BIOMOLECULES & THERAPEUTICS

Review
Biomol Ther, 569-577 (2017)

Potential Immunotherapeutics for Immunosuppression in Sepsis

Jinwook Shin[1] and Mirim Jin[2,*]

[1]*Department of Microbiology, College of Medicine, Inha University, Incheon 22212,*
[2]*Department of Microbiology, College of Medicine, Gachon University, Incheon 21999, Republic of Korea*

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,

Open Access **https://doi.org/10.4062/biomolther.2017.193**

Received Sep 26, 2017 **Revised** Oct 10, 2017 **Accepted** Oct 10, 2017
Published Online November 1, 2017

***Corresponding Author**
E-mail: mirimj@gachon.ac.kr
Tel: +82-32-899-6080, Fax: +82-32-899-6029

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

surgical approaches for eliminating the source of infection, administration of intravenous fluids to restore and maintain adequate intravascular volume, and vasoconstriction 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 immunomodulatory 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

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 punctuation (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 medicated 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 (Pastille *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; Riccardi *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 programed 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 Veerdonk *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; Unsinger *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 (Petit *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-

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. Naïve 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 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.

Table 2. Immune modulatory therapeutics

Modulator	Therapeutic effects
Recombinant GM-CSF	Increased leukocyte maturation Enhanced antigen presentation Restored TNF-α production Reduced ventilation days
Recombinant G-CSF	Increased leukocyte maturation
Recombinant IFN-γ	Elevated monocytic HLA-DR expression Th17 skewing No further cytokine storm
Recombinant IL-7	Increased T cell diversity Increased T cell proliferation and survival Reduced Treg population Restored IFN-γ production of T cells Restored loss of delayed-type hypersensitivity
PD-1/PD-L1 antagonist	Prevention of T cell exhaustion

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 (Hutchins *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

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 monocytic 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.

ACKNOWLEDGMENTS

We thank SJ Yoon for supporting manuscript preparation. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1D-1A1B03935883), INHA University Research Grant INHA-53348-01 for J.S, the Bio-Synergy Research Project (NRF-2012M3A9C4048758) and Global Frontier Project grants (NRF-2015M3A6A4065732) of the Ministry of Science, ICT and Future Planning through the National Research Foundation.

REFERENCES

Alves-Filho, J. C., Freitas, A., Souto, F. O., Spiller, F., Paula-Neto, H., Silva, J. S., Gazzinelli, R. T., Teixeira, M. M., Ferreira, S. H. and Cunha, F. Q. (2009) Regulation of chemokine receptor by Toll-like receptor 2 is critical to neutrophil migration and resistance to polymicrobial sepsis. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 4018-4023.

Alves-Filho, J. C., Spiller, F. and Cunha, F. Q. (2010) Neutrophil paralysis in sepsis. *Shock* **34 Suppl 1**, 15-21.

Angus, D. C. (2012) Drotrecogin alfa (activated)...a sad final fizzle to a roller-coaster party. *Crit Care* **16**, 107.

Benjamim, C. F., Lundy, S. K., Lukacs, N. W., Hogaboam, C. M. and Kunkel, S. L. (2005) Reversal of long-term sepsis-induced immunosuppression by dendritic cells. *Blood* **105**, 3588-3595.

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T. B., Oukka, M., Weiner, H. L. and Kuchroo, V. K. (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* **441**, 235-238.

Biedermann, T., Kneilling, M., Mailhammer, R., Maier, K., Sander, C. A., Kollias, G., Kunkel, S. L., Hultner, L. and Rocken, M. (2000) Mast cells control neutrophil recruitment during T cell-mediated delayed-type hypersensitivity reactions through tumor necrosis factor and macrophage inflammatory protein 2. *J. Exp. Med.* **192**, 1441-1452.

Biswas, S. K. and Lopez-Collazo, E. (2009) Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trends Immunol.* **30**, 475-487.

Bo, L., Wang, F., Zhu, J., Li, J. and Deng, X. (2011) Granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) for sepsis: a meta-analysis. *Crit. Care* **15**, R58.

Boomer, J. S., To, K., Chang, K. C., Takasu, O., Osborne, D. F., Walton, A. H., Bricker, T. L., Jarman, S. D., 2nd, Kreisel, D., Krupnick, A. S., Srivastava, A., Swanson, P. E., Green, J. M. and Hotchkiss, R. S. (2011) Immunosuppression in patients who die of sepsis and multiple organ failure. *JAMA* **306**, 2594-2605.

Camacho-Gonzalez, A., Spearman, P. W. and Stoll, B. J. (2013) Neonatal infectious diseases: evaluation of neonatal sepsis. *Pediatr. Clin. North Am.* **60**, 367-389.

Cavaillon, J. M. and Adib-Conquy, M. (2006) Bench-to-bedside review: endotoxin tolerance as a model of leukocyte reprogramming in sepsis. *Crit. Care* **10**, 233.

