumor efficacy [58-60]. Jonathan S et al. demonstrated increased expression of inhibitory receptor LAG-3 in T lymphocytes, accompanied by decreased expression of the IL-7 receptor over the course of sepsis [61]. Li J identified that anti-LAG-3 antibody improved prognosis and reversed T-cell dysfunction in mice with experimental sepsis [62]. These studies have indicated that anti-LAG-3 is effective when given via a therapeutic approach. Thus, the therapeutic potential of anti-LAG-3 in human sepsis remains seductive.

TIGIT

TIGIT (T-cell Ig and ITIM domain), a member of the immunoglobulin (Ig) superfamily, first identified in 2009 by three different groups through bioinformatics, has recently gained attention in cancer immunotherapy [63-65]. Its expression is limited to lymphocytes, including T-cells, regulatory T-cells (Tregs), and natural killer (NK) cells [66]. TIGIT binds two ligands, CD112 and CD155, which are expressed in APCs, T-cells, and tumor cells. Joller et al. suggested that not only is TIGIT majorly expressed on natural Tregs, but also can promote induced Tregs differentiation. Intriguingly, TIGIT marks a functionally distinct subset of human nTregs with superior suppressive activity *in vitro* (134). Inhibitory assay results revealed that TIGIT+ Tregs inhibited T-cell differentiation and response of Th1 and Th17 subsets but promoted Th2 immunity through a fibrinogen-like 2-dependent mechanism [67]. In melanoma patients, Julien F et al. showed that Tregs exhibited increased TIGIT expression and decreased expression of its competing costimulatory receptor CD226, compared with $CD4^+$ effector T-cells, resulting in an increased TIGIT/CD226 ratio [68]. Alice L. H et al. recently found that late expression of TIGIT allows for delayed treatment efficacy. Addition of anti-PD-1 confers increased survival benefits. However, contrary to expectations, anti-TIGIT therapy alone showed no significant effects on survival at early and late time-points. Anti-PD-1 and Anti-TIGIT Co-blockade reduced tumor infiltrating dendritic cells [69]. Yan M et al. found that upregulation of CD155 in DCs decreased proinflammatory cytokines and increased anti-

inflammatory cytokines, contributing to immunosuppression in septic mice. However, little is known about the intrinsic molecular programs that control this. It is believed that novel therapeutic strategies, targeting TIGIT in sepsis, could be promising strategies in preventing the development and progression of sepsis.

Conclusion

Although substantial advances have been made in the understanding of sepsis, none of the promising therapeutic approaches for sepsis targeting inflammatory response have been successfully translated to the clinical setting. Rates of sepsis mortality have not decreased. Sepsis-induced immune dysfunction and evidence of immune depression in severe sepsis and persistent critical illness have been increasingly recognized in many studies. They are now reaching the stage of formal clinical assessment as suitable targets for intervention. Many trials using immune-modulatory agents have yielded discouraging results in human clinical trials for sepsis [70]. In the future, immunotherapy will probably be tailored to individual patients, based on specific laboratory or clinical findings. Immunosuppression induced by sepsis is very common. Co-inhibitory molecules expressed on the T-cell surface play a crucial role in this mechanism. Several studies have shown that targeting these molecules could improve survival in sepsis, indicating that these co-inhibitory molecules may be potential targets for sepsis treatment. However, precision immunotherapy based on the immune status of patients is necessary, while immunosuppression is reversed. In the right patients and at the right times, immunotherapy improves the survival of patients with sepsis.

Disclosure of conflict of interest

None.

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