Potential Immunotherapeutics for Immunosuppression in Sepsis

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Abstract

Sepsis is a syndrome characterized by systemic inflammatory responses to a severe infection. Acute hyper-inflammatory reactions in the acute phase of sepsis have been considered as a primary reason for organ dysfunction and mortality, and advances in emergency intervention and improved intensive care management have reduced mortalities in the early phase. However, it has been recognized that increased deaths in the late phase still maintain sepsis mortality high worldwide. Patients recovered from early severe illness are unable to control immune system with sepsis-induced immunosuppression such as immunological tolerance, exhaustion and apoptosis, which make them vulnerable to nosocomial and opportunistic infections ultimately leading to threat to life. Based on strategies to reverse immunosuppression, recent developments in sepsis therapy are focused on molecules having immune enhancing activities. These efforts are focused on defining and revising the immunocompromised status associated with long-term mortality.

Key Words: Sepsis, Immunosuppression, Immune modulators, Immunotherapy, Precision medicine, Theranostics

INTRODUCTION

Sepsis is a catastrophic illness occurring when severe infection leads to a systemic inflammatory response (Hotchkiss et al., 2013b; Kaukonen et al., 2015; Kim et al., 2016). “Sepsis-3” is the third iteration of the International Consensus Definition for Sepsis and Septic Shock in 2016, defined sepsis as a life-threatening organ dysfunction due to a dysregulated host response to infection. In the absence of early diagnosis and prompt treatment, sepsis progresses to septic shock, defined as a subset of sepsis where underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality (Singer et al., 2016). Despite marked advances in emergency medicine and improved intensive care unit (ICU) management, sepsis is still a leading cause of death in critically ill patients (Mayr et al., 2014). Sepsis-related worldwide mortality rates remain at 20-50%, while the cost of sepsis management is enormous (Coopersmith et al., 2012; Gaieski et al., 2013). Deaths occur in three phases: an initial peak at several days, a late peak at several weeks owing to persistent organ injury and failure, and the third peak 60 to 90 days after sepsis (Winters et al., 2010; Needham et al., 2012; Delano and Ward, 2016). A robust pro-inflammatory response, such as a cytokine storm, is a distinct feature for death in early-phase sepsis (Moore and Moore, 1995); however, it is accepted that most sepsis patients commit a significant immunosuppressive status with immune cell dysfunction through the concomitant occurrence of pro- and anti-inflammatory mechanisms (Munford and Pugin, 2001; Hotchkiss et al., 2013b). Immunocompromised patients acquire nosocomial and opportunistic infections as well as additional organ failure and protracted events, resulting in death in the late phase. Moreover, more than 70% of deaths occur after day 3 and many deaths with unresolved septic foci detected at postmortem, followed by weeks and months after sepsis onset (Otto et al., 2011). Accordingly, many epidemiological studies confirm both recent reductions in 30-day sepsis mortality rates and increases in both long-term sepsis mortality (Delano and Ward, 2016) and sepsis-induced disability after severe illness associated with immunosuppression (Gaieski et al., 2013; Hutchins et al., 2014).

Sepsis patients exhibit some of the following manifestations: fever, anorexia, tachycardia, leukocytosis or leukocytopenia, hypotension, coagulopathy, metabolic alteration, organ damage, and death. Current strategies for symptomatic treatment of sepsis include administration of antibiotics,
surgical approaches for eliminating the source of infection, administration of intravenous fluids to restore and maintain adequate intravascular volume, and vasocostriction and/ or inotropic drugs including norepinephrine or vasopressin, and mechanical ventilation. Since sepsis has been historically considered as a hyper-inflammatory syndrome caused by host immune responses to pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharides (LPS), (Fink and Warren, 2014) most research on development of sepsis therapy has focused on inhibiting the initial hyper-inflammatory responses (Hotchkiss et al., 2013a). Numerous drug candidates including corticosterone, toll-like receptor (TLR) 4 antagonist, anti-LPS (Ziegler et al., 1982), anti-interleukin (IL)-1β and anti-tumor necrosis factor (TNF)-α antibodies (Panacek et al., 2004; Lorente and Marshall, 2005) have been assessed in clinical trials; however, none appeared to significantly improve sepsis-related mortality (Rice et al., 2010; Qiu et al., 2013). In addition, Xigris (a systemic anticoagulant known as “activated protein C”) was the only FDA-approved therapeutic agent for sepsis (Riewald and Ruf, 2005; Ward and Bosmann, 2012); however, it was withdrawn in 2010 because of hemorrhagic side effects and no resulting improvement in mortality (Angus, 2012; Ward and Bosmann, 2012; Williams, 2012). Therefore, no drugs for sepsis exist currently. Reasons for no successful therapeutic developments include the following: i) inactive compounds, ii) inadequate animal models of sepsis, iii) inappropriate clinical trials owing to heterogeneity in the patient population, iv) arbitrarily determined treatment durations, and v) incomplete understanding of the complex pathophysiology of sepsis (van Deuren et al., 1998; Kellum et al., 2007; Fink and Warren, 2014), rather than incorrect targeting of the hyper-inflammatory pathway itself.