Chang, K., Svabek, C., Vazquez-Guillamet, C., Sato, B., Rasche, D., Wilson, S., Robbins, P., Ulbrandt, N., Suzich, J., Green, J., Patera, A. C., Blair, W., Krishnan, S. and Hotchkiss, R. (2014) Targeting the programmed cell death 1: programmed cell death ligand 1 pathway reverses T cell exhaustion in patients with sepsis. *Crit. Care* **18**, R3.

Chang, K. C., Burnham, C. A., Compton, S. M., Rasche, D. P., Mazuski, R. J., McDonough, J. S., Unsinger, J., Korman, A. J., Green, J. M. and Hotchkiss, R. S. (2013) Blockade of the negative co-stimulatory molecules PD-1 and CTLA-4 improves survival in primary and secondary fungal sepsis. *Crit. Care* **17**, R85.

Chen, L. and Flies, D. B. (2013) Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat. Rev. Immunol.* **13**, 227-242.

Coopersmith, C. M., Wunsch, H., Fink, M. P., Linde-Zwirble, W. T., Olsen, K. M., Sommers, M. S., Anand, K. J., Tchorz, K. M., Angus, D. C. and Deutschman, C. S. (2012) A comparison of critical care research funding and the financial burden of critical illness in the United States. *Crit. Care Med.* **40**, 1072-1079.

Cummings, C. J., Martin, T. R., Frevert, C. W., Quan, J. M., Wong, V. A., Mongovin, S. M., Hagen, T. R., Steinberg, K. P. and Goodman, R. B. (1999) Expression and function of the chemokine receptors CXCR1 and CXCR2 in sepsis. *J. Immunol.* **162**, 2341-2346.

Day, C. L., Kaufmann, D. E., Kiepiela, P., Brown, J. A., Moodley, E. S., Reddy, S., Mackey, E. W., Miller, J. D., Leslie, A. J., DePierres, C., Mncube, Z., Duraiswamy, J., Zhu, B., Eichbaum, Q., Altfeld, M., Wherry, E. J., Coovadia, H. M., Goulder, P. J., Klenerman, P., Ahmed, R., Freeman, G. J. and Walker, B. D. (2006) PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* **443**, 350-354.

Delano, M. J., Thayer, T., Gabrilovich, S., Kelly-Scumpia, K. M., Winfield, R. D., Scumpia, P. O., Cuenca, A. G., Warner, E., Wallet, S. M., Wallet, M. A., O'Malley, K. A., Ramphal, R., Clare-Salzer, M., Efron, P. A., Mathews, C. E. and Moldawer, L. L. (2011) Sepsis induces early alterations in innate immunity that impact mortality to secondary infection. *J. Immunol.* **186**, 195-202.

Delano, M. J. and Ward, P. A. (2016) Sepsis-induced immune dysfunction: can immune therapies reduce mortality? *J. Clin. Invest.* **126**, 23-31.

Docke, W. D., Randow, F., Syrbe, U., Krausch, D., Asadullah, K., Reinke, P., Volk, H. D. and Kox, W. (1997) Monocyte deactivation in

septic patients: restoration by IFN-gamma treatment. *Nat. Med.* **3**, 678-681.

Dries, D. J., Jurkovich, G. J., Maier, R. V., Clemmer, T. P., Struve, S. N., Weigelt, J. A., Stanford, G. G., Herr, D. L., Champion, H. R., Lewis, F. R., Hoyt, D., Hansbrough, J., Yellin, A. E., Berne, T. V., Trunkey, D. D., Jaffe, H. S., Munera, C., Fisher, P. and Starko, K. M. (1994) Effect of interferon gamma on infection-related death in patients with severe injuries. A randomized, double-blind, placebo-controlled trial. *Arch. Surg.* **129**, 1031-1041; discussion 1042.

Ertel, W., Kremer, J. P., Kenney, J., Steckholzer, U., Jarrar, D., Trentz, O. and Schildberg, F. W. (1995) Downregulation of proinflammatory cytokine release in whole blood from septic patients. *Blood* **85**, 1341-1347.

Fehervari, Z. and Sakaguchi, S. (2004) $CD4^+$ Tregs and immune control. *J. Clin. Invest.* **114**, 1209-1217.

Felmet, K. A., Hall, M. W., Clark, R. S., Jaffe, R. and Carcillo, J. A. (2005) Prolonged lymphopenia, lymphoid depletion, and hypoprolactinemia in children with nosocomial sepsis and multiple organ failure. *J. Immunol.* **174**, 3765-3772.

Fink, M. P. and Warren, H. S. (2014) Strategies to improve drug development for sepsis. *Nat. Rev. Drug Discov.* **13**, 741-758.

Francisco-Cruz, A., Aguilar-Santelises, M., Ramos-Espinosa, O., Mata-Espinosa, D., Marquina-Castillo, B., Barrios-Payan, J. and Hernandez-Pando, R. (2014) Granulocyte-macrophage colony-stimulating factor: not just another haematopoietic growth factor. *Med. Oncol.* **31**, 774.

Gaieski, D. F., Edwards, J. M., Kallan, M. J. and Carr, B. G. (2013) Benchmarking the incidence and mortality of severe sepsis in the United States. *Crit. Care Med.* **41**, 1167-1174.

