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Sepsis-Induced Immunosuppression

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Abstract

Sepsis is expected to have a substantial impact on public health and cost as its prevalence increases. Factors contributing to increased prevalence include a progressively aging population, advances in the use of immunomodulatory agents to treat a rising number of diseases, and immune-suppressing therapies in organ transplant recipients and cancer patients. It is now recognized that sepsis is associated with profound and sustained immunosuppression, which has been implicated as a predisposing factor in the increased susceptibility of patients to secondary infections and mortality. In this review, we discuss mechanisms of sepsis-induced immunosuppression and biomarkers that identify a state of impaired immunity. We also highlight immune-enhancing strategies that have been evaluated in patients with sepsis, as well as therapeutics under current investigation. Finally, we describe future challenges and the need for a new treatment paradigm, integrating predictive enrichment with patient factors that may guide the future selection of tailored immunotherapy.

INTRODUCTION

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection (1). The recent Global Burden of Diseases, Injuries, and Risk Factors Study estimated that the burden of cases of sepsis incidence and deaths is twice what was previously thought (2). Although the precise incidence of sepsis is unknown, estimates indicate that it is a leading cause of mortality and critical illness worldwide (3). As the prevalence of sepsis increases, it is expected to have greater public health and cost implications. An aging population, who have impaired immunity from immunosenescence (4), and advances in the use of immunomodulatory agents to treat patients with autoimmune diseases and cancer, or immune-suppressing therapies in solid-organ transplant recipients are major contributors because these populations are more vulnerable to infections.

The host immune response during sepsis involves complex pathophysiology and is now recognized to consist of both pro- and anti-inflammatory mechanisms (5, 6). Infection triggers an initial cytokine-mediated host inflammatory response that may be excessive and associated with tissue damage, endothelial cell dysfunction, and organ failure (5, 6). The intensity of the hyperinflammatory response is variable and dependent on factors such as patient comorbidities, nutritional status, microorganism load, and virulence (6). A concurrent, albeit less evident, anti-inflammatory response may be characterized by metabolic dysfunction of leukocytes, changes in gene expression impacting immune cell function, cellular anergy or death. Moreover, persistence of the anti-inflammatory milieu promotes sustained immunosuppression, associated with susceptibility for secondary infections and a litany of potential complications that ultimately increase morbidity and mortality.

In the absence of targeted therapies to modulate the complex pathophysiology of the host immune response, sepsis has traditionally been treated with timely implementation of supportive therapies such as fluids, vasopressors, oxygen, mechanical ventilation, prompt source control, and antibiotics (7). Previous clinical trials of mainly anti-inflammatory therapies to counteract excessive inflammation in patients with sepsis were unsuccessful. Many factors have been proposed as explanations for the failure of these earlier trials (6). Importantly, sepsis is heterogeneous, and identification of subgroups of patients that are more likely to benefit from a certain intervention (i.e., predictive enrichment of the study population) was not performed. In addition, certain therapeutic targets, even if effectively inhibited, may not drive pathology in real-life sepsis despite providing benefit in preclinical settings (6). Partially as a consequence of the negative trials testing anti-inflammatory agents, but also following increased insight into the relevance of the sustained immunosuppression, therapeutic approaches aimed at stimulating immune function have become the focus of sepsis trials more recently (5, 8).

In this review, we discuss mechanisms of sepsis-induced immunosuppression, biomarker use, clinical consequences, and complications. In addition, we consider immune-stimulating therapeutics under investigation and issues with prognostic enrichment-only approaches. Finally, we present a new paradigm for treatment in the critically ill septic patient, integrating predictive enrichment with baseline patient factors that may guide future selection of tailored immunotherapy.

MECHANISMS

At the time of hospital admission, most patients with sepsis show signs of immunosuppression, which is prolonged in those who remain in need of intensive care for extended periods of time. The term immunosuppression in sepsis relates to different cell types and features, in particular, increased apoptosis of T and B cells, T cell exhaustion, reprogramming of monocytes and macrophages through epigenetic changes, and reduced expression of activating cell surface

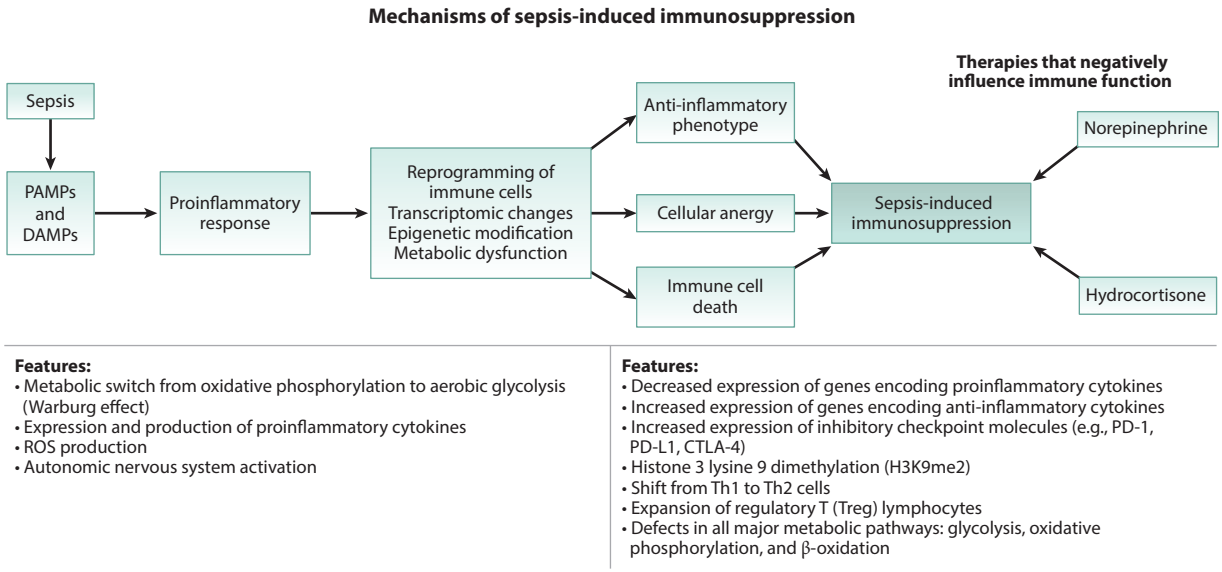

Figure 1

Mechanisms of sepsis-induced immunosuppression. The host response during sepsis consists of both pro- and anti-inflammatory mechanisms. Infection triggers an initial cytokine-mediated host inflammatory response initiated by an interaction between pattern recognition receptors, PAMPs, and DAMPs that is associated with reprogramming of immune cells, transcriptomic changes, epigenetic modification, and metabolic dysfunction. The inflammatory reactions are excessive in patients with sepsis and result in tissue damage and organ failure. Concurrently, and likely initiated by inflammation, anti-inflammatory responses are induced, characterized by cellular anergy and cell death, among others. Persistence of the anti-inflammatory milieu promotes sustained immunosuppression. Certain therapies administered to patients with sepsis can further enhance immunosuppression, most notably norepinephrine and hydrocortisone. Abbreviations: CTLA-4, cytotoxic T lymphocyte antigen-4; DAMP, damage-associated molecular pattern; PAMP, pathogen-associated molecular pattern; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; ROS, reactive oxygen species; Th, T helper.

molecules. An overview of mechanisms that drive sepsis-induced immunosuppression is provided in **Figure 1**. Factors influencing immune cell gene expression, cellular metabolism, and apoptosis have been well established in both animal and human models. Common therapies used in the management of sepsis and septic shock, such as use of vasopressors (9) and hydrocortisone (10), may also have detrimental effects on immune cell function and are under investigation.

Pathogen-Associated Molecular Patterns, Damage-Associated Molecular Patterns, and Immune Cell Death

Pathogens are recognized by the host innate immune system via an array of pattern recognition receptors (PRRs) (11). These PRRs recognize molecules released by both pathogens, i.e., pathogen-associated molecular patterns (PAMPs) and injured cells from the human host, i.e., damage-associated molecular patterns (DAMPs). In most cases of infection, the burden of pathogen is diminished and eventually eliminated by the innate immune system. However, pathogen persistence will lead to dysregulation of the host immune response. As injured cells continue to release DAMPs, they can activate PRRs that also recognize PAMPs, initiating a detrimental cycle of sustained immune activation and organ dysfunction (12).

Apoptosis of immune cells in sepsis is particularly prominent in $CD4^+$ T cells, $CD8^+$ T cells, B cells, natural killer (NK) cells, and follicular dendritic cells. Postmortem studies of patients who died of sepsis showed depleted populations of splenic T cells compared with patients who died of

noninfectious causes (13). Furthermore, T cells obtained from subjects who died from sepsis were found to have increased expression of programmed death ligand 1 (PD-L1) (13). Sepsis-induced apoptosis occurs through both death receptor– and mitochondrial-mediated pathways, suggesting that multiple cell death stimuli are activated during sepsis. The pathophysiological importance of enhanced apoptosis in sepsis has been documented in several animal models, demonstrating improved survival through interventions inhibiting apoptosis (5, 8). The increased apoptosis in immune cells is observed to a lesser extent in parenchymal cells, and the relevance of parenchymal apoptosis in organ dysfunction (e.g., acute kidney injury) is unclear. Of note, sustained lymphopenia in sepsis may be caused by not only enhanced apoptosis but increased extravasation and recruitment to sites of inflammation may also contribute. Autophagy is a physiological mechanism of cells to remove redundant or dysfunctional components. Mice with a reduced autophagy capacity in lymphocytes due to cell-specific deletion of *Atg5* or *Atg*7 showed an increased mortality together with immune dysfunction in abdominal sepsis (14). Deletion of *Atg5* in T cells resulted in higher release of the anti-inflammatory cytokine interleukin (IL)-10 by these cells during sepsis, suggesting that impaired autophagy can contribute to immunosuppression (14). Sepsis is also associated with depletion of B lymphocytes, due to deficient T cell support or enhanced apoptosis, and surviving B lymphocytes have an exhausted phenotype characterized by decreased major histocompatibility complex II (MHC II) expression and increased IL-10 production (15). T cell dysfunction contributes to B cell dysfunction in sepsis through impairment of T cell–dependent B cell maturation. Recently identified pathways of programmed cell death, such as necroptosis or pyroptosis, have not yet been described in the literature of sepsis-induced immunosuppression.

Transcriptomic Changes

The recent developments using transcriptomic analysis in sepsis studies have facilitated greater understanding of gene expression and their canonical signaling pathways. A body of evidence has now emerged that patients with sepsis have marked alterations in leukocyte transcriptomes and, as a result, different patient phenotypes are becoming evident. Leukocytes of critically ill patients show a profoundly altered transcriptome with 70–80% of all RNA transcripts differentially expressed relative to health (6). Expression of genes involved in pro-/anti-inflammatory and mitochondrial dysfunction pathways is typically increased in sepsis, while expression of genes with functions in translation initiation, mechanistic target of rapamycin (mTOR) signaling, adaptive (mainly T cell) immunity, and antigen presentation is decreased (6). Additionally, transcriptomic and functional analyses of circulating monocytes in patients with gram-negative sepsis and 1–3 months after sepsis recovery revealed functional plasticity or reprogramming, with expression and activity of hypoxia-inducible factor (HIF)-1α upregulated in sepsis monocytes compared with recovery monocytes (16). This observation was associated with upregulation of IL-1 receptor-associated kinase M in monocytes and concurrent downregulation of expression of tumor necrosis factor (TNF) and IL-6 following ex vivo lipid A exposure [a component of gram-negative endotoxin and a specific Toll-like receptor (TLR) 4 ligand (16)]. HIF-1α was thus implicated as a key mediator of cellular reprogramming from a proinflammatory to an immunosuppressive state during sepsis (16).

More recent studies have attempted to identify patient phenotypes of immune response. Interestingly, two distinct sepsis response signatures (SRS) were described in transcriptomic analysis of circulating leukocytes of intensive care unit (ICU) patients with community-acquired pneumonia, one of which (named SRS1) identified patients with an immunosuppressed phenotype, including attributes of endotoxin tolerance, T cell exhaustion, and downregulation of HLA class II, and an associated higher 14-day mortality (17). Additionally, the majority of the SRS was concluded to be expressed independent of the sepsis source, when comparing transcriptional responses in

circulating leukocytes of critically ill patients with community-acquired pneumonia or fecal peritonitis (18). Another study identified four distinct molecular subgroups in patients with sepsis based on gene expression profiles in blood leukocytes, termed endotypes Mars1–4 (19). Akin to the SRS1 group, the Mars1 endotype was associated with decreased expression of genes associated with innate and adaptive immune signaling and an increased mortality (19). A recent investigation used single-cell RNA sequencing to profile peripheral blood mononuclear cells and dendritic cells to show that sepsis was associated with an expansion of a unique $CD14^+$ monocyte population, named MS1, which (relative to other $CD14^+$ monocytes) displayed major features of immunosuppression, i.e., reduced MHC II expression and a reduced capacity to activate nuclear factor-kappa B (NF-κB) and produce TNF upon ex vivo stimulation with lipopolysaccharide (LPS) (20). This study exemplifies that single-cell analyses can be highly valuable in obtaining insight into distinct cellular phenotypes (proinflammatory versus immunosuppressive) in sepsis (20). It is important to recognize, however, the potential limitations of gene expression data, which may not necessarily reflect protein levels due to posttranscriptional regulation (discussed in further detail below).

Epigenetic Modification

Epigenetic modification of immune cells in sepsis has been identified as an important mechanism by which transcriptional regulation takes place (6). It can occur through one of three mechanisms: DNA methylation, histone modification, and posttranscriptional regulation by microRNA (miRNA) and long-noncoding RNA (21, 22). Transcriptional regulation results in the organization of gene loci on chromatin into transcriptionally active or transcriptionally silent states (22). The results are stable and potentially heritable alterations in gene expression and cellular function without changes to the DNA sequence (22). Epigenetic modifications thus have a prominent role in potential long-term consequences of transcriptional changes that may extend to subsequent generations of cells (21, 22).

One of the earliest examples of epigenetic regulation contributing to an immunosuppressive phenotype came from a study showing that downregulation of marks of open chromatin from histone modification histone 3 lysine 4 trimethylation (H3K4me3), a permissive epigenetic mark, led to LPS-induced tolerance in murine macrophages (23). Furthermore, increased levels of a repressive histone modification known as H3K9 dimethylation (H3K9me2) were noted in the promoter regions of genes for proinflammatory cytokines IL-1β and TNF in immune-tolerant leukocytes (24). Evidence for epigenetic modification in organ-specific tissues was supported by a study of lung-resident dendritic cells in septic mice. It showed permissive modification with decreased methylation of histone H3K4 and repressive modification with increased methylation of H3K27 at the promoter of *Il12* that correlated with decreased IL-12 production after stimulation with TLR agonists tripalmitoyl-*S*-glyceryl-cysteine (Pam3cys), LPS, and CpG-DNA (25). Histone deacetylation is an additional mechanism for inhibiting proinflammatory cytokine transcription during endotoxin tolerance; e.g., the p50-recruited HDAC-containing nuclear receptor corepressor complex inhibits expression of NF-κB-target proinflammatory genes (26).

In the adaptive immune response, repressive histone modification H3K27 methylation after sepsis occurs at the promoter region of the gene for interferon (IFN)-γ in T helper (Th)1 cells and the gene encoding GATA-binding protein 3 (GATA3) in Th2 cells (27). Additionally, an increase in permissive histone modification of the gene Forkhead box protein 3 (*FOXP3*), the transcription factor for regulatory T (Treg) cells, may influence differentiation toward an anti-inflammatory milieu, as discussed below (28).

Recent evidence has emerged that epigenetic alterations take place within cells of bone marrow. A murine cecal ligation and puncture (CLP) model was used to show that after sepsis recovery,

bone marrow–derived macrophages (BMDMs) and wound macrophages of mice later subjected to wound healing displayed decreased expression of inflammatory cytokines essential for wound repair (IL-1β, IL-12, and IL-23) when compared to control mice (29). Chromatin immunoprecipitation of these postsepsis BMDMs and wound macrophages revealed decreased expression of mixed-lineage leukemia 1 (*Mll1)*, an epigenetic enzyme, and impaired H3K4me3 at NF-κB-binding sites on inflammatory gene promoters (29). Bone marrow implantation from postsepsis donor mice into postsepsis recipient mice showed impaired wound healing, decreased expression of inflammatory cytokines in peripheral blood monocytes, and decreased H3K4me3 on the NF-κB-binding sites of *Il1b* and *Il12* in peripheral monocytes compared with implantation from sham donor mice. This suggests that epigenetic modifications initiate in bone marrow progenitor cells following sepsis and may lead to lasting impairment in macrophage function (29). Thus, persistent immunosuppressive changes in subsequent generations of leukocytes may have the capacity to adversely impact outcomes related to morbidity and mortality.

Several functional links between metabolic and epigenetic regulation of the immune response have been described. As an example, the α-ketoglutarate to succinate ratio can modulate the activation of macrophages. While macrophage activation increases the tricarboxylic acid intermediate succinate, which stabilizes HIF-1α and promotes transcription of *IL1B* (30), α-ketoglutarate can increase the expression of M2 macrophage–associated genes during endotoxin tolerance through epigenetic regulation by lysine demethylase 6B, which requires α-ketoglutarate as a cofactor for H3K27me3 demethylation (31).

Metabolic Dysfunction

Alterations in immune cell metabolism have become apparent as significant drivers of sepsis-induced immunosuppression (8, 32, 33). Under inactivated, aerobic conditions, immune cells utilize mitochondrial oxidative phosphorylation as their main source of ATP production. This oxygen-dependent process is very efficient, yielding 36 molecules of ATP from each glucose molecule. Upon stimulation with purified bacterial components, immune cells undergo a metabolic switch to anaerobic glycