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Single versus combined immunoregulatory approach using PD-1 and CTLA-4 modulators in controlling sepsis

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Abstract

Introduction: Sepsis is a disease process characterized by an extreme inflammatory response followed by a period of severe immunosuppression. In recent years, there has been improved survival in the initial immune response during systemic inflammatory response syndrome, and compensatory anti-inflammatory response, yet is mostly unchanged with 18–30% mortality during the later stage of sepsis despite numerous Phase 3 clinical trials.

Areas covered: This review article presents a critical evaluation of the most promising newer studies aimed at improving the immunosuppressive stage of sepsis. Administration of DHEA/AED/AET show promise in improving survival. Blockade of signaling pathways for PD-1/PD-L1/CTLA-4, and partial blockade of TREM-1 signaling, and modification to sTREM-1 and the JAK/STAT pathway are promising methods of restoring host immune response and improving survival. While there has been significant progress, currently no findings have been translated into effective clinical interventions.

Expert Commentary: Clinical success by immunomodulation with individual immune mediator is encouraging and should progress to evaluating combined methods of immunoregulation. Since most of the animal studies do not reproduce human sepsis, development of better animal models and moving toward human studies for intervention will lead to the most beneficial findings in translational science.

Keywords

Androstenediol; Androstenediol; CTLA-4; Dehydroepiandrosterone; Hemorrhagic Shock; PD-1; PD-L1; Sepsis; sTREM-1; TREM-1

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Declaration of Interest

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1. Introduction

Sepsis is a condition characterized by a vigorous inflammatory response to bacterial, viral, fungal, or other pathogen, accompanied by an intense dysregulation of the host immune response - typically resulting in severe immunosuppression, septic shock, and multiple organ failure [1,2]. Sepsis affects millions of individuals worldwide with over 750,000 cases annually in the United States and the number of occurrences is increasing [3,2]. Severe sepsis affects 1 in 3 patients in the Intensive Care Unit [4]. While mortality rates have declined in the last 30 years, the 28-day mortality rate is still 18–30% with an increase to 60–80% if the cause of secondary infection is peritonitis [2,5]. Accordingly, sepsis is listed in the top ten causes of death in the United States [4].

The initial signs of sepsis begin as early as 30 minutes after trauma (injury, surgery, or ischemia-reperfusion injury), with a detectable rise in systemic inflammation [6]. The magnitude of hyper-inflammation is determined by several factors, including pathogen virulence, bacterial/viral load, host genetics, and comorbidities (i.e. TNF- α and IL-1 β antagonists taken by individuals with autoimmune disease impact the development and survival in sepsis) [7]. A systemic hyper-inflammatory response syndrome (SIRS) is due a cytokine storm that causes an increase in pro-inflammatory cytokines and chemokines which cause fever, rapid heart rate and breathing, and is followed quickly by shock and multiple organ failure [8] (Figure 1). The magnitude of initial hyper-inflammation corresponds with increased mortality [8,7]. In 2013, the Surviving Sepsis Campaign updated their recommendations for the treatment of sepsis [3]. In the first six hours of sepsis diagnosis, all patients undergo the following procedures: pressure resuscitation with balanced crystalloid fluids (not saline), normalization of lactate, screening for infection (with cultures to determine the source of infection), administration of broad spectrum antibiotics until a specific pathogen is identified, source control for infection (e.g. change catheters, surgical drainage or removal of abscesses), and administration of preventive oral chlorohexidine gluconate (in cases of mechanical ventilation) and vasopressors (specifically norepinephrine, with the addition of epinephrine as needed) for hypotension that is non-responsive to adequate fluid resuscitation [3].

With these guidelines, there has been an improved survival rate for patients during SIRS, the initial hyper-inflammatory syndrome of sepsis [9]. However, there has been a corresponding increase in negative outcomes from the subsequent immunosuppressive response, which occurs initially to compensate the inflammation, hence referred to as compensatory anti-inflammatory response (CARS). But, due to severe suppression of the immune system there is a development of hyporesponsiveness that can also be produced by severe trauma, burns, and sepsis. During this second stage of sepsis, there is a significant reduction in splenocyte cytokine production (specifically IFN- γ , TNF- α , IL-1 β) within five hours of the onset of sepsis [9]. Monocytes become hypo-responsive to stimuli (known as immune cell exhaustion due to persistent antigen presentation) with less than 5% of monocytes producing cytokines in septic patients [2,7]. There is an increase in apoptosis of CD4⁺ lymphocytes and B cells, natural killer cells, and follicular dendritic cells [2,1]. Problems arising during this severe immunosuppression include secondary infections (e.g. ventilator associated pneumonia, nosocomial pneumonia, infection at catheter or *i.v.* sites), viral reactivation, and peritonitis

[9,10]. Following proper surgical intensive care, there is a significant increase in the intensive care unit patient population to survive initial SIRS and CARS stages, and these patients progress to the stage of persistent inflammation, immunosuppression and catabolism (PICS), and thus reducing the incidence of late multiple-organ failure and death [11]. Treatment recommendations for the later stage of sepsis include supportive/adjunctive measures, such as continued administration of fluids, vasopressors, inotropic therapy, limited use of corticosteroids (continuous *i.v.* hydrocortisone 200mg/day that is tapered when vasopressors are no longer needed – only to be used if patient is unresponsive to adequate fluid resuscitation), and if all else fails - mechanical ventilation and sedation which are weaned as soon as possible as the patient responds [3]. During this time, the patients undergo the evaluation for glucose levels, renal function, and possible development of deep vein thromboses and have nutritional intake monitored [3]. The immunological responses with the release of immune response mediators in the initial and later stages in sepsis progression are shown in Figure 1. It should be noted that sepsis can present clinically with characteristics of both hyper- and hypo-inflammatory dysregulation with significant overlap.

Another consideration in septic patients is determining the cause of inflammation. During the initial stage of SIRS, inflammation can be caused by infection: bacterial, viral, fungal; or the inflammation can be sterile: trauma, burns, surgery, autoimmune disorders [12,13,14]. Because of shared cellular signaling pathways, most of the downstream signaling events for sterile and infectious inflammation could be difficult to distinguish. Therefore, in this article we focused our investigation primarily on infectious sepsis. A comparison of sterile and infectious SIRS is shown schematically in Figure 2.

Due to the multi-variate nature of immunology, and the heterogeneity of patients in hospital settings who develop sepsis, over 25 interventions aimed at targeting certain immune response mediators have recently failed in phase 3 trials [8,9,10]. These failed trials include modulation of Toll-like Receptor (TLR)-4, Recombinant Protein-C, Nitric Oxide, Tumor Necrosis Factor (TNF)- α , and Interleukin (IL)-1 [8,10]. Additional phase 2 trials targeting Interferon (IFN)- γ , Granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-7 have produced encouraging results, similarly a new review of a 1997 phase 3 clinical trial shows promise for effective use of IL-1Ra in a specific subset of patients demonstrating macrophage activation syndrome-like symptoms; however, these findings are not yet established or generalizable to the larger population, and as such are not discussed further in this article [15,16]. As such, novel methods to control sepsis have been widely studied. This manuscript focuses on the most recent interventions and methods in the current literature on the comparisons of survival advantage and immunomodulation. These studies are summarized in Table 1 and Table 2, respectively.

While not further examined in this article, there have been several studies with conflicting findings regarding the importance of gender on the development of and survival from sepsis [17,18,19]. In general, there appears to be a slight protection for women in the premenstrual phase of life when estrogen levels are the highest [19]. Premenopausal women tend to have shorter stays in the Intensive Care Unit with a lower incidence of nosocomial infections and multiorgan failure [19]. This protection could be due to lower levels of IL-1 β or increased

immune and cardiac function in women with higher levels of estrogen. More inquiry is warranted to determine specific protective mechanisms, if any [17,18].

2. Steroid Treatment in Sepsis

Previous clinical studies have found impaired response to cortisol paired with adrenal insufficiency in patients with sepsis. Because of these findings, administration of cortisone in low levels was recommended as a novel treatment for immunosuppression in septic patients. After large-scale trials failed to show improved mortality rates, the recommendation to use hydrocortisone was limited, and the use of glucocorticoids was largely abandoned due to possible negative pleiotropic effects of steroid administration [3,20].

Glucocorticoids alleviate inflammation through a multi-faceted approach of either direct or indirect inhibition in transmembrane signaling pathways to regulate gene transcription and inhibiting enzymes, such as phospholipase A1 via the generation of lipocortins. Glucocorticoids can block prostaglandin secretion by activating annexin I, mitogen-activated protein kinases (MAPK) phosphatase-1, and inhibiting cyclooxygenase transcription. The proteins also inhibit the actions of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and activator protein-1 (AP-1). This diverse approach lends to unwanted side-effects if beneficial signaling pathways are modified in a way which prevents healing [21]. Of special note to sepsis, glucocorticoids can lead to hypertension, poor wound healing, immunosuppression, glaucoma, thrombosis, adrenal atrophy, and disturbances in metabolic pathways. These negative repercussions can have an inflated effect in septic patients who may already suffer from such comorbidities [21]. Glucocorticoids function along the hypothalamic-pituitary-adrenal axis to regulate the inflammatory response. Dysfunction of this pathway (exaggeration or suppression) is correlated with poor patient outcomes [21].

In the highly-referenced Hydrocortisone Polytraumatise (HYPOLYTE) study, 149 patients among several trauma centers were assigned to receive continuous corticosteroid treatment with *i.v.* hydrocortisone, or a placebo, for seven days [22]. This random, double-blinded study determined that stress-doses of hydrocortisone had a protective effect against nosocomial pneumonia. In this study, 35.6% of patients receiving hydrocortisone developed hospital-acquired pneumonia during the 28-day study compared to 51.3% of patients receiving placebo. The study also determined that administration of stress-doses of hydrocortisone increased mechanical-ventilation free days (16 compared to 12), led to fewer days in the hospital (18 compared to 24), and led to a 10% decrease in cases of acute respiratory distress syndrome. Interestingly, death rates were slightly elevated in the hydrocortisone groups (8.2% and 10.7%) compared to the placebo groups (5.3% for each placebo group) [22]. Two authors editorialize that the HYPOLYTE study does not have a large enough sample size to adequately determine any statistically significant impact on mortality rates [23]. A 2014 meta-analysis by Wang and colleagues also showed that low-dose hydrocortisone therapy may be beneficial in attenuating septic shock, but provides no benefit to 28-day mortality rates [24].

More recently, dehydroepiandrosterone (DHEA; 5-androsten-3 β -ol-17-one), an abundant steroid hormone, and its downstream steroid metabolites androstenediol (AED; androstene-3 β ,17 β -diol) and androstetriol (AET; 5-androstene-3 β ,7 β ,17 β -triol) have shown promise in mitigating the negative effects of sepsis and improving survival. In a murine model study, DHEA restored splenocyte proliferation to pre-trauma levels, while there was 25% reduction in proliferation in the vehicle resuscitation control group [25]. Similar trends were noted for IL-2 and IFN- γ production. Vehicle control mice had a 52% decrease in IL-2 levels and a 43% reduction in IFN- γ production, whereas DHEA-treated mice resumed IL-2 and IFN- γ production at pre-trauma levels. Administration of DHEA to sham-operated mice did not increase any measured variables to supra-physiological levels. In their earlier report, many members of the same research team demonstrated improved survival (77% compared to 20% of controls) in male C3H/HeN mice treated with DHEA for three days after the induction of sepsis by cecal ligation and puncture (CLP) [26]. In 2010, a research group found similar results of survival and splenocyte proliferation in murine studies [27]. DHEA was administered in male NMRI mice either one hour before, or eight hours after, CLP-induced sepsis [27]. The group measured survival rates, splenocyte proliferation, apoptosis, and levels of circulating IL-1 β and IL-6. Survival was improved for both groups of mice receiving DHEA pre-or post-operatively (75% vs. 44% and 78% vs. 47% respectively). Along with improved survival, this study demonstrates improved survival regardless of the timing of DHEA administration [27]. The group noted increased splenocyte proliferation and decreased apoptosis in DHEA-treated mice independent of timing of the administration. Among DHEA treated mice, circulating levels of IL-1 β were increased and levels of IL-6 were reduced; again this finding was independent of timing [27]. In similar, but conflicting, findings Lichte and colleagues found DHEA administration to reduce levels of both IL-6 and IL-1 β during a murine model bilateral femoral fracture experiment [28]. This group also found reduced levels of MCP-1 in treated mice [28].

Along with DHEA, downstream metabolites, AED and AET, elicit protective effects in sepsis. One group demonstrated the ability of DHEA and AED to improve survival against lethal doses of gram-positive (*Enterococcus faecalis*) and gram-negative (*Pseudomonas aeruginosa*) bacteria [29]. Compared to no treatment, mice treated with DHEA or AED after exposure to *P. aeruginosa* had decreased mortality (97%, 57%, and 33%, respectively). With exposure to *E. faecalis*, 100% of mice treated with either DHEA or AED survived, whereas 57% of animals with no treatment died. The study also showed that DHEA suppressed the production of TNF- α and IL-1. AED did not elicit such modifications in cytokine production in this study [29].

Recently, several studies have supported the use of AED and AET in sepsis treatment to improve survival. One group of investigators determined that AED after trauma hemorrhage and CLP-induced sepsis decreased IL-6, IL-10, myeloperoxidase (MPO), and inducible nitrous oxide synthase levels [30]. Another group found AED to decrease circulating IL-6 and TNF- α levels and revert suppressed IL-2 and IFN- γ levels to normal after a two-hit model of hemorrhage and sepsis in adult male Sprague-Dawley rats [31]. A third group determined that AET was also effective in improving survival, this time in radiation murine model, which develops a similar risk of infection. The major significance of this work was to demonstrate the protective effects of AET at 10x lower dose than those needed to produce

similar results with AED intervention [32]. Previously, studies showed AET to increase the production of IL-2 and IL-3 in immunosuppressed mice, improving cytokine regulatory functions [33]. Another lab showed a 25% survival advantage after 72 hours (100% vs. 75% for controls) in adult male Sprague-Dawley Rats treated with AET after hemorrhagic shock [34]. In a follow-up study, results demonstrated improved rates of survival in adult male Sprague-Dawley rats upon administration of AET after severe trauma-hemorrhage [35]. Rats treated with AET had a 69% survival rate compared to 31% in the control group. Secondary findings from this study show AET to reduce Th2 (IL-4, IL-6, IL-10, and IL-18) and increase Th1 (IFN- γ and IL-2) cytokines [35].