During the last three decades, researchers have recognized that the pathophysiological mechanisms resulting from organ damage and death consist of a complex yet ill-defined immune/inflammatory process, which varies during the disease progresses and presents a heterogeneous immunological status. Recent studies focus on not only defining the heterogeneity by characterizing subgroups of patients but also understanding underlying alterations in innate and adaptive immunity, which enable development of precise and personalized therapy. In this review, we present brief overview of sepsis-induced immune cell dysfunction and recent efforts to overcome sepsis-induced immunosuppression using immuno-modulatory molecules.

### IMMUNOSUPPRESSION AND IMMUNE CELL ALTERATION IN SEPSIS

Although it has been controversial whether low-grade local inflammation or immunosuppression could be a main cause for late deaths after overcoming the initial episode; currently, researchers have accepted the immunosuppression which may be responsible for sepsis-related morbidity and mortality rather than hyper-/low-grade local inflammation, (Xiao et al., 2011; Hotchkiss et al., 2013b). Immunosuppression is especially common among the elderly because of age-related impairment of the immune system (Martin et al., 2003), and elderly sepsis patients with comorbidities often do not show any prominent signs of sepsis and do not display a prominent inflammatory response to infections or anti-inflammatory reactions; hence, a consistently high mortality rate is prevalent among individuals aged over 65 years (Reber et al., 2012). Furthermore, many unresolved septic foci have been reported in many organs in non-survivors of sepsis (Torgersen et al., 2009; Otto et al., 2011) owing to not only relatively non-virulent pathogens, e.g., Acinetobacter spp, Enterococcus spp, Stenotrophomonas spp, Pseudomonas spp (Kollef et al., 2008; Otto et al., 2011), but also reactivation of latent viruses, predominantly herpes simplex virus and cytomegalovirus (Limaye et al., 2008; Luyt and Kaiser, 2012), which is consistent with impaired host immunity. Moreover, increasing evidence suggests that sepsis is an immunosuppressive disorder at the cellular and molecular level (Hotchkiss et al., 2013b; Venet et al., 2013; Delano and Ward, 2016). Sepsis affects most innate and adaptive immune cells to be reprogrammed to display functionally defective phenotypes including tolerance, anergy, exhaustion, and apoptosis. Following is an overview of sepsis-induced alterations in innate and adaptive immune cell types along with possible molecular mechanisms.

### NEUTROPHILS

Neutrophils as a fundamental component of the innate immune responses mediate the prompt eradication of foreign pathogens (Nathan, 2006). They comprise the majority of the cells in bone marrow (BM) and are produced and released into peripheral blood daily. Neutrophil levels can be rapidly and significantly elevated in response to an infection (Tamayo et al., 2012), and the cells die within 6-24 hours. Importantly, immature neutrophils are significantly released from BM (Delano et al., 2011) and circulating neutrophils show delayed apoptosis (Hotchkiss and Nicholson, 2006) in sepsis patients. Neutrophils with varying degrees of maturity show diverse functional

### Table 1. Sepsis-induced alterations of immune system

| Innate immunity                          | Adaptive immunity                  |
|-----------------------------------------|------------------------------------|
| Extensive apoptotic cell death           | Apoptotic lymphocyte death         |
| Endotoxin tolerance                     | T cell anergy                      |
| Exhaustion phenotypes                   | Exhaustion phenotypes              |
| Release of immature myeloid cells       | Decreased T cell activation        |
| Reduced pro-inflammatory cytokines      | Unbalanced Th polarization         |
| Decreased antigen presentation capacity | Enhanced Treg function and survival|
|                                        | Decreased antibody production      |
defects: i) reduced production of reactive oxygen species, diminished nitric oxide release (Kovach and Standiford, 2012), and the oxidative burst (Delano et al., 2011), ii) loss of chemotactic activity (Alves-Filho et al., 2009), reduced expression of cell surface molecules including C-X-C chemokine receptor 2 (CXCR2) (Cummings et al., 1999) and decreased recruitment to sites of infection (Alves-Filho et al., 2010), and iii) reduced activation of the complement system (Morris et al., 2011). These defects are reported to lead to failure in bacterial clearance. In a mouse model of sepsis induced by cecum ligation and punctation (CLP), both reduced neutrophil function and an increased susceptibility to infection was reported (Delano et al., 2011). Consequently, sepsis patients with severely dysfunctional neutrophils are increasingly susceptible to nosocomial and secondary infections (Stephan et al., 2002). Although the underlying mechanisms have been poorly understood, it is suggested that alterations in TLR expression and signaling are associated with the functional defects in neutrophils (Lerman et al., 2014). Considering that immunocompromised sepsis patients have comorbid infections and protracted illness owing to unresolved septic foci, molecules modulating neutrophil function may be potential therapeutic candidates.

**MONOCYTES AND MACROPHAGES**

Monocytes and macrophages play pivotal roles in orchestrating host immune responses during sepsis (Parihar et al., 2010). They not only participate in the initiation of the cytokine storm but also contribute to immunosuppression. The most well-known functional defect in monocytes and macrophages is “endotoxin tolerance” (Biswas and Lopez-Collazo, 2009), which refers to diminished capacity of release of pro-inflammatory cytokines in response to bacterial components such as LPS and other TLR stimuli (Cavaillon and Adib-Conquy, 2006; Biswas and Lopez-Collazo, 2009). Blood analysis from sepsis patients showed decreased production of TNF-α, Interleukin (IL)-1β and IL-6 upon LPS treatment (Munoz et al., 1991; Ertel et al., 1995). When splenocytes obtained promptly after septic death were exposed to LPS, the induction of pro- and anti-inflammatory cytokines was markedly reduced to less than 10–20% in comparison with those from patients without sepsis (Boomer et al., 2011). Recent system biology approaches using monocytes from sepsis patients revealed a more complex feature of their alteration rather than a simple immunosuppressive phenotype. In vivo septic monocytes were reprogrammed to recover from overt inflammation, thereby impairing the capacity to sustain further inflammation and immune activation and promoting protective responses including phagocytosis, anti-microbial activity, and tissue remodeling (Shalova et al., 2015) while still with ex vivo LPS challenges septic monocytes exhibited “endotoxin tolerance”; blunting in chemokine and cytokine secretion. Monocytes and macrophages exhibiting endotoxin tolerance displayed decreased antigen presentation capacity along with decreased human leukocyte antigen (HLA)-DR expression (Docke et al., 1997) and intracellular signaling via anti-inflammatory mediators (Delano and Ward, 2016), thereby indicating the development of anergy (Monneret et al., 2004; Lukaszewicz et al., 2009), consistent with an increased risk of nosocomial infections and death (Monneret et al., 2008; Venet et al., 2013). Furthermore, transcriptome analysis of sepsis monocytes suggested that endotoxin tolerance is mediated by IL-1 receptor-associated kinase (IRAk), an inhibitor of TLR signaling via hypoxia inducible factor-1α (HIF-1α), which was suggested as a key regulator for monocyte reprogramming in sepsis (Tannahill et al., 2013; Shalova et al., 2015).