Gautier, E. L., Huby, T., Saint-Charles, F., Ouzilleau, B., Chapman, M. J. and Lesnik, P. (2008) Enhanced dendritic cell survival attenuates lipopolysaccharide-induced immunosuppression and increases resistance to lethal endotoxic shock. *J. Immunol.* **180**, 6941-6946.

Gow, N. A., van de Veerdonk, F. L., Brown, A. J. and Netea, M. G. (2011) Candida albicans morphogenesis and host defence: discriminating invasion from colonization. *Nat. Rev. Microbiol.* **10**, 112-122.

Guignant, C., Lepape, A., Huang, X., Kherouf, H., Denis, L., Poitevin, F., Malcus, C., Cheron, A., Allaouchiche, B., Gueyffier, F., Ayala, A., Monneret, G. and Venet, F. (2011) Programmed death-1 levels correlate with increased mortality, nosocomial infection and immune dysfunctions in septic shock patients. *Crit. Care* **15**, R99.

Guisset, O., Dilhuydy, M. S., Thiebaut, R., Lefevre, J., Camou, F., Sarrat, A., Gabinski, C., Moreau, J. F. and Blanco, P. (2007) Decrease in circulating dendritic cells predicts fatal outcome in septic shock. *Intensive Care Med.* **33**, 148-152.

Hall, M. W., Knatz, N. L., Vetterly, C., Tomarello, S., Wewers, M. D., Volk, H. D. and Carcillo, J. A. (2011) Immunoparalysis and nosocomial infection in children with multiple organ dysfunction syndrome. *Intensive Care Med.* **37**, 525-532.

Heffernan, D. S., Monaghan, S. F., Thakkar, R. K., Machan, J. T., Cioffi, W. G. and Ayala, A. (2012) Failure to normalize lymphopenia following trauma is associated with increased mortality, independent of the leukocytosis pattern. *Crit Care* **16**, R12.

Heidecke, C. D., Hensler, T., Weighardt, H., Zantl, N., Wagner, H., Siewert, J. R. and Holzmann, B. (1999) Selective defects of T lymphocyte function in patients with lethal intraabdominal infection. *Am. J. Surg.* **178**, 288-292.

Hoeller, C., Michielin, O., Ascierto, P. A., Szabo, Z. and Blank, C. U. (2016) Systematic review of the use of granulocyte-macrophage colony-stimulating factor in patients with advanced melanoma. *Cancer Immunol. Immunother.* **65**, 1015-1034.

Hotchkiss, R. S., Monneret, G. and Payen, D. (2013a) Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. *Lancet Infect. Dis.* **13**, 260-268.

Hotchkiss, R. S., Monneret, G. and Payen, D. (2013b) Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat. Rev. Immunol.* **13**, 862-874.

Hotchkiss, R. S. and Nicholson, D. W. (2006) Apoptosis and caspases regulate death and inflammation in sepsis. *Nat. Rev. Immunol.* **6**, 813-822.

Hotchkiss, R. S., Tinsley, K. W., Swanson, P. E., Grayson, M. H., Osborne, D. F., Wagner, T. H., Cobb, J. P., Coopersmith, C. and Karl, I. E. (2002) Depletion of dendritic cells, but not macrophages, in patients with sepsis. *J. Immunol.* **168**, 2493-2500.

Hotchkiss, R. S., Tinsley, K. W., Swanson, P. E., Schmieg, R. E., Jr., Hui, J. J., Chang, K. C., Osborne, D. F., Freeman, B. D., Cobb, J. P., Buchman, T. G. and Karl, I. E. (2001) Sepsis-induced apoptosis causes progressive profound depletion of B and $CD4^+$ T lymphocytes in humans. *J. Immunol.* **166**, 6952-6963.

Huang, X., Chen, Y., Chung, C. S., Yuan, Z., Monaghan, S. F., Wang, F. and Ayala, A. (2014) Identification of B7-H1 as a novel mediator of the innate immune/pro-inflammatory response as well as a possible myeloid cell prognostic biomarker in sepsis. *J. Immunol.* **192**, 1091-1099.

Huang, X., Venet, F., Wang, Y. L., Lepape, A., Yuan, Z., Chen, Y., Swan, R., Kherouf, H., Monneret, G., Chung, C. S. and Ayala, A. (2009) PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 6303-6308.

Hutchins, N. A., Unsinger, J., Hotchkiss, R. S. and Ayala, A. (2014) The new normal: immunomodulatory agents against sepsis immune suppression. *Trends Mol. Med.* **20**, 224-233.

Kasten, K. R., Prakash, P. S., Unsinger, J., Goetzman, H. S., England, L. G., Cave, C. M., Seitz, A. P., Mazuski, C. N., Zhou, T. T., Morre, M., Hotchkiss, R. S., Hildeman, D. A. and Caldwell, C. C. (2010) Interleukin-7 (IL-7) treatment accelerates neutrophil recruitment through $\gamma\delta$ T-cell IL-17 production in a murine model of sepsis. *Infect. Immun.* **78**, 4714-4722.

