Mini Review Article

Immunoadjuvant therapy in sepsis: novel strategies for immunosuppressive sepsis coming down the pike

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Recent efforts have focused on immunoadjuvant therapies for sepsis. The host inflammatory response consequent to initial exposure to pathogens is often followed by anti-inflammatory forces, resulting in increased morbidity and mortality in such critically ill patients. In the subacute stage of sepsis, apoptosis (type I programmed cell death) and subsequently autophagy (type II programmed cell death) have been attracting recent research interest. Although many patients may die during the initial cytokine storm, those who survive this phase might acquire defining characteristics of profound immunosuppression, including failure to clear the primary infection, development of secondary opportunistic infections, and reactivation of latent viruses. Both types of cell death are currently thought to be associated with this subacute immunosuppressive phase of sepsis, and acceleration of autophagy might alleviate immunosuppression through regulation of apoptosis of key immune effector cells. Programmed cell death 1 (PD-1) and its corresponding ligand play a major pathological role in immunosuppression not only in cancer but also in sepsis. Positive costimulatory pathways in T cells, such as CD28 signaling, permit the effector T cell to expand, persist, and effectively clear antigen. However, PD-1 is a negative costimulatory pathway on T cells that broadly enhances immunosuppressive signals across the innate and adaptive immune system. To counter this immunosuppression in sepsis, checkpoint blockade has garnered attention in an area of clinical research. In this review, we introduce some approaches of immunotherapy using anti-PD-1 antibody in infectious diseases and share our future perspectives.

Key words: Apoptosis, autophagy, clinical trial, immunosuppression, programmed cell death, sepsis

BACKGROUND

Sepsis remains a leading cause of mortality in intensive care units worldwide. Despite promising preclinical research advances, over one hundred clinical trials have failed to identify a single effective therapy for this devastating syndrome. Recent efforts have focused on immunoadjuvant therapies. The host inflammatory response consequent to initial exposure to pathogens is often followed by anti-inflammatory forces, resulting in increased morbidity and mortality in such critically ill patients. Although many patients may die during the initial cytokine storm, those who survive this phase might acquire defining characteristics of profound immunosuppression including failure to clear the primary infection, development of secondary opportunistic infections, and reactivation of latent viruses. Extensive apoptosis (type I programmed cell death) occurs in splenic CD4+ and CD8 T cells in patients with sepsis, thereby leading to an acquired immune deficiency syndrome that resembles the immunosuppression occurring with AIDS. Additionally, those who survive to discharge have an increased risk of readmission due to new secondary infections. In fact, sepsis is the most common cause for hospital readmissions, and 90-day mortality in septic patients can be as high as 45%. Autophagy (type II programmed cell death) appears to play an active role in splenic CD4+ T cells in early sepsis. These two types of cell death pathways might be associated with immunosuppression subsequent to the acute resuscitation phase of sepsis.

IMMUNOTHERAPY AS AN EVOLVING APPROACH TO SEPSIS

Given the profound immunosuppression that occurs during sepsis, a current focus in the treatment
of the disorder is to restore or augment host immunity. Although a number of immunoadjuvant therapies are being tested, one of the most promising agents is interleukin-7 (IL-7), a pluripotent cytokine that is essential for survival of CD4 and CD8 T cells. Interleukin-7 is an attractive therapy in sepsis because it blocks sepsis-induced apoptosis of immune effector cells and increases γ-interferon (IFN-γ), a cytokine that is critical for an effective host response against invading pathogens. Our group recently completed a small, randomized, double-blind, placebo-controlled trial of IL-7 in patients with septic shock and lymphopenia. Interleukin-7 was well-tolerated without evidence of inducing cytokine storm or worsening inflammation or organ dysfunction. Interleukin-7 caused a threefold to fourfold increase in absolute lymphocyte counts and in circulating CD4 and CD8 T cells that persisted for weeks after the end of IL-7 treatment. This trial of IL-7 represents the first trial of an immunoadjuvant therapy targeting defects in adaptive immunity in patients with sepsis. Interleukin-7 successfully reversed the marked loss of CD4 and CD8 immune effectors cells, a hallmark of sepsis and a likely key mechanism in its morbidity and mortality. In our opinion, IL-7 represents a potential new way forward in the treatment of patients with sepsis by restoring adaptive immunity. Such immune-based therapy should be broadly protective against numerous diverse bacterial and fungal pathogens.

PROGRAMMED CELL DEATH INHIBITORY PATHWAYS AND THE MECHANISMS

A CENTRAL PATHOPHYSIOLOGICAL event in sepsis is widespread apoptosis of key immune effector cells. Further driving immunological imbalance are suppressive cells, such as regulatory T cells and myeloid-derived suppressor cells, which subsequently occupy the available immunological niche. Programmed cell death 1 (PD-1) and its corresponding ligand (PD-L1) also play a major pathological role in immunosuppression not only in cancer but also in infectious diseases, including sepsis. Positive costimulatory pathways in T cells, such as CD28 signaling, permit the effector T cell to expand, persist, and effectively clear antigen. Programmed cell death-1, in contrast, is a negative costimulatory pathway on T cells that “puts the brakes” on effector function and broadly enhances immunosuppressive signals across the innate and adaptive immune system (Fig. 1). Whereas PD-1 surface expression is restricted to activated immune cells such as T cells, PD-L1 can be found on the surface of a broad variety of cells, notably tumors and antigen-presenting cells (APCs). Ligation of PD-1 to PD-L1 initiates T-cell growth arrest, decreased cytokine secretion, and eventually apoptotic cell death. While “exhausted” T cells are known to chronically express PD-1, investigators have found PD-L1 expressed on these same subsets of T cells. Whether or not this suggests an additional autocrine inhibitory mechanism is unknown. As such, targeting the PD-1/PD-L1 axis in patients with defective immunity, that is, immunosuppressed septic patients, promises major clinical benefits. The immunostimulatory effects of blocking PD-1/PD-L1 are presented in Table 1.

Effector T cells, when stimulated by antigen, produce pro-inflammatory cytokines such as IFN-γ and IL-2. Physiological IFN-γ signaling augments antimicrobial responses by inducing macrophage activation. A secondary effect of IFN-γ is induction of the inhibitory ligand PD-L1 on macrophages and other APCs. When antigen is not effectively cleared, such as in a chronic viral infection like lymphocytic choriomeningitis or cancer, persistent stimulation can impair IFN-γ signaling and globally shift immune cells to suppressive, anti-inflammatory phenotypes. Thus, IFN-γ may have both immunostimulatory and immunosuppressive effects that are likely dependent on cell context and timing.

In the context of protracted, unresolved infection, excessive PD-1 and PD-L1 upregulation and ligation can lead to functional deficits in host immunity driven by epigenetic, metabolic shifts toward immunosuppression: defective phagocytosis, effector T-cell exhaustion or apoptosis, and enhanced regulatory T-cell function are some of the many immunosuppressive features of this pathway. Monoclonal blocking antibodies against either PD-1 or PD-L1 have shown major survival benefits in murine models of sepsis as well as improved T cell and monocyte cytokine production in human ex vivo studies. Additionally, anti-PD-1 treatment in hepatitis C virus (HCV)-infected chimpanzees potently enhanced antiviral T-cell responses, as evidenced by decreased viremia. Therefore, checkpoint blockade might at least partially reverse immune defects caused by chronic infection.

In sepsis, positive costimulatory molecules such as CD28 and antigen-presenting proteins such as HLA-DR are significantly downregulated, likely impairing the host’s ability to mount effective responses. Recently, investigators have identified that T-cell PD-1 signaling, initiated by binding PD-L1 on APCs, results in abrogation of CD28 signaling, decreased cytokine production, and reduced survival of these critical immune effectors (Fig. 2, left panel). Several lines of evidence indicate that the presence of such costimulatory signals on T cells could predict responses to PD-1/PD-L1 blockade (Fig. 2, right panel). For instance, Krophorst et al. conditionally knocked out the CD28 gene in tumor-bearing mice, and this intervention eliminated the antitumor response following checkpoint blockade.
Additionally, they showed that the subset of proliferating CD8 T cells in peripheral blood existing after anti-PD-1/PD-L1 therapy expressed increased levels of CD28 compared to those present prior to treatment. These findings have major therapeutic implications: given the heterogeneous patient responses to checkpoint blockade observed to date, more research needs to be done to completely define the exhausted repertoire.\textsuperscript{15}

Whereas PD-1/PD-L1 interactions are thought to occur between T cells and APCs, a recent study by Patera et al. reported that a subset of neutrophils from septic patients can be induced to participate as well.\textsuperscript{13} Termed low density neutrophils, these cells display a suppressor phenotype that can be reversed following blockade of either PD-I or PD-L1. This particular study showed that neutrophils, in addition to T cells and macrophages, can undergo a phenotypic switch to become suppressive. The investigators found that reduced neutrophil and monocyte activity correlated with levels of PD-1 on CD8 T cells and natural killer cells, indicating broad defects in both innate and adaptive immunity.\textsuperscript{13}