**DENDRITIC CELLS**

Dendritic cells (DCs) are short-lived immune cells and continuously replenished from DC precursor (Pastilie et al., 2011). Antigen-presenting DCs induce T cell immune responses and cytokine-releasing DCs activate innate and adaptive immunity (Steinman and Hemmi, 2006). Induction of marked apoptosis in conventional and plasmacytoid DCs particularly contributes to protracted immunosuppression in sepsis. Reduced numbers of circulating DCs were reported in patients with sepsis (Poehlmann et al., 2009; Ricardi et al., 2011) and septic shock (Guisset et al., 2007) and a significantly reduced number of DCs was observed in the spleen of patients who experienced septic death in comparison with death caused by burns (Hotchkiss et al., 2002). Moreover, septic DCs express low levels of HLA-DR and increasingly produce IL-10 (Hotchkiss et al., 2013b), indicating decreased antigen presentation capacity. Co-culturing of DCs from sepsis patients with T cells could not induce proper T cell effector function, but instead facilitated T cell anergy or regulatory T cell (Treg) proliferation (Delano and Ward, 2016). These alterations in DCs are also associated with nosocomial infections and mortality. Blocking apoptosis of DCs by increased expression of the anti-apoptotic factor B cell lymphoma-2 (BCL-2) improved survival in animal models of endotoxin shock (Gautier et al., 2008) and treatment of DCs with growth factor FMS-like tyrosine kinase 2 ligand (FLT3L), which increases the secretion of cytokines including IL-12, IL-15, and IFN-γ from DCs and strengthen CD4+ T cell function (Hotchkiss et al., 2013b), also reduced mortality in an animal model. Further studies indicated that increased expression of MHC-II and the costimulatory molecules CD80 and CD86 (Delano and Ward, 2016) owing to augmentation of DC function by TLR agonists improved the survival of mice with pneumonia (Benjamim et al., 2005). A recent study indicated that DCs are dysfunctional owing to diminished antigen-presenting capacity and cytokine release after a severe primary infection. Dysfunctional DCs secreted TGF-β and induced local Treg accumulation, which is associated with high amount of B lymphocyte-induced maturation protein (Blimps) that is a transcription factor associated with tolerogenic function and low expression of interferon regulatory factor 4 (IRF4) that is a transcription factor for antigen presentation (Roquilly et al., 2017).

**T CELLS**

When antigen-presenting cells present an antigenic peptide by MHC-II molecules, CD4+ T cells react the peptide/MHC complex through their T cell receptors (TCRs) and are activated. Once activated, CD4+ T cells can rapidly proliferate and differentiate to diverse effector T helper (Th) cell lineages such as Th1, Th2 and Th17 cells, which are defined by specific transcription factors and signature cytokine expressions. The most notable immunosuppressive features in
septic T cells include i) development of apoptosis, ii) anergy and exhaustion, and iii) an increased percentage of Treg cells. Many investigators have reported reductions in circulating and tissue-resident T cells in sepsis (Hotchkiss et al., 2001; Hotchkiss and Nicholson, 2006). Profound loss of CD4+ T cells in the spleen from sepsis patients was observed (Toti et al., 2004), and marked increase in caspase-3 mediated apoptosis in CD4+ and CD8+ T cells was determined in septic shock patients, which was accompanied by upregulation of programmed death 1 (PD-1) expression on T cells and monocytes (Wynn et al., 2007; Zhang et al., 2011), thereby displaying a marked lymphocytopenia (Toti et al., 2004; Felmet et al., 2005), which is particularly serious because clonal expansions are critical to overcome potentially lethal infections (Hotchkiss et al., 2013a). Of note, the proliferative defects together with upregulation of PD-1 expression in T cells were significantly correlated with nosocomial infections and mortality in sepsis (Guignant et al., 2011). According to the loss of CD4+ T cells, both Th1 and Th2 cytokine productions are diminished in sepsis patients following reduced master transcription factors, T-bet for Th1 cells and GATA-binding protein 3 (GATA3) for Th2 cells (Heidecke et al., 1999; Wick et al., 2000; Pachot et al., 2005). Anergy has been described as the loss of delayed-type hypersensitivity reaction to skin test recall antigens in sepsis patients, which is associated with diminished T cell proliferation and cytokine productions including IL-2 and IFN-γ (Meakins et al., 1982; Heidecke et al., 1999; Monneret et al., 2008). The concept of “exhaustion” originated from study on mice with a chronic viral infection with severely impaired T cell effector function (Zajac et al., 1998). Postmortem study with spleens that experienced septic death showed a typical exhaustion phenotype including suppression of IFN-γ and TNF-α production following T cell stimulation (Boomer et al., 2011). The dysregulation of T cell functions has been found in neonatal and pediatric sepsis patients (Camacho-Gonzalez et al., 2013). Reduced Th17 cell function and cytokine response were also reported in sepsis (van de Veroendk et al., 2012), which were accompanied by a diminished expression of retinoic acid receptor related orphan receptor-γt (RORγt) that is a crucial transcription factor for Th17 cell differentiation (Pachot et al., 2005; Venet et al., 2010). As Th17 cells play an important role in defending against fungal infections by producing IL-17 and IL-22, decreased Th17 cells can lead to secondary fungal infections (Gow et al., 2011; Monneret et al., 2011; Romani, 2011). Indeed, it has been reported that augmented Th17 cell function reduce the number of deaths from secondary infection with Candida albicans in an animal model (Kasten et al., 2010; Unsingier et al., 2012).