3. Triggering Receptor Expressed on Myeloid Cells-1

Immunomodulation by triggering receptor expressed on myeloid cells-1 (TREM-1) is a most recent addition to the sepsis treatment regimen, but already shows great promise. Sepsis research involving TREM-1 began with the notion that levels of TREM-1 and soluble-TREM-1 (sTREM-1) could be used as biomarkers in patients who presented with sepsis or SIRS to determine disease differentiation, progression, and prognosis [36]. Indeed, modulation of TREM-1 or sTREM-1 during sepsis could produce an improved survival advantage [37–42]. However, the studies for both areas of translational application have conflicting findings and remain controversial.

There have been several studies suggesting that the levels of circulating sTREM-1 can be used to differentiate SIRS from later stages of sepsis, as well as determine degree of sepsis and predict favorable or unfavorable outcomes. In general, higher levels of sTREM-1 (especially levels over 62 pg/ml, or levels that remain elevated over time) show the progression to more severe stage of sepsis and are prognostic of increased mortality [43–47]. There have also been studies that show temporal levels of peripheral sTREM-1 are more accurate prognostic indicators than blood levels of either C-reactive protein or procalcitonin in determining mortality [44,48].

Two studies showed a distinct inverse correlation between TREM-1 expressed on monocytes with circulating levels of sTREM-1 [45,47]. This correlation could be due to sTREM-1 proteolytic cleavage from membrane-bound TREM-1 molecules during cell necrosis or proteinase activity [49]. A noted decrease in TREM-1 expression over time was investigated, as both survivors and non-survivors showed similar expression levels upon arrival to the hospital; however, by day three TREM-1 levels in non-survivors were still elevated, while the surviving counterparts showed a great reduction in TREM-1 expression on monocytes. Among patients with trauma but not sepsis, or healthy volunteers, TREM-1 expression started low and remained low when monitored over the same duration of time as the septic patients [47].

Conflicted with these studies, are examples where sTREM-1 levels are lower in non-surviving patients upon admission to the hospital, or studies which show no difference in TREM-1 levels between survivors or non-survivors over time [450]. A literature review evaluating TREM-1/sTREM-1 as a diagnostic marker in pediatric patients determined that the current body of knowledge is too heterogeneous in methodology and findings to be able

to make any verifications regarding the diagnostic or prognostic quality of sTREM-1 levels as a sepsis biomarker [50]. A meta-analysis of adult patients found that most research showed TREM-1 levels decreasing over time as sepsis ameliorates [36]. Therefore, the authors posit TREM-1 as a much more valuable marker temporally than as a single marker upon admission. The analysis also showed sTREM-1 elevation to be a moderately effective marker for the diagnosis and prognosis of sepsis [36].

In experimental survival models of TREM-1 or sTREM-1 modulation it has been loosely determined that the correct ratio of TREM-1 blockage improves survival, however complete blockage increases mortality rates [37–42]. In the first renowned study, one group developed a clonal homolog of murine TREM-1 antibody from mouse and human components [42]. The team also developed an antagonistic antibody (mTREM-1/IgG1) to conduct blockade studies and determined survival statistics and immune responses. The findings showed an upregulation in TREM-1 expression on peritoneal neutrophils during lipopolysaccharide (LPS)-induced sepsis. Administration of the antagonist mTREM-1/IgG1 one hour before or 1, 2, or 4 hours after sepsis showed improved survival (76% vs. 6% and 80%, 60%, 40% respectively) [42]. In CLP-induced septic shock there was a smaller, but still significant survival advantage (45%) with the administration of antagonist. One group measured cytokine and chemokine secretion and determined that ligation of TREM-1 leads to a sustained pro-inflammatory release of TNF- α , IL-1 β , IL-8, and monocyte chemotactic protein (MCP)-1 [42]. Despite their promising findings, the authors noted that complete blockage of TREM-1 *in vivo* could potentially be harmful by limiting the host's ability to generate an appropriate immune response [42]. That instinct was verified by another lab, who determined that TREM-1 knockout mice showed a much lower survival rate than wild type controls (28.6% vs. 61.9%, respectively) when exposed to *Klebsiellapneumonia* infection. The impaired survival was due to an increase in liver damage paired with diminished bacterial clearance in the TREM-1^{-/-} mice [39]. This notion was again confirmed in a study where TREM-1 expression in the presence of *Pneumococcal pneumonia* infection led to an improved bacterial clearance and improved survival [40]. Another study regarding this relationship using siRNA TREM-1 silenced mice in a fecal peritonitis model survival study also showed an increased mortality with complete TREM-1 silencing (100% mortality vs. 77% in controls) due to decreased bacterial clearance [41]. The group then evaluated the effects of partial TREM-1 blockade and found promising results. With partial silencing, the group established a 75% survival rate in mice with fecal peritonitis induced sepsis [41]. When measuring immune response, it was found that TREM-1 expression up-regulated TNF- α , IL-1 β , GM-CSF, and inhibited secretion of IL-10. It was concluded that TREM-1 was not involved in phagocytosis regulation but it was imperative in producing the neutrophil oxidative burst required for adequate bacterial clearance [41].

Other studies have shown TREM-1 blockade to be successful in improving survival [37,38]. One study showed an 80% survival improvement in BALB/c mice treated with TREM-1 Fc fusion protein probably due to a significant decrease in IL-1 β , TNF- α , MCP-1, and IFN- γ , as well as a decrease in costimulatory molecules CD40 and CD86 [37]. Yet another study showed time-dependent improved survival upon infection with *E. coli* and treatment with LP-17 (a competitive antagonist TREM-1 peptide) [38]. The group determined a single dose of LP17 (100 μ g) one hour before infection led to a 100% survival rate, with single doses at 4

and 6 hours after infection improving survival with ~40% and ~20% survival, respectively. The study also showed a 200µg dose improving survival by 50% with repeated injections further improving survival[38]. The group also determined that LP17-induced inhibition of TREM-1 expression led to an inhibition in NF-κB activation, as well as a 30% reduction in cytokine production and an increase in circulating sTREM-1 levels. The group determined that LP17 showed improved prognoses independent of bacterial clearance (upon which the peptide had no effect) [38].