Kaukonen, K. M., Bailey, M., Pilcher, D., Cooper, D. J. and Bellomo, R. (2015) Systemic inflammatory response syndrome criteria in defining severe sepsis. *N. Engl. J. Med.* **372**, 1629-1638.

Kellum, J. A., Kong, L., Fink, M. P., Weissfeld, L. A., Yealy, D. M., Pinsky, M. R., Fine, J., Krichevsky, A., Delude, R. L. and Angus, D. C. (2007) Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. *Arch. Intern. Med.* **167**, 1655-1663.

Kim, S. J., Park, J. S., Lee, D. W. and Lee, S. M. (2016) Trichostatin A protects liver against septic injury through inhibiting toll-like receptor signaling. *Biomol. Ther. (Seoul)* **24**, 387-394.

Kollef, K. E., Schramm, G. E., Wills, A. R., Reichley, R. M., Micek, S. T. and Kollef, M. H. (2008) Predictors of 30-day mortality and hospital costs in patients with ventilator-associated pneumonia attributed to potentially antibiotic-resistant gram-negative bacteria. *Chest* **134**, 281-287.

Kovach, M. A. and Standiford, T. J. (2012) The function of neutrophils in sepsis. *Curr. Opin. Infect. Dis.* **25**, 321-327.

Lerman, Y. V., Lim, K., Hyun, Y. M., Falkner, K. L., Yang, H., Pietropaoli, A. P., Sonnenberg, A., Sarangi, P. P. and Kim, M. (2014) Sepsis lethality via exacerbated tissue infiltration and TLR-induced cytokine production by neutrophils is integrin $\alpha 3\beta 1$-dependent. *Blood* **124**, 3515-3523.

Lewkowicz, P., Lewkowicz, N., Sasiak, A. and Tchorzewski, H. (2006) Lipopolysaccharide-activated CD4+CD25+ T regulatory cells inhibit neutrophil function and promote their apoptosis and death. *J. Immunol.* **177**, 7155-7163.

Limaye, A. P., Kirby, K. A., Rubenfeld, G. D., Leisenring, W. M., Bulger, E. M., Neff, M. J., Gibran, N. S., Huang, M. L., Santo Hayes, T. K., Corey, L. and Boeckh, M. (2008) Cytomegalovirus reactivation in critically ill immunocompetent patients. *JAMA* **300**, 413-422.

Liston, A. and Gray, D. H. (2014) Homeostatic control of regulatory T cell diversity. *Nat. Rev. Immunol.* **14**, 154-165.

Lorente, J. A. and Marshall, J. C. (2005) Neutralization of tumor necrosis factor in preclinical models of sepsis. *Shock* **24 Suppl 1**, 107-119.

Lukaszewicz, A. C., Grienay, M., Resche-Rigon, M., Pirracchio, R., Faivre, V., Boval, B. and Payen, D. (2009) Monocytic HLA-DR expression in intensive care patients: interest for prognosis and secondary infection prediction. *Crit. Care Med.* **37**, 2746-2752.

Lundstrom, W., Fewkes, N. M. and Mackall, C. L. (2012) IL-7 in human health and disease. *Semin. Immunol.* **24**, 218-224.

Luyt, C. E. and Kaiser, L. (2012) Virus detection in patients with severe pneumonia: still more questions than answers? *Am. J. Respir. Crit. Care Med.* **186**, 301-302.

Mackall, C. L., Fry, T. J. and Gress, R. E. (2011) Harnessing the biology of IL-7 for therapeutic application. *Nat. Rev. Immunol.* **11**, 330-342.
Mandai, M., Hamanishi, J., Abiko, K., Matsumura, N., Baba, T. and Konishi, I. (2016) Dual faces of IFNγ in cancer progression: a role of PD-L1 induction in the determination of pro- and antitumor immunity. *Clin. Cancer Res.* **22**, 2329-2334.
Martin, G. S., Mannino, D. M., Eaton, S. and Moss, M. (2003) The epidemiology of sepsis in the United States from 1979 through 2000. *N. Engl. J. Med.* **348**, 1546-1554.
Mayr, F. B., Yende, S. and Angus, D. C. (2014) Epidemiology of severe sepsis. *Virulence* **5**, 4-11.
Meakins, J. L., Christou, N. V., Bohnen, J. and MacLean, L. D. (1982) Failure of delayed hypersensitivity skin testing to predict postoperative sepsis and mortality. *Br. Med. J. (Clin. Res. Ed.)* **285**, 1207-1208.
Meisel, C., Schefold, J. C., Pschowski, R., Baumann, T., Hetzger, K., Gregor, J., Weber-Carstens, S., Hasper, D., Keh, D., Zuckermann, H., Reinke, P. and Volk, H. D. (2009) Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. *Am. J. Respir. Crit. Care Med.* **180**, 640-648.
Monneret, G., Debard, A. L., Venet, F., Bohe, J., Hequet, O., Bienvenu, J. and Lepape, A. (2003) Marked elevation of human circulating CD4+CD25+ regulatory T cells in sepsis-induced immunoparalysis. *Crit. Care Med.* **31**, 2068-2071.
Monneret, G., Finck, M. E., Venet, F., Debard, A. L., Bohe, J., Bienvenu, J. and Lepape, A. (2004) The anti-inflammatory response dominates after septic shock: association of low monocyte HLA-DR expression and high interleukin-10 concentration. *Immunol. Lett.* **95**, 193-198.
Monneret, G., Venet, F., Kullberg, B. J. and Netea, M. G. (2011) ICU-acquired immunosuppression and the risk for secondary fungal infections. *Med. Mycol.* **49 Suppl 1**, S17-S23.
Monneret, G., Venet, F., Pachot, A. and Lepape, A. (2008) Monitoring immune dysfunctions in the septic patient: a new skin for the old ceremony. *Mol. Med.* **14**, 64-78.
Moore, F. A. and Moore, E. E. (1995) Evolving concepts in the pathogenesis of postinjury multiple organ failure. *Surg. Clin. North Am.* **75**, 257-277.
Morris, A. C., Brittan, M., Wilkinson, T. S., McAuley, D. F., Antonelli, J., McCulloch, C., Barr, L. C., McDonald, N. A., Dhaliwal, K., Jones, R. O., Mackellar, A., Haslett, C., Hay, A. W., Swann, D. G., Anderson, N., Laurenson, I. F., Davidson, D. J., Rossi, A. G., Walsh, T. S. and Simpson, A. J. (2011) C5a-mediated neutrophil dysfunction is RhoA-dependent and predicts infection in critically ill patients. *Blood* **117**, 5178-5188.
Munford, R. S. and Pugin, J. (2001) Normal responses to injury prevent systemic inflammation and can be immunosuppressive. *Am. J. Respir. Crit. Care Med.* **163**, 316-321.
Munoz, C., Carlet, J., Fitting, C., Misset, B., Bleriot, J. P. and Cavaillon, J. M. (1991) Dysregulation of in vitro cytokine production by monocytes during sepsis. *J. Clin. Invest.* **88**, 1747-1754.
Nalos, M., Santner-Nanan, B., Parnell, G., Tang, B., McLean, A. S. and Nanan, R. (2012) Immune effects of interferon gamma in persistent staphylococcal sepsis. *Am. J. Respir. Crit. Care Med.* **185**, 110-112.
Nathan, C. (2006) Neutrophils and immunity: challenges and opportunities. *Nat. Rev. Immunol.* **6**, 173-182.
Needham, D. M., Davidson, J., Cohen, H., Hopkins, R. O., Weinert, C., Wunsch, H., Zawistowski, C., Bemis-Dougherty, A., Berney, S. C., Bienvenu, O. J., Brady, S. L., Brodsky, M. B., Denehy, L., Elliott, D., Flatley, C., Harabin, A. L., Jones, C., Louis, D., Meltzer, W., Muldoon, S. R., Palmer, J. B., Perme, C., Robinson, M., Schmidt, D. M., Scruth, E., Spill, G. R., Storey, C. P., Render, M., Votto, J. and Harvey, M. A. (2012) Improving long-term outcomes after discharge from intensive care unit: report from a stakeholders' conference. *Crit. Care Med.* **40**, 502-509.
Nelson, S., Belknap, S. M., Carlson, R. W., Dale, D., DeBoisblanc, B., Farkas, S., Fotheringham, N., Ho, H., Marrie, T., Movahhed, H., Root, R. and Wilson, J. (1998) A randomized controlled trial of filgrastim as an adjunct to antibiotics for treatment of hospitalized patients with community-acquired pneumonia. CAP Study Group. *J. Infect. Dis.* **178**, 1075-1080.
Otto, G. P., Sossdorf, M., Claus, R. A., Rodel, J., Menge, K., Reinhart, K., Bauer, M. and Riedemann, N. C. (2011) The late phase of sepsis is characterized by an increased microbiological burden and death rate. *Crit. Care* **15**, R183.
Pachot, A., Monneret, G., Voirin, N., Leissner, P., Venet, F., Bohe, J., Payen, D., Bienvenu, J., Mougin, B. and Lepape, A. (2005) Longitudinal study of cytokine and immune transcription factor mRNA expression in septic shock. *Clin. Immunol.* **114**, 61-69.
Panacek, E. A., Marshall, J. C., Albertson, T. E., Johnson, D. H., Johnson, S., MacArthur, R. D., Miller, M., Barchuk, W. T., Fischkoff, S., Kaul, M., Teoh, L., Van Meter, L., Daum, L., Lemeshow, S., Hicklin, G. and Doig, C. (2004) Efficacy and safety of the monoclonal anti-tumor necrosis factor antibody F(ab')2 fragment afelimomab in patients with severe sepsis and elevated interleukin-6 levels. *Crit. Care Med.* **32**, 2173-2182.
Parihar, A., Eubank, T. D. and Doseff, A. I. (2010) Monocytes and macrophages regulate immunity through dynamic networks of survival and cell death. *J. Innate. Immun.* **2**, 204-215.
Pastille, E., Didovic, S., Brauckmann, D., Rani, M., Agrawal, H., Schade, F. U., Zhang, Y. and Flohe, S. B. (2011) Modulation of dendritic cell differentiation in the bone marrow mediates sustained immunosuppression after polymicrobial sepsis. *J. Immunol.* **186**, 977-986.
Perales, M. A., Goldberg, J. D., Yuan, J., Koehne, G., Lechner, L., Papadopoulos, E. B., Young, J. W., Jakubowski, A. A., Zaidi, B., Gallardo, H., Liu, C., Rasalan, T., Wolchok, J. D., Croughs, T., Morre, M., Devlin, S. M. and van den Brink, M. R. (2012) Recombinant human interleukin-7 (CYT107) promotes T-cell recovery after allogeneic stem cell transplantation. *Blood* **120**, 4882-4891.
Petit, I., Szyper-Kravitz, M., Nagler, A., Lahav, M., Peled, A., Habler, L., Ponomaryov, T., Taichman, R. S., Arenzana-Seisdedos, F., Fujii, N., Sandbank, J., Zipori, D. and Lapidot, T. (2002) G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat. Immunol.* **3**, 687-694.
Poehlmann, H., Schefold, J. C., Zuckermann-Becker, H., Volk, H. D. and Meisel, C. (2009) Phenotype changes and impaired function of dendritic cell subsets in patients with sepsis: a prospective observational analysis. *Crit. Care* **13**, R119.
Qiu, P., Cui, X., Sun, J., Welsh, J., Natanson, C. and Eichacker, P. Q. (2013) Antitumor necrosis factor therapy is associated with improved survival in clinical sepsis trials: a meta-analysis. *Crit. Care Med.* **41**, 2419-2429.
Reber, A. J., Chirkova, T., Kim, J. H., Cao, W., Biber, R., Shay, D. K. and Sambhara, S. (2012). Immunosenescence and challenges of vaccination against influenza in the aging population. *Aging Dis* **3**, 68-90.
Riccardi, F., Della Porta, M. G., Rovati, B., Casazza, A., Radolovich, D., De Amici, M., Danova, M. and Langer, M. (2011) Flow cytometric analysis of peripheral blood dendritic cells in patients with severe sepsis. *Cytometry B Clin. Cytom.* **80**, 14-21.
Rice, T. W., Wheeler, A. P., Bernard, G. R., Vincent, J. L., Angus, D. C., Aikawa, N., Demeyer, I., Sainati, S., Amlot, N., Cao, C., Ii, M., Matsuda, H., Mouri, K. and Cohen, J. (2010) A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis. *Crit. Care Med.* **38**, 1685-1694.
Riewald, M. and Ruf, W. (2005) Protease-activated receptor-1 signaling by activated protein C in cytokine-perturbed endothelial cells is distinct from thrombin signaling. *J. Biol. Chem.* **280**, 19808-19814.
Romani, L. (2011) Immunity to fungal infections. *Nat. Rev. Immunol.* **11**, 275-288.
Ronchetti, S., Zollo, O., Bruscoli, S., Agostini, M., Bianchini, R., Nocentini, G., Ayroldi, E. and Riccardi, C. (2004) GITR, a member of the TNF receptor superfamily, is costimulatory to mouse T lymphocyte subpopulations. *Eur. J. Immunol.* **34**, 613-622.
Root, R. K., Lodato, R. F., Patrick, W., Cade, J. F., Fotheringham, N., Milwee, S., Vincent, J. L., Torres, A., Rello, J. and Nelson, S. (2003) Multicenter, double-blind, placebo-controlled study of the use of filgrastim in patients hospitalized with pneumonia and severe sepsis. *Crit. Care Med.* **31**, 367-373.
Roquilly, A., McWilliam, H. E. G., Jacqueline, C., Tian, Z., Cinotti, R., Rimbert, M., Wakim, L., Caminschi, I., Lahoud, M. H., Belz, G. T.,

Kallies, A., Mintern, J. D., Asehnoune, K. and Villadangos, J. A. (2017) Local modulation of antigen-presenting cell development after resolution of pneumonia induces long-term susceptibility to secondary infections. *Immunity* **47**, 135-147.e5.

Scumpia, P. O., Delano, M. J., Kelly-Scumpia, K. M., Weinstein, J. S., Wynn, J. L., Winfield, R. D., Xia, C., Chung, C. S., Ayala, A., Atkinson, M. A., Reeves, W. H., Clare-Salzler, M. J. and Moldawer, L. L. (2007) Treatment with GITR agonistic antibody corrects adaptive immune dysfunction in sepsis. *Blood* **110**, 3673-3681.

Shalova, I. N., Lim, J. Y., Chittezhath, M., Zinkernagel, A. S., Beasley, F., Hernandez-Jimenez, E., Toledano, V., Cubillos-Zapata, C., Rapisarda, A., Chen, J., Duan, K., Yang, H., Poidinger, M., Melillo, G., Nizet, V., Arnalich, F., Lopez-Collazo, E. and Biswas, S. K. (2015) Human monocytes undergo functional re-programming during sepsis mediated by hypoxia-inducible factor-1α. *Immunity* **42**, 484-498.

Sharpe, A. H., Wherry, E. J., Ahmed, R. and Freeman, G. J. (2007) The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat. Immunol.* **8**, 239-245.

Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., Bellomo, R., Bernard, G. R., Chiche, J. D., Coopersmith, C. M., Hotchkiss, R. S., Levy, M. M., Marshall, J. C., Martin, G. S., Opal, S. M., Rubenfeld, G. D., van der Poll, T., Vincent, J. L. and Angus, D. C. (2016) The third international consensus definitions for sepsis and septic shock (sepsis-3) *JAMA* **315**, 801-810.

Steinman, R. M. and Hemmi, H. (2006) Dendritic cells: translating innate to adaptive immunity. *Curr. Top. Microbiol. Immunol.* **311**, 17-58.

Stephan, F., Yang, K., Tankovic, J., Soussy, C. J., Dhonneur, G., Duvaldestin, P., Brochard, L., Brun-Buisson, C., Harf, A. and Delclaux, C. (2002) Impairment of polymorphonuclear neutrophil functions precedes nosocomial infections in critically ill patients. *Crit. Care Med.* **30**, 315-322.

Tamayo, E., Gomez, E., Bustamante, J., Gomez-Herreras, J. I., Fonteriz, R., Bobillo, F., Bermejo-Martin, J. F., Castrodeza, J., Heredia, M., Fierro, I. and Alvarez, F. J. (2012) Evolution of neutrophil apoptosis in septic shock survivors and nonsurvivors. *J. Crit. Care* **27**, 415.e1-415.e11.

Tannahill, G. M., Curtis, A. M., Adamik, J., Palsson-McDermott, E. M., McGettrick, A. F., Goel, G., Frezza, C., Bernard, N. J., Kelly, B., Foley, N. H., Zheng, L., Gardet, A., Tong, Z., Jany, S. S., Corr, S. C., Haneklaus, M., Caffrey, B. E., Pierce, K., Walmsley, S., Beasley, F. C., Cummins, E., Nizet, V., Whyte, M., Taylor, C. T., Lin, H., Masters, S. L., Gottlieb, E., Kelly, V. P., Clish, C., Auron, P. E., Xavier, R. J. and O'Neill, L. A. (2013) Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* **496**, 238-242.

Topalian, S. L., Hodi, F. S., Brahmer, J. R., Gettinger, S. N., Smith, D. C., McDermott, D. F., Powderly, J. D., Carvajal, R. D., Sosman, J. A., Atkins, M. B., Leming, P. D., Spigel, D. R., Antonia, S. J., Horn, L., Drake, C. G., Pardoll, D. M., Chen, L., Sharfman, W. H., Anders, R. A., Taube, J. M., McMiller, T. L., Xu, H., Korman, A. J., Jure-Kunkel, M., Agrawal, S., McDonald, D., Kollia, G. D., Gupta, A., Wigginton, J. M. and Sznol, M. (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* **366**, 2443-2454.

Torgersen, C., Moser, P., Luckner, G., Mayr, V., Jochberger, S., Hasibeder, W. R. and Dunser, M. W. (2009) Macroscopic postmortem findings in 235 surgical intensive care patients with sepsis. *Anesth. Analg.* **108**, 1841-1847.

Toti, P., De Felice, C., Occhini, R., Schuerfeld, K., Stumpo, M., Epistolato, M. C., Vatti, R. and Buonocore, G. (2004) Spleen depletion in neonatal sepsis and chorioamnionitis. *Am. J. Clin. Pathol.* **122**, 765-771.

Unsinger, J., Burnham, C. A., McDonough, J., Morre, M., Prakash, P. S., Caldwell, C. C., Dunne, W. M., Jr. and Hotchkiss, R. S. (2012) Interleukin-7 ameliorates immune dysfunction and improves survival in a 2-hit model of fungal sepsis. *J. Infect. Dis.* **206**, 606-616.

Unsinger, J., McGlynn, M., Kasten, K. R., Hoekzema, A. S., Watanabe, E., Muenzer, J. T., McDonough, J. S., Tschoep, J., Ferguson, T. A., McDunn, J. E., Morre, M., Hildeman, D. A., Caldwell, C. C. and Hotchkiss, R. S. (2010) IL-7 promotes T cell viability, trafficking, and functionality and improves survival in sepsis. *J. Immunol.* **184**, 3768-3779.

van de Veerdonk, F. L., Mouktaroudi, M., Ramakers, B. P., Pistiki, A., Pickkers, P., van der Meer, J. W., Netea, M. G. and Giamarellos-Bourboulis, E. J. (2012) Deficient Candida-specific T-helper 17 response during sepsis. *J. Infect. Dis.* **206**, 1798-1802.

van Deuren, M., Frieling, J. T., van der Ven-Jongekrijg, J., Neeleman, C., Russel, F. G., van Lier, H. J., Bartelink, A. K. and van der Meer, J. W. (1998) Plasma patterns of tumor necrosis factor-α (TNF) and TNF soluble receptors during acute meningococcal infections and the effect of plasma exchange. *Clin. Infect. Dis.* **26**, 918-923.

Venet, F., Chung, C. S., Kherouf, H., Geeraert, A., Malcus, C., Poitevin, F., Bohe, J., Lepape, A., Ayala, A. and Monneret, G. (2009) Increased circulating regulatory T cells (CD4+CD25+CD127-) contribute to lymphocyte anergy in septic shock patients. *Intensive Care Med.* **35**, 678-686.

Venet, F., Davin, F., Guignant, C., Larue, A., Cazalis, M. A., Darbon, R., Allombert, C., Mougin, B., Malcus, C., Poitevin-Later, F., Lepape, A. and Monneret, G. (2010) Early assessment of leukocyte alterations at diagnosis of septic shock. *Shock* **34**, 358-363.

Venet, F., Foray, A. P., Villars-Mechin, A., Malcus, C., Poitevin-Later, F., Lepape, A. and Monneret, G. (2012) IL-7 restores lymphocyte functions in septic patients. *J. Immunol.* **189**, 5073-5081.

Venet, F., Lukaszewicz, A. C., Payen, D., Hotchkiss, R. and Monneret, G. (2013) Monitoring the immune response in sepsis: a rational approach to administration of immunoadjuvant therapies. *Curr. Opin. Immunol.* **25**, 477-483.

Venet, F., Pachot, A., Debard, A. L., Bohe, J., Bienvenu, J., Lepape, A. and Monneret, G. (2004) Increased percentage of CD4+CD25+ regulatory T cells during septic shock is due to the decrease of CD4+CD25- lymphocytes. *Crit. Care Med.* **32**, 2329-2331.

Ward, P. A. and Bosmann, M. (2012) A historical perspective on sepsis. *Am. J. Pathol.* **181**, 2-7.

Wick, M., Kollig, E., Muhr, G. and Koller, M. (2000) The potential pattern of circulating lymphocytes TH1/TH2 is not altered after multiple injuries. *Arch. Surg.* **135**, 1309-1314.

Williams, S. C. (2012) After Xigris, researchers look to new targets to combat sepsis. *Nat. Med.* **18**, 1001.

Winters, B. D., Eberlein, M., Leung, J., Needham, D. M., Pronovost, P. J. and Sevransky, J. E. (2010) Long-term mortality and quality of life in sepsis: a systematic review. *Crit. Care Med.* **38**, 1276-1283.

Wynn, J. L., Scumpia, P. O., Delano, M. J., O'Malley, K. A., Ungaro, R., Abouhamze, A. and Moldawer, L. L. (2007) Increased mortality and altered immunity in neonatal sepsis produced by generalized peritonitis. *Shock* **28**, 675-683.

Xiao, W. Z., Mindrinos, M. N., Seok, J., Cuschieri, J., Cuenca, A. G., Gao, H., Hayden, D. L., Hennessy, L., Moore, E. E., Minei, J. P., Bankey, P. E., Johnson, J. L., Sperry, J., Nathens, A. B., Billiar, T. R., West, M. A., Brownstein, B. H., Mason, P. H., Baker, H. V., Finnerty, C. C., Jeschke, M. G., Lopez, M. C., Klein, M. B., Gamelli, R. L., Gibran, N. S., Arnoldo, B., Xu, W. H., Zhang, Y. P., Calvano, S. E., McDonald-Smith, G. P., Schoenfeld, D. A., Storey, J. D., Cobb, J. P., Warren, H. S., Moldawer, L. L., Herndon, D. N., Lowry, S. F., Maier, R. V., Davis, R. W. and Tompkins, R. G. (2011) A genomic storm in critically injured humans. *J. Exp. Med.* **208**, 2581-2590.

Zajac, A. J., Blattman, J. N., Murali-Krishna, K., Sourdive, D. J. D., Suresh, M., Altman, J. D. and Ahmed, R. (1998) Viral immune evasion due to persistence of activated T cells without effector function. *J. Exp. Med.* **188**, 2205-2213.

Zhang, Y., Li, J., Lou, J., Zhou, Y., Bo, L., Zhu, J., Zhu, K., Wan, X., Cai, Z. and Deng, X. (2011) Upregulation of programmed death-1 on T cells and programmed death ligand-1 on monocytes in septic shock patients. *Crit. Care* **15**, R70.

Zhang, Y., Zhou, Y., Lou, J., Li, J., Bo, L., Zhu, K., Wan, X., Deng, X. and Cai, Z. (2010) PD-L1 blockade improves survival in experimental sepsis by inhibiting lymphocyte apoptosis and reversing monocyte dysfunction. *Crit. Care* **14**, R220.

Ziegler, E. J., McCutchan, J. A., Fierer, J., Glauser, M. P., Sadoff, J. C., Douglas, H. and Braude, A. I. (1982) Treatment of gram-negative bacteremia and shock with human antiserum to a mutant Escherichia coli. *N. Engl. J. Med.* **307**, 1225-1230.