Tregs constitute an important component of the adaptive immune response, and play roles in immunological homeostasis (Fehervari and Sakaguchi, 2004) such as development of tolerance to self-antigens. These cells are involved in the pathogenesis of autoimmune diseases and cancer, as well as infectious diseases (Liston and Gray, 2014), via suppression of activation of other effector T cell subsets (Bettelli et al., 2006). It has known that Tregs are resistant to sepsis-induced apoptosis compared to other effector T cells (Venet et al., 2004). The increased number and percentage of Treg cells were observed immediately after the onset of sepsis, mainly due to a reduction in other effector T cells population (Venet et al., 2004). These phenotypes are persistently maintained in patients with septic shock (Monneret et al., 2003). Consistently, the expression of factor forkhead box P3 (Foxp3) that is a Treg-associated master transcription factor was increased or not altered during sepsis (Venet et al., 2004). Treg is intrinsically immunosuppressive in both innate and adaptive immunity. This induced apoptotic cell death of monocytes and neutrophils (Biedermann et al., 2000; Lewkowicz et al., 2006), as well as delayed type hypersensitivity reactions (Unsinger et al., 2010). Based on the evidences from the studies that Foxp3 targeting siRNA technology and glucocorticoid-induced TNF-receptor related protein (GITR) inhibitory antibody efficiently blocking Treg generation and function, could enhance immune responses and reduce mortalities in sepsis animal models (Ronchetti et al., 2004; Venet et al., 2009), it is proposed that Treg-targeting therapy to recover the effector T cell activities may be promising strategy for new drug development of sepsis (Scumpia et al., 2007).

**POTENTIAL IMMUNOMODULATORY THERAPIES FOR SEPSIS-INDUCED IMMUNOSUPPRESSION**

Considering the contribution of sepsis-induced immunosuppression to sepsis-associated disability and mortality, immunomodulatory molecules enhancing immunity against infections can be considered as potential drug candidates, although anti-inflammatory drugs are probably effective in patients with increased pro-inflammatory cytokines in early sepsis. We briefly review the currently considered immunomodulatory therapies along with outcomes of clinical studies.

**GM-CSF AND G-CSF**

Granulocyte macrophage colony stimulating factor (GM-CSF) is a cytokine that accelerates stem cell precursors to differentiate into neutrophils, monocytes, and macrophages (Francisco-Cruz et al., 2014); hence, investigators tested whether administration of GM-CSF is beneficial for sepsis management. In a biomarker-guided clinical trial, wherein sepsis patients were selected on the basis of decreased HLA-DR expression, GM-CSF treatment reversed inactivation of monocyte function (Meisel et al., 2009) owing to increased HLA-DR expression and cytokine production, and yielded shorter periods for mechanical ventilation and fewer days in the ICU (Meisel et al., 2009) in comparison with untreated patients. In other clinical trials for pediatric and neonatal sepsis patients GM-CSF therapy also restored TNF-α production and diminished nosocomial infections (Hall et al., 2011). Granulocyte colony stimulating factor (G-CSF) is administered to immunocompromised patients undergoing BM transplantation and to those with cancer to prevent infection through an increase in the number of neutrophils. With the same rationale, researchers conducted clinical trials for sepsis, using G-CSF (Pett et al., 2002). There was a significant increase in the number of leukocytes, including neutrophils, although improvement of 28-day mortality was not determined (Nelson et al., 1998; Root et al., 2003). Furthermore, a meta-analysis of twelve clinical trials using GM-CSF or G-CSF for sepsis also demonstrated increased resolution of infection (Bo et al., 2011). Considering that most patients who died in the protracted phase have persistent infection, GM-CSF and/or G-CSF, in combination with other immunomodulatory agents, may effi-