Other studies have shown that bacterial sepsis strongly induces TREM-1 expression on neutrophils, monocytes, and macrophages at the location of infection and distally in circulation [52]. There has also been an inverse correlation between TREM-1 and IL-6, and increase in plasma sTREM-1 levels negatively correlated with TNF-α and IL-1β [45,52]. Moreover, TREM-1 engagement was demonstrated to co-localize with TLR-4 as well as up-regulate Jak2, and anti-TREM-1 activity led to STAT5 phosphorylation [53]. These assertions warrant further scientific inquiry.

4. Programmed Cell Death-1 and the PD-ligands

Discovered in 1992, programmed cell death-1 (PD-1) is a type-I transmembrane immunoreceptor glycoprotein which belongs to the CD28/CTLA-4 family. While PD-1 can be found in the cytoplasm of T cells, it is typically analyzed as a cell-surface marker and is up-regulated within 24–72 hours of T cell activation [54,55]. After interaction with either ligand PD-L1 (B7-H1; CD274) or PD-L2 (B7-DC; CD273), PD-1 recruits tyrosine phosphatase to attenuate antigen T cell receptor (TCR) signaling [54,55]. PD-1 is expressed on both peripheral T and B cells throughout the body. This wide dispersal allows PD-1 to have a regulatory effect on many immune response pathways from cancer to transplant rejection, and sepsis [54]. Normally, PD-1 prevents autoimmune reactions by downregulation of the host immune response after the initial infection has been cleared; however, in cases of sepsis or autoimmune disorders, the PD-1 pathway negatively affects the host through excessive immunosuppression [54].

PD-L1 is expressed on T and B cells, macrophages, and dendritic cells (DCs). PD-L1 and PD-L2 transcripts are expressed in high levels in tissues of the placenta, heart, liver and lungs, and to a lesser extent in the spleen, lymph nodes, and thymus. However, PD-L2 proteins are typically found only in lymph tissues. PD-L1 proteins are present in both lymphoid and non-lymphoid tissues indicating that the PD-1/PD-L1 pathway is responsible for immune responses in non-target organs [54]. PD-L1 is upregulated by LPS, IFN-γ, and IL-12 as well as many other mediators. PD-L2 is rarely induced in T and B cells, but can be induced on DCs and macrophages by IL-4 and IFN-γ, and IL-12 [56]. Several studies have evaluated PD-1/PD-L1 antibody levels in patients with various degrees of sepsis progression and immunosuppression.

One study found that certain viruses (specifically human rhinovirus) could induce PD-L1 expression on DCs creating a rampant hypoproliferative state in T cells known as cell exhaustion. Proliferation is only restored after the addition of anti-PD-L1 antibodies [57].

Recently PD-1 expression has been documented on exhausted CD8 T cells in the presence of chronic viral infection, and by blocking of PD-1, T cell function can be restored [58].

In CLP-induced sepsis, PD-1^{-/-} mice had a much higher rate of survival than controls with survival rates of 77% and 22%, respectively, 7 days post-CLP [59]. In these studies, PD-1^{-/-} mice did not show the distinctive pathologic changes of CLP-induced sepsis such as villus shortening, loss of epithelial cells, mucosal sloughing, and mucosal thinning in the jejunum and kidneys. There was no pathologic tissue changes in the spleen or thymus tissues of the PD-1^{-/-} mice post-sepsis. Also, the levels of circulating pro- and anti-inflammatory cytokines were closer to baseline in PD-1^{-/-} mice compared to the wild type controls at 6 and 24 hours after sepsis induction. The group also found that macrophages in PD-1^{-/-} mice were more resistant to dysfunction throughout the course of CLP-induced sepsis and continued to produce higher levels of IFN- γ and IL-12 and lower levels of IL-10 when compared to their wild type counterparts. The group posits that increased PD-1 expression on macrophages and monocytes could be used as a marker of dysfunction during sepsis [59].

Another experiment determined survival rates in a murine model of CLP-induced sepsis [60]. The study evaluated adult male C57BL/6 mice for the expression of PD-1 and PD-L1 on peripheral T cells, B cells, and monocytes. As secondary findings, the team evaluated the effects of an anti-PD-L1 antibody on: the total number of lymphocytes, cellular apoptosis, cytokine production, bacterial clearance rates, and survival. The group determined that PD-1 was upregulated on peripheral T cells, B cells, and monocytes, and that PD-L1 expression was also increased on B cells and monocytes. After introducing the anti-PD-L1 antibody, there was an increase in TNF- α and IL-6 production and a decrease in IL-10 production. The increase in Th1 cytokines led to an improved bacterial clearance in treated animals compared to controls [60].

Similar results were found when another research team conducted a study to determine if administration of anti-PD-1 antibodies could produce the same survival advantage as PD-1^{-/-} knockout mice. They believed that the results from this study would be more relevant to translational medicine as therapy was delivered after the development of sepsis, not prior-to, more closely resembling the course of real-life sepsis development. The study used 8–10-week old CD-1 mice that underwent CLP to induce polymicrobial peritonitis. After the induction of sepsis, mice were given injections of anti-PD-1 antibody, isotype control antibody, or saline injections by tail vein or intraperitoneally administered at 24 and 48 hours after surgery. The group also evaluated PD-1 expression in spleen cells, apoptosis rates, and plasma levels of IL-6, IL-10, TNF- α , and IFN- γ . Elevated PD-1 levels were found in septic animals, and blocking PD-1 inhibited lymphocyte apoptosis, thereby increasing the number of viable immune cells (CD4 T cells, CD8 T and B cells, and DCs). There was an increase in IL-6 production in mice treated with anti-PD-1 antibody, however the group did not find an increase in IL-10, TNF- α , or IFN- γ . For overall survival, mice treated with anti-PD-1 antibodies had an increased survival rate (70.6%) compared with saline controls (33.3%) or isotype control antibody treated mice (28.6%) [61].

After the successful murine model, many members of the same research team conducted an experiment to assess similar aspects in human populations. In a prospective study, 19

patients with septic shock and 22 healthy controls (age-matched) had blood samples drawn and measured for levels of lymphocyte apoptosis and PD-1/PD-L1 expression on peripheral T cells, B cells, and monocytes [62]. The study found an increase in total number of leukocytes, but a dramatically reduced number of lymphocytes in septic patients. The group measured an increase in apoptosis rates in CD4⁺ T cells, CD8⁺ T cells, and CD19⁺ B cells among septic patients (based on detectable caspase-3 levels), but discovered that the addition of PD-1/PD-L1 blockade in vitro could decrease this accelerated rate of apoptosis by as much as 50%. The group also noted that the levels of PD-1 on peripheral T cells and PD-L1 on monocytes were significantly upregulated in septic patients compared to healthy controls [62]. After blocking PD-1/PD-L1, LPS-induced TNF- α and IL-6 production increased dramatically and IL-10 production by monocytes was greatly decreased. The limitations in this study included limited sample size and failure to assess morbidity/mortality rates of the patients. Another limitation was the fact that blood samples were drawn only on the day of recruitment in the study which prevents analysis of the immune response over the course of sepsis progression [62].

Another group evaluated the effects of PD-1 on patients with sepsis by conducting a prospective and observational study on 126 patients in Lyon, France; this cohort included 64 patients with septic shock, 13 non-septic trauma patients, and 49 healthy volunteers. Along with information regarding diagnosis, subsequent infections, and survival collected from all patients, blood samples were drawn from patients with septic shock at days 1–2, 3–5, and 6–10 after diagnosis. The group found that 30% of septic patients developed a secondary infection along with a 17% mortality rate at 28 days. The group found dramatic increase in the levels of PD-1, PD-L1, and PD-L2 on circulating CD4⁺ and CD8⁺ lymphocytes and monocytes in septic patients compared to either trauma patients or healthy volunteers. The group found higher levels of circulating IL-10 among non-survivors coinciding with higher levels of PD-1, PD-L1, and PD-L2 expression. Lymphocyte proliferation was also greatly decreased in septic patients which had a significant negative-correlation with the levels of PD-1, PD-L1 or PD-L2. Patients who developed a secondary viral infection showed trademark signs of CD8⁺ T cell exhaustion with an overexpression of PD-1 causing a decrease in cytotoxic activity, proliferation, and IFN- γ production when stimulated with the appropriate antigens [63].

A recent study determined the effect of anti-PD-1 and anti-PD-L1 antibodies on blood samples (collected four times over 21 days) from 43 sepsis and 15 non-sepsis critically ill patients [64]. In this study, septic patients had decreased production of IFN- γ and IL-2 but had an increased CD8⁺ T cell expression of PD-1. An increase in PD-1 expression on CD4⁺ T cells positively correlated with the rate of apoptosis. Patients who presented with the highest PD-1 expression coupled with the lowest PD-L1 expression on CD8⁺ T cells had the lowest levels of human leukocyte antigen - antigen D-related (HLA-DR) monocyte expression and showed significantly increased rate of secondary nosocomial infection. As expected, treatment with anti-PD-1 or anti-PD-L1 antibodies decreased levels of cellular apoptosis and increased production of IFN- γ and IL-2. Overall, diminishing PD-1/PD-L1 expression attenuated the cellular dysfunction and exhaustion typical in septic patients [64].

5. Cytotoxic T-Lymphocyte Antigen-4

Cytotoxic T-lymphocyte antigen-4 (CTLA-4, also known as CD152) has been most extensively studied in melanoma and other malignancies; however, like PD-1, the findings could be translated to the immunomodulation in sepsis [65,66]. CTLA-4 augments host T cell-mediated immunity by preventing down-regulation and enhancing signaling [65]. Naïve T cells are primed for the immune response by an antigen presenting cell (APC), typically a dendritic cell [65]. APCs are recognized by T cell receptor complexes along with major histocompatibility complexes (MHCs) I or II. In a costimulatory second signal, CD80 or CD86 ligands bind to the surface of the APC via CD28 receptor molecules on the T cell. This process activates the T cell to secrete IL-2 which causes further T cell proliferation and differentiation as well as cytokine secretion [65,67]. CTLA-4 acts as an inhibitory molecule along this pathway. CTLA-4 is a homolog of CD28 (a member of the immunoglobulin superfamily), and is induced upon T cell activation. CTLA-4 up-regulation persists for as much as 2–3 days post-induction. CTLA-4 binds with the CD80 B7 ligand at a much higher affinity (100x) than CD28 (specifically on CD4⁺ and CD8⁺ T cells), thereby inhibiting CD80 binding on APCs, effectively impeding further T cell activation and dampening the immune response [65,68]. In cancer research, CTLA-4 blockade was shown to prolong T cell activation and restore proliferation levels to enhance the host immune response [67]. While this process naturally benefits the host by preventing an over-exaggerated immune response due to pathogen infection, in cases of uncontrolled immunosuppression, impeding the effects of CTLA-4 could re-activate the immunocapacity of the host to restore health.

In response to these findings Ipilimumab (a fully human anti-CTLA-4 monoclonal antibodies) was developed and studied in Phase 1 and 2 trials and was approved by the FDA in 2011 for patients suffering from unresectable or metastatic melanoma [65,67]. In one study, a single dose of Ipilimumab (3mg/kg) resulted in significant tumor necrosis and immune response upregulation with minimum or no secondary toxicities [49]. Further studies found that increased dosage could potentially lead to immune-related toxicity, such as diarrhea, dermatitis, vitiligo, and hyperthyroidism [69,70]. These immune-related adverse events (irAEs) have been reported in subsequent studies and have been summarized in the review articles [65,67]. Even as such, the possible auto-immune side effects are usually controllable and reversible, leading to a documented manageable safety profile for Ipilimumab [71]. While these irAEs are of vital consideration in the longer-term dosing seen in cancer therapy, as a treatment for sepsis-related severe immunosuppression the consequences are mitigated by the short-term or single use needed to show improvement in morbidity and mortality.

One team set out to determine the effects of CTLA-4 blockade in a murine model of CLP-induced sepsis in a one- or two-hit model with *Candida albicans* [68]. For the experiment, male CD-1 or C57BL/6 mice underwent sham surgery or CLP, or CLP flowed by tail vein injection of *C. albicans*. Mice were then administered a control, or anti-CTLA-4 antibody at the 2nd, 5th and 7th days. In these studies, the administration of low dose anti-CTLA-4 antibodies significantly improved survival in a one-hit (33% vs. 5.6% and tested again at 40% vs. 0% with controls) or two-hit (around 30% compared to 0% in controls) model, but high doses decreased overall survival rates. Anti-CTLA-4 antibodies also reduced CD4⁺ and

CD8+ T cell apoptosis by 50%; however, the antibody had no effect on either pro- or anti-inflammatory cytokine production [68].

5.1 PD-1 and CTLA-4 Immunoregulation

PD-1 and CTLA-4 are both negative regulators in T cell activation and adaptive immunity by means of CD28 blockade which prevents increases in T cell response; however, the two molecules appear to play different, yet complementary, roles in the regulatory process. PD-1 regulates immunity through peripheral T cell exhaustion (and seems to block CD28 binding more readily than CTLA-4), moreover PD-1 is also present on B cells: implying a broader role in the immune response than that of CTLA-4 [66,71]. CTLA-4 prevents naïve T cells from being activated by competitively binding CD80 [64].

Blocking CTLA-4 or PD-1 has been found to be useful in cancer therapy for metastatic melanoma, as well as murine models of fungal sepsis survival [72]. One lab studied the effects of anti-PD-1, anti-PD-L1, or anti-CTLA-4 molecules on survival outcomes in fungal sepsis. The study determined that all three interventional methods were successful in improving survival in a one-hit (*C. albicans*) or two-hit (CLP plus *C. albicans*) model compared to controls. In the two-hit model, anti-PD-1 and anti-PD-L1 showed a two-fold improvement in survival while anti-CTLA-4 antibodies showed a 57.9% survival compared to 27.8% in the control group. In the one-hit model, anti-PD-1 interventions showed 73.3% survival, anti-PD-L1 interventions showed 70% survival, and anti-CTLA-4 showed 68.4% survival compared to 31.5% survival in control groups [72]. In one study, a concurrent blockade of PD-1 and CTLA-4 provided an unprecedented level of tumor reduction (80%) when using a safely combined dose of 1mg/kg Nivolumab (anti-PD-1) and 3mg/kg Ipilimumab (anti-CTLA-4) [71]. While the researchers caution the generalizability due to small sample size, findings from additional investigations could potentially be more broadly applied as interventional strategies in both cancer and sepsis-induced immunosuppression.

Given the fact that PD-1 and CTLA-4 immunologic checkpoints could be non-redundant distinctly prohibiting T cell activation, proliferation, and function, each therapy should be studied more thoroughly (independently and concurrently) to mitigate the excessive immunosuppression seen in cases of severe sepsis and septic shock.

6. Signal Transducer and Activator of Transcription Pathways (STAT) Proteins

Many cytokines involved in the inflammatory process use complicated intracellular signaling pathways to enact or promote their biological functions. Several of these cytokines employ Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathways [73]. JAK/STAT signaling pathways are important in regulating and augmenting immune function and can be disrupted, if necessary, by the modulation of STAT expression and suppressor of cytokine signaling (SOCS) proteins [73].

In recent years, the JAK/STAT signaling pathway has been the focus of several research projects to understand the effects of immunomodulation on the inflammatory/immune response, and subsequently to determine how the modulation could positively impact

morbidity and mortality rates in inflammatory conditions such as sepsis. Cytokine responses can be categorized into two groups: Type-1 and Type-2. Type-1 cytokine response is essential to combat bacterial infection and is considered a local pro-inflammatory defense of cell-mediated immunity which typically presents with high levels of IL-12, IFN- γ and TNF- α expression, as well as IL-2, IL-4, IL-5, IL-7, IL-9, IL-11, IL-13, and IL-15 and hemoproteins which induce growth and differentiation of red blood cells and leukocytes (especially T and B lymphocytes) [5]. Type-2 cytokine response involves a more systemic response with an up-regulation of IL-4, IL-5, IL-10, and IL-13 cytokines as well as IFN- γ , IFN- β , IFN- α , which activate macrophages and enhance cell mediated immune responses (especially to viral infections) [5,73]. Most of the damage done in septic patients comes from an overzealous inflammatory response followed by a period of host-immunosuppression and secondary infection. With severe sepsis typically having a predominant Type-2 response [5]. There is typically considerable regulation between Type-1 and Type-2 responses, an overzealous response of either can lead to the tissue damage and organ failure common to septic infections [5]. Finding a way to synthetically balance Type-1 and Type-2 cytokine responses could alleviate some of the host burden in recovering from the initial infection and improve clearance of any secondary infection [5].

Cytokines are low weight molecular polypeptides or glycoproteins that regulate numerous cellular functions and allow autocrine and paracrine signaling, influence cell differentiation, proliferation, activation, and modulate pro-/anti-inflammatory responses through Type-1 or Type-2 cytokine signaling and immunomodulation [5]. Cytokines bind to corresponding receptors and initiate intracellular signaling cascades which cause a change in gene expression; this process is accomplished when cytokines bind to receptors and cause dimerization. Dimerization enables JAKs (which have a functional catalytic kinase domain and a pseudokinase domain, both having kinase like activity) to phosphorylate other JAKs (homodimerization) or other cytokine receptors (heterodimerization) [5]. Phosphorylation allows STAT binding (through Src homology 2 domain (SH2)-phosphotyrosine interactions) at a conserved tyrosine residue 700 aminoacids from the N terminus, which is, in turn, phosphorylated by other JAK proteins. STATs dissociate from receptors and dimerize. The STAT dimers are stabilized by reciprocal phosphotyrosine and Src homology 2 domain interactions. The STAT dimer translocate to the nucleus, bind to specific palindromic sequences within the promoter region of target genes, and thus regulate transcription and produce altered gene expression [5]. In mammalian cells, there have so far been four JAKs (JAK 1–3 and Tyk2) and seven STATs (STAT-1, –2, –3, –4, –5a, –5b, and –6) proteins identified in the JAK/STAT signaling pathway [5].

Suppressor of Cytokine Signaling (SOCS) proteins are SH2 domain-containing cytoplasmic proteins that complete negative feedback loops to diminish signal transduction of cytokines utilizing JAK/STAT signaling pathways [73]. SOCS proteins include a kinase inhibitory region and extended SH2 subdomain creating a myriad of possible interventions in the JAK/STAT pathway [5]. SOCS proteins are regulated through a classical feedback loop where SOCS expression is induced via STATs and the respective cytokines [5]. STAT proteins also can be dephosphorylated by tyrosine phosphatases or degraded by ubiquitin-proteasome pathway disrupting signaling capabilities [5]. SOCS3 is induced by IL-10 and IL-6, which could be the mechanism responsible for the reported IL-10 inhibition of pro-inflammatory

macrophage function [5]. The JAK/STAT signaling pathway is the major induction pathway for IFN- γ and other lipopolysaccharide microbial signals critical in activating macrophages and monocytes [5].

Table 3 presents the information on the concise effects of each JAK/STAT protein within the signaling pathway [5,73,74]. In 2001, it was determined that STAT4 and STAT6 were of interest in sepsis-related immunomodulation. Of note, STAT6 has been shown to modify host susceptibility to infection, specifically blocking STAT6 decreases the prevalence of sepsis in knockout mice, but increases neutrophil numbers as well as TNF- α and IL-12 production to augment host defenses during a period typically categorized as being one of severe immunosuppression. STAT4 blockade leads to a decrease in Type-1 cytokine production (specifically IL-12) and may ameliorate systemic organ damage without shifting the bacterial load in measured peritoneal fluid or peripheral blood samples [74]. In the same 2001 study, it was determined that the 14-day survival rate of STAT6^{-/-} knockout mice was 15–20%, while the survival of STAT4^{-/-} mice was even better at 42%. More importantly, knockout mice showed much better survival than wild-type mice with all WT mice having died by the 5th day post CLP-induced sepsis [74]. This finding shows promise in the regulation of STAT4 and STAT6 as possible targets for combined therapy to improve sepsis mortality rates in conjunction with other immunomodulation interventions.

7. The Use of Animal Models in Sepsis Research

Recently there has been much controversy regarding the use of animal models (especially murine models) in clinical studies in sepsis [75–79]. The controversies and current findings are summarized below.

One study evaluated the use of CLP in mouse models in comparison to human inflammatory and immune response to reveal translatability or limitations with the procedure [79]. Benefits of CLP include the ability to modify the level of polymicrobial peritonitis through the number of cecum punctures and size of needle used. The authors also determined that CLP-induced sepsis follows the same progression of hemodynamic and metabolic phases as human sepsis with a hyper-inflammatory state followed by distinct immunosuppression. The major downside of CLP is that the process is exceedingly variable based on clinician experience and technique chosen, making study comparisons more difficult. Other mouse models such as bacteria, fungi, or viral endotoxemia are beneficial in that they are easily reproducible and highly standardized [79]. The main drawback of these models is that they cannot reproduce the complete and complex human pathophysiological responses needed to adequately evaluate novel sepsis interventions. The authors had several recommendations to bridge the gap between murine and human models. The recommendations include more research into the pathogenic mechanisms of human sepsis and an attempt to more accurately reflect human clinical scenarios in murine models of sepsis research. Accurate depiction of human sepsis in murine models could be achieved by using aged rodents instead of young, and more heterogeneous population of mice, and subjecting study mice to the same clinical interventions human receive in clinic scenarios (e.g. Fluid resuscitation and antibiotic therapy) [79].

Another lab set out to determine if mouse or rat models more closely resembled human conditions of sepsis progression [75]. The researchers intraperitoneally injected male Wistar rats and C57 black mice with a fecal slurry and monitored metabolic and cardiovascular pathways. The team discovered that both rodent species had similar mortality curves, around 75%, fatality with similar dispersion among mild (33–38%) and severe (62–66%) sepsis classifications. Rats showed better metabolic control, with all mice becoming significantly hypothermic during the experiment [75]. Mice also showed greater reductions in oxygen consumption and carbon dioxide production compared to rats. Echocardiography showed lower measured variables (e.g. peak velocity, stroke volume, etc.) in mice with sepsis compared to healthy mice; rats, however, did not show significant variation between healthy and septic animals [75]. Based on the findings, the authors suggest caution in applying rodent models of sepsis to human outcomes, specifically in regards to differing metabolic profiles between the two-rodent species and humans [75].

In an expansive, collaborative effort genomic responses in mouse models compared to human inflammatory diseases were assessed [76]. These authors developed the Inflammation and Host Response to Injury, Large Scale Collaborative Research Program to conduct multiple studies on genomic responses of patients in clinical settings as well as healthy controls and murine models. In summary, the studies compared gene expression between trauma, burns, and endotoxemia between human subjects and between human subjects and mouse models under the same conditions. The group also compared these changes in gene expression over time, up to one-year post-incident for human burn victims. The group focused on identifying major signaling pathways within the human inflammatory response compared to an in-vivo model and three murine models [76]. And finally, researchers evaluated their findings compared to other available representative human and mouse studies. The major finding from all this collaborative effort is that human inflammatory responses are all very similar between acute inflammatory stresses, but that these responses are not adequately reproduced in mouse models for any of the categories of genomic changes, temporal response patterns, or inflammatory response pathways. The researchers posit this difference could be due to the evolutionary distance between mice and humans, or the extreme heterogeneity of human patients with varying comorbidities, lifestyles, and treatments when compared to the intentional homogeneity of most experimental lab mice [76].

Other research compiled all the animal trials that have been conducted in the evaluation and survival of sepsis [77]. While many trials involve mouse or rat models, Fink [77] also notes studies in baboons and rabbits. It was concluded that very few animal models translated to reproducible, beneficial, outcomes in human models. Based on the compiled information, the use of the “humanized” mice model was recommended. These γ -irradiated, neonatal NOD-scidIL2r γ null mice utilize transplanted human CD34+stem cells to develop into adult mice with a complete set of human innate and adaptive immune response cells [80]. These mice would bring the pathophysiological responses of mouse models closer to those evaluated in human models, but the process of creating such mice is time-consuming and expensive [77]. Other solutions include using baboons or other similar monkey species which closely resemble human immunophysiology. However, the associated cost and sentimental attachment of the governing bodies for these animals make the option less viable [77].

Based on the in-depth evaluations, new approaches to clinical research designs regarding sepsis, as it applies to humans, need to be developed. Murine models such as CLP may allow for beginning investigations in a timely and feasible manner. However, researchers must be aware that results from these studies, more than likely, might not be directly applicable to human models.

8. Conclusion

Sepsis and its different stages, SIRS, CARS, and PICS, are complicated conditions with a high mortality and increasing incidence in the United States. While there has been much scientific inquiry into treatment regimens, there have been no viable interventions that have proven efficacious without adverse side-effects in Phase 3 trials. Given the new clinical research into AED/AET, TREM-1/sTREM-1, PD-1/PD-L1, CTLA-4, and the JAK/STAT pathway hopefully soon new or combined approaches will lead to translatable findings to improve human outcomes.

9. Expert Commentary

Sepsis, SIRS, CARS, and PICS are widely variable disease processes that involve a host of inflammatory and immune response signaling pathways combined with possible immunosuppression, tissue damage, secondary infection, and multiple organ failure. Even though improvements in evaluation and treatment have been seen through the surviving sepsis campaign, there is still an increasing incidence and steady mortality associated with the disease. While patients are now, more than ever, surviving the initial cytokine storm involved with the initial inflammatory response seen in the SIRS stage of sepsis, many are dying during the later immunosuppressive stage of CARS due to secondary infections or organ failure. Despite the growing body of knowledge on the progression and immune responses associated with sepsis, there have been very few translatable findings to human models. With several failed Phase 3 trials for sepsis interventions, new studies are under more scrutiny to accurately reflect human conditions.

Recently, findings from several investigations have shown promise in modulating host immune response and hopefully will show translatability to improve human survival to the same degree as documented murine survival. Interventions modulating DHEA/AED/AET have also shown promise in improving survival especially in murine models of radiation or burn trauma with or without sepsis. Blockers of PD-1 and PD-L1 have shown marked survival improvements compared to controls, with an immune response shift from Th2 to Th1 cytokine production. While impressive independently, CTLA-4 blockade concurrent to PD-1/PD-L1 modulation shows even more promise in ameliorating the negative impact of sepsis immunosuppression. Partial TREM-1 blockade also shows promise in improving survival while maintaining host immune response and the ability to adequately clear bacterial infections secondary to sepsis. Modifications along the JAK/STAT signaling pathway, specifically interventions blocking STAT4 or STAT6, show great potential in improving sepsis outcomes with limited negative effects from unforeseen complications.

10. Five-year View

While the recent interventions are promising, the approach with a combination of immunomodulators could prove more beneficial. Indeed, further research is warranted to develop a treatment strategy with the capability to restore the host immune function while limiting tissue damage due to a hyper-inflammatory response. Through modulation and effector cell profile switch to a more favorable T_H1 response survival appears to be improved. Due to chronological sequence and complexity, effective treatment options will require early and late phase alteration/modulation through a combined treatment approach. For sepsis, treatment of the immune response requires a modulating approach to maintain homeostasis in a favorable healthy state, neither hyper- nor hypo-inflammatory. Another important facet of sepsis treatment is personalized medicine. Once effective treatment modalities have been established, it is crucial for health care providers to determine which treatments will be most effective for which patients. As further methods are under investigation, it would be better to focus the research efforts on developing not only the interventional strategy, but also determining the most effective diagnostic markers to ensure success of each specific treatment strategy. By determining which patients are in need of immunoregulation or immunostimulation to prevent secondary infections and improve the patient's ability to clear the initial infection, all while not compromising organ function, the effects of hyper-inflammation and severe immunosuppression seen in sepsis could no longer be the life-threatening condition as it is today.

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Reference annotations

*Of interest

**Of considerable interest

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Key Issues

- Sepsis is a disorder with high incidence and mortality which has proven difficult to effectively diagnose and treat despite years of research and a robust body of knowledge.
- While recommendations for steroid use have been limited in sepsis therapy, new interventions using DHEA/AED/AET have shown clinical promise.
- PD-1 expression correlates with sepsis severity and anti-PD-1/PD-L1 antibodies can partially attenuate the negative outcomes of sepsis-related immunosuppression.
- Anti-CTLA-4 antibodies have demonstrated some effect in ameliorating the negative effects of sepsis and returning exhausted immune function to normal levels.
- PD-1 in conjunction with blocking CTLA-4 should be considered as novel approaches to sepsis mitigation.
- The JAK/STAT pathway has shown promising targets for intervention in reducing sepsis mortality, specifically with the downregulation of STAT4 and STAT6 to decrease tissue damage and improve host immune response, respectively.
- TREM-1 and sTREM-1 have shown promise as both an evaluative marker and immunomodulatory in sepsis survival; however, more research is warranted.
- While CLP is considered the “gold-standard” for animal models of sepsis; in general, animal models vary significantly from the human immune response which develops during sepsis.

Figure 1: Immune Response in different stages of Sepsis and corresponding mediators depending on the time from insult.

CARS: compensatory anti-inflammatory response; CD: cluster of differentiation;

CTLA-4: cytotoxic T-lymphocyte-associated protein 4; **HMGB-1:** high mobility group box 1 protein; **HLA-DR:** human leukocyte antigen-d related; **IFN:** interferon; **IL:**

interleukin; **MHC:** major histocompatibility complex; **MIF:** macrophage migration inhibitory factor; **NF-κB:** nuclear factor kappa-light-chain-enhancer of activated B cells;

PICS: persistent inflammation, immunosuppression and catabolism; **PD-1:** programmed cell death 1 protein; **sTREM-1:** soluble triggering receptor expressed on myeloid cells 1; **SIRS:** systemic hyper-inflammatory response syndrome; **TNF:** tumor necrosis factor.

It should be noted that sepsis can present clinically with characteristics of both hyper- and hypo-inflammatory dysregulation with significant overlap.

Figure 2: Immune Response of Sterile and Infectious Systemic Inflammatory Response Syndrome.

DAMPs: Damage-associated molecular patterns; **NLRs:** NOD-like receptors; **PAMPs:** Pathogen-associated molecular patterns; **PRRs:** Pattern recognition receptors; **RLRs:** RIG-like receptors; **TLRs:** Toll-like receptors.

Table 1

Survival advantage.

Intervention	Survival Advantage (%)	Reference
PD-1 Block	55 (N=22)	Huang et al. 2009
PD-1 Block	37.6 (N=17)	Brahmamdham et al. 2010
PD-1 Block	41.8 (N=35)	Chang et al. 2013
PD-L1 Block	38.5 (N=39)	Chang et al. 2013
CTLA-4 Block	40 (N=5)	Inoue et al. 2011
CTLA-4 Block	27.4 (N=18)	Inoue et al. 2011
CTLA-4 Block	30 (N=7)	Inoue et al. 2011
CTLA-4 Block	30.1 (N=19)	Chang et al. 2013
CTLA-4 Block	36.9 (N=19)	Chang et al. 2013
STAT4 Block	42 (N=24)	Matsukawa et al. 2001
STAT6 Block	15–20 (N=26)	Matsukawa et al. 2001
TREM-1 Block (Partial)	75 (N=12)	Gibot et al. 2007
TREM-1 Block (Partial)	50 (N=30)	Gibot et al. 2004
TREM-1 Block (Partial)	80 (N=5)	Wang et al. 2012
TREM-1 Block (Partial)	40 (N=48)	Gibot et al. 2004
DHEA Administration	57 (N=28)	Angele et al. 1998
DHEA Administration	31 (N=72)	Schmitz et al. 2010
DHEA Administration	40 (N=14)	Ben-Nathan et al. 1999
DHEA Administration	43 (N=16)	Ben-Nathan et al. 1999
AED Administration	64 (N=14)	Ben-Nathan et al. 1999
AED Administration	43 (N=16)	Ben-Nathan et al. 1999
AET Administration	25 (N=12)	Marcu et al. 2006
AET Administration	38 (N=16)	Marcu et al. 2007

PD-1: programmed cell death-1, **PD-L1:** programmed cell death ligand-1, **CTLA-4:** Cytotoxic T-lymphocyte antigen-4, **STAT4 and STAT6:** Signal Transducer and Activator of Transcription 4 and 6, **TREM-1:** triggering receptor expressed on myeloid cells-1, **DHEA:** Dehydroepiandrosterone, **AED:** Androstenediol, **AET:** Androstenediol

Table 2

Immunomodulatory capabilities.

Intervention	Mediators and Cellular Response	
	Increased	Decreased
PD-1 Block	IFN- γ , TNF- α , IL-2, IL-6, IL-12, Bacterial Clearance, T Cell Function and Proliferation	IL-10, Apoptosis: CD4+ T Cells, CD8+ T Cells, B Cells, and DC (50%)
CTLA-4 Block	T Cell Activation and Proliferation,	Apoptosis: CD4+ T Cells, CD8+ T Cells (50%)
TREM-1 Block	Circulating sTREM-1, IL-6, Phosphorylation of STAT5	IL-1 β , IFN- γ , TNF- α , MCP-1, CD40, CD86, NF- κ B, Overall 30% reduction in Cytokine Production
Increase in sTREM-1	--	IL-1 β , TNF- α
DHEA Administration	Splenocyte Proliferation, IL-2, IFN- γ , IL-1 β	Lymphocyte Apoptosis, IL-1, IL-6, MCP-1, TNF- α
AED Administration	IL-2, IFN- γ	IL-6, TNF- α
AET Administration	IL-2, IL-3, IFN- γ	IL-4, IL-6, IL-10, IL-18

Table 3

STAT proteins.

STAT Protein	Cells and Mediators	
	Upregulated	Downregulated
STAT1 ^{-/-}	Peritoneal Macrophages, IFN- γ	IFN- α signaling, IFN- β signaling, IL-6, IL-10, HMGB-1
STAT2 ^{-/-} Relatively Insignificant	--	Response to IFN- α , IFN- β and IFN- γ signaling
STAT3 ^{-/-} Do not survive embryonic stage	IFN- γ , NK cells	TNF- α , LPS levels, IL-6, IL-10, T Cell Proliferation
STAT4 ^{-/-}	--	IFN- γ , TNF- α , IL-10, IL-13, MIP-2, MPO, AST/ALT/BUN/Creatinine Levels, hepatic inflammation
STAT5a/b ^{-/-} Relatively Insignificant	--	NK Cell number and function, IL-2 responsiveness
STAT6 ^{-/-}	IL-12, TNF- α , Chemokine C10, Total Neutrophil Count, Pro-inflammatory Cytokines	IL-4, IL-13, MIP-2, MPO, Bacteria levels