Another study by Gordon et al., although in the context of cancer, used Rag\textsuperscript{−}\textsuperscript{−}/c0\textsuperscript{−}\textsuperscript{−}/c0\textsuperscript{−}\textsuperscript{−}, tumor-bearing mice to show that tumor-associated macrophages also express PD-1 and are responsive to PD-1 blockade.\textsuperscript{17} Furthermore, the authors used a blocking antibody which was specific for phagocytosis and this abrogated the antitumor response. Further complicating the picture, investigators have also detected a soluble form of PD-L1 (sPD-L1) circulating in peripheral blood of septic patients.\textsuperscript{18} Intriguingly, cancer patients also have higher than normal levels of circulating sPD-L1.\textsuperscript{19} Together, these observations support the notion that the PD-1/PD-L1 axis can play a significant role in immunosuppression and that clinical targeting could reverse immune defects caused by sepsis.\textsuperscript{20}

\begin{figure}
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\includegraphics[width=\textwidth]{Figure1.png}
\caption{Programmed cell death inhibitory pathways. CD4 and CD8 T-cell functions, such as cytokine production and cell proliferation, are inhibited by engagement of programmed cell death 1 (PD-1) receptor with its ligands (PD-L1 and PD-L2) that are expressed on numerous cells, for example, macrophages (MACØs) and dendritic cells (DCs), as well as on activated endothelial cells. There is suggestive evidence that PD-L1 and PD-L2 might also transmit inhibitory signals to MACØs and DCs, respectively. PD-L1 can also bind to the CD80 receptor that is expressed on antigen-presenting cells. PMN, polymorphonuclear cell. Figure reproduced from Moldawer et al. (2018),\textsuperscript{11} with permission from Wiley publishers.}
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\textbf{Table 1.} Immunostimulatory effects of blocking programmed cell death 1/programmed cell death 1 ligand 1 & \\
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\textsuperscript{↑} T-cell production of IFN-γ & \textsuperscript{↓} T-cell apoptosis \\
\textsuperscript{↓} T regulatory cell formation & \textsuperscript{↑} T-cell motility \\
\textsuperscript{↑} MDSC production of immunosuppressive IL-10 & \textsuperscript{↓} Suppressive effect of low density neutrophils \\
\textsuperscript{↓} Neutrophil phagocytosis and oxidative burst & \\
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\textsuperscript{IFN-γ, γ-interferon; IL-10, interleukin-10; MDSC, myeloid-derived suppressive cell.} & \\
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CROSS-TALK BETWEEN TWO TYPES OF PROGRAMMED CELL DEATH IN SEPSIS

**A**

POPTOSIS IS KNOWN as type 1 programmed cell death, and autophagy as type 2 programmed cell death. The second type of programmed cell death, autophagy, has recently gained increased attention from researchers in the field of critical care. Autophagy is defined as the mechanism by which cell components are transferred to lysosomes within the same cell and degraded, and the degradation products, such as amino acids and fatty acids, are reused. However, inhibition of autophagy is reported to trigger apoptosis in cells. Autophagy-related protein 5 (Atg5) is cleaved by calpain, a protease that triggers necrosis, and induces apoptosis by binding to Bcl-xL, an anti-apoptotic transmembrane protein component of mitochondria. It is also known that the anti-apoptotic Bcl2/Bcl-xL complex conjugates with Beclin 1 to inhibit autophagy. Recently, we observed enhanced expression of *PDCD1*, a pro-apoptotic gene, a significant reduction in *BCL2*, an anti-apoptotic gene, and enhanced apoptotic activity in CD4+ T cells in CD4-Cre/Atg5fl mice. We also reported that the mRNA expression of the human immunity-related GTPase family M protein (*IRGM*) is significantly lower in the TT genotype of rs10065172 SNP in *ex vivo* experiments, rendering it defective in autophagy. The *IRGM* gene has been found to play an important role in the autophagic degradation of *Mycobacterium bovis* in cultured human macrophages. Another group showed that autophagy-related *ATG5* expression levels decreased with the severity of sepsis, and rs506027 T>C and rs510432 G>A were associated with sepsis progression and mortality. Furthermore, the rs506027 TT and rs510432 GG carriages also exhibited increased expression levels of *ATG5*. Although mRNA expression in a model of an acute disease such as sepsis greatly varies over time and depends on cell type, we propose that substantial cross-talk might exist between autophagy and apoptosis following the initiation of these two processes. In summary, blocking autophagy is thought to lead to apoptosis and immunosuppression regardless of an insufficient autophagy process in T cells. Taking those into consideration, acceleration of autophagy might alleviate immunosuppression through regulation of apoptosis.

**CLINICAL TRIALS**

CHECKPOINT BLOCKADE IS an area of active clinical research in oncology and has recently been implemented in infectious disease. Partly driving this success of immunotherapy in infectious disease have been
serendipitous outcomes that bridged the two fields. In one trial, an HIV-positive patient with recurrent lung cancer received anti-PD-1 (nivolumab) as second-line treatment and experienced a precipitous decline in HIV reservoirs and decrease in number of exhausted CD4 and CD8 T cells. In another case study, an HCV-positive patient with malignant melanoma, who was treated with anti-CTLA4 (ipilimumab), checkpoint blockade resulted in a multiple-log reduction of viral levels, from 398,938 IU/mL to 12 IU/mL, just 4 weeks after therapy. Significantly, a single dose of nivolumab resulted in clearing of viral titers in 11–15% of patients with HCV, including those who were non-responders to standard IFN therapy. Although there are now effective therapies for hepatitis C that result in approximately 90% cure, the studies with checkpoint inhibitors highlight the ability of immunotherapy to enhance host immunity leading to pathogen control. One of most striking examples of the potential for immunotherapy is illustrated in a recent case study published in The Lancet Infectious Disease. A patient with life-threatening, refractory mucormycosis was treated on a compassionate basis with nivolumab, a humanized immunoglobulin G4 blocking antibody against PD-1, and IFN-γ. This immunotherapeutic approach led to successful eradication of the invasive mucormycosis and patient survival. A phase Ib/IIa trial of anti-PD-1 antibody, an immune checkpoint inhibitor, in severe sepsis/septic shock completed in the USA (https://clinicaltrials.gov/, NCT02960854; accessed 25 May 2018), has been expanded to Japan to evaluate the pharmacokinetics as well as safety of the drug (http://www.clinicaltrials.jp/ctDetail.jsp, JapicCTI-173600; accessed 5 June 2018). These remarkable results together show that immunotherapy can be effective against a diverse array of pathogens. Given that sepsis is a heterogeneous, complex syndrome presenting with multiple immune defects across innate and adaptive arms, immunotherapy has the greatest potential to attenuate the significant mortality in intensive care units due to sepsis.

CLINICAL TRIALS WITH CHECKPOINT INHIBITORS

There is increasing interest in the use of checkpoint inhibitors to treat sepsis. Clinical trials of anti-PD-L1 and anti-PD-1 antibodies are registered on the clinicaltrials.gov website but results of these small exploratory trials have not been formally presented in detail. An abstract presentation of the anti-PD-L1 trial at the 2018 Society of Critical Care Medicine annual meeting revealed that a single dose of anti-PD-L1 was well tolerated with no clinical evidence of induction of a hyperinflammatory state by the immune modulator. There was also no clinical evidence of serious adverse effects or autoimmune side-effects with anti-PD-L1 therapy. Importantly, there was evidence of enhanced host immunity as indicated by a non-statistically significant trend toward increased monocyte HLA-DR expression at higher doses of anti-PD-L1. Furthermore, the safety, tolerability, and pharmacokinetics of single doses of ONO-4538 (nivolumab) in patients with sepsis or septic shock have already been evaluated in Japan and will be presented soon.

CONCLUSIONS

Sepsis is a complex syndrome that can be triggered by infection by any pathogen and encompasses a wide variety of pathological conditions. To address and overcome sepsis, it is imperative for intensivists to carefully consider the patient’s immune status, nutritional status, interindividual differences, and disease stage after proper stratification and to not miss the therapeutic window, which can be very narrow. Monitoring and controlling programmed cell death as described in the present article could provide a breakthrough approach that will improve survival in septic patients, especially in their subacute phase. Possible means to monitor the extent of immune dysfunction due to PD-1/PD-L1-mediated mechanisms include flow cytometric expression of PD-1 or PD-L1 on immune effector cells. Recently, circulating levels of sPD-L1 have been reported to be a marker for persistent immune suppression in patients who develop chronic critical illness after sepsis. In summary, checkpoint blockade is one of the most promising strategies to counter immunosuppressive sepsis in the future.

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DISCLOSURE

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Informed consent: N/A.
Registry and the registration no. of the study/trial: N/A.
Animal studies: N/A.
Conflict of interest: Richard S Hotchkiss received research funding from Bristol Myers Squibb, GlaxoSmithKline, and Revimmune. The other authors have no conflict of interest.
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