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ciently eradicate infection and improve mortality. However, the effects of GM-CSF seem weaker than those of IFN-γ and there is a concern that GM-CSF may have an immunosuppressive effect, thereby inducing the proliferation of immunosuppressive cells such as myeloid-derived suppressive cells (MDSCs) (Hoeller et al., 2016).

**IFN-γ**

IFN-γ is a cytokine potentiating monocyte and macrophage activities and plays pivotal roles in defending against bacterial, viral, and fungal infections (Docke et al., 1997). It has been reported that application of IFN-γ to sepsis patients with low monocytic HLA-DR expression reversed the inactivation of monocytes and resulted in the clearance of sepsis (Docke et al., 1997), and several case studies have indicated that IFN-γ treatment induced eradication of persistent bacterial infections (Dries et al., 1994) and potentially lethal fungal infections in patients with low monocyte HLA-DR expression (Hotchkiss et al., 2013b), indicating that IFN-γ therapy may be useful for sepsis patients with persistent infections. IFN-γ therapy increased monocyte HLA-DR expression and the number of IL-17 producing CD4+ T cells (Nalos et al., 2012), and in addition mediated clinical resolution of sepsis. Furthermore, it seems to be promising because it is safe and does not induce cytokine storm (Hotchkiss et al., 2013b). IFN-γ may be more efficacious if administered in a time-phased approach in conjunction with GM-CSF. IL-7, or other immunomodulatory molecules to enhance specific immune functions and reduce secondary infections. However, as an immunosuppressive cytokine, IFN-γ may have limitations. Recently, it has been reported that long-term treatment with IFN-γ induced increased expression of PD-1 and PD-L1 ligand (Mandai et al., 2016).

**IL-7**

IL-7 is a 25-kDa glycoprotein produced by stromal cells and the IL-7 receptor is expressed by most resting human T cells. Naive and memory T cells express IL-7 at high levels, while Tregs express IL-7 at low levels (Lundstrom et al., 2012). IL-7 receptor mediated signaling is critical for development, proliferation, and homeostasis (Mackall et al., 2011) in T cells. Considering severe impairments in T cells during sepsis, IL-7 may be a suitable therapeutic candidate. The advantage of IL-7 as an immune modulator in sepsis is that IL-7 not only significantly broadens circulating T cell repertoire diversity (Perales et al., 2012), but also reduces the proportions of circulating Tregs (Unsinger et al., 2010). In a murine model of peritonitis, IL-7 administration inhibited cell apoptosis, restored IFN-γ production, induced delayed-type hypersensitivity, and finally improved host survival (Unsinger et al., 2010). Furthermore, an animal model of 2 hit (second fungal infection after the first bacterial infection) showed that IL-7 administration restored loss of delayed-type hypersensitivity, a hallmark of sepsis, and improved survival against secondary infections (Unsinger et al., 2012). Consistently, ex vivo treatment of cells of sepsis patients with human recombinant IL-7 treatment induced improved lymphocyte functions including CD4+ and CD8+ T cell proliferation, diminished IFN-γ production, impaired phosphorylation of signal transducer and activator of transcription 5 (STAT5), and reduced BCL-2 levels (Venet et al., 2012). Clinical trials with IL-7 have been performed on patients with viral infections and cancers and the results have shown increased circulating CD4+ and CD8+ T cell levels and spleen and lymph node augmentation (Mackall et al., 2011); hence, it is plausible that IL-7 administration may be a promising therapeutic strategy for sepsis, wherein T cells continuously undergo apoptosis.

**PD-1 and PD-L1**

PD-1 and PD-L1 have been recognized in co-inhibition of T cell function (Sharpe et al., 2007). PD-1 is expressed on T cells, B cells, myeloid cells, and DCs; PD-L1 is expressed in epithelial cells, endothelial cells, and antigen-presenting cells including monocytes/macrophages, and DCs (Chen and Flies, 2013). Under the environment of prolonged antigen exposure such as chronic viral infection and cancer, T cells undergo “T cell exhaustion” via upregulation of PD-1 and PD-L1. The interaction of PD-1 with PD-L1 produces inhibitory signals and negatively regulates immune cell effector functions (Day et al., 2006). Therefore, anti-PD-1 or anti-PD-L1 antibody treatments have shown success in chronic viral infection and cancer (Hutcheson et al., 2014). Since sepsis presents an immunosuppression similar to that observed in cancer, it has been suggested that anti–PD-1 and anti–PD-L1 therapies could have similar beneficial effects in sepsis patients with immune cell dysfunction (Topalian et al., 2012). Indeed, increased PD-1 expression was reported both in monocytes and lymphocytes from septic shock patients in the ICU (Boomer et al., 2011) and in peritoneal macrophages and T/B lymphocytes in sepsis mice (Huang et al., 2009), which was consistent with mortality and nosocomial infections (Unsinger et al., 2010; Perales et al., 2012). Furthermore, animal models of sepsis and sepsis patients showed increased expression of PD-L1 in neutrophils, macrophages, and peripheral blood (Huang et al., 2014), resulting in immune cell apoptosis (Heffernan et al., 2012). As expected, it has been reported that treatment

**Table 2. Immune modulatory therapeutics**

| Modulator | Therapeutic effects |
|-----------|---------------------|
| Recombinant GM-CSF | Increased leukocyte maturation |
| Recombinant G-CSF | Increased leukocyte maturation |
| Recombinant IFN-γ | Elevated monocyte HLA-DR expression |
| Recombinant IL-7 | Th17 skewing |
| Recombinant PD-1/PD-L1 antagonist | Prevention of T cell exhaustion |

and reduced BCL-2 levels (Venet et al., 2012). Clinical trials with IL-7 have been performed on patients with viral infections and cancers and the results have shown increased circulating CD4+ and CD8+ T cell levels and spleen and lymph node augmentation (Mackall et al., 2011); hence, it is plausible that IL-7 administration may be a promising therapeutic strategy for sepsis, wherein T cells continuously undergo apoptosis.
with PD-1 and PD-L1-blocking antibodies protected mice from sepsis-induced mortality (Zhang et al., 2010) and reduced incidence of secondary fungal infection (Chang et al., 2013). PD-1 and PD-L1 have suggested as biomarkers for immunomodulatory therapy, and blocking of the inhibitory molecules may be a reasonable strategy for sepsis treatment (Chang et al., 2014).

CONCLUSION AND FUTURE PROSPECTIVE

Most researchers and clinicians agree that sepsis patients should be treated using personalized precision medicine strategies because sepsis induces heterogeneous and complex immune dysfunction during illness. To fulfill this, first, the cellular and molecular mechanisms by which therapeutic reagents have effects on immune functions need to be precisely elucidated. Second, the patients’ immune dysfunction should be diagnosed using innovative biomarkers. Although there have been advancements in the definition of the host immune status, e.g., decreased expression of monotypic HLA-DR and increased IL-10 production for GM-CSF or IFN-γ trials, phenotyping of patient’s immune status should be further sophisticated using modern molecular biotechnology, e.g., omics and system biology, and a combination of biomarkers can be established for goal-directed application of immunomodulatory therapies. Considering the previous trials for drug development for sepsis, we currently understand that a single agent targeting a single pathway would be ineffective. Therefore, the new development of combination therapy to correct multiple defects in individuals in a time-phased approach is definitely needed. In summary, we propose here that with concept of theranostic that is treating patients with both a diagnostic test to identify patients most likely to be treated by new drugs and a targeted therapy based on the test’s results, immune modulatory intervention to overcome sepsis-induced immune alterations can be a prospective strategy for sepsis therapy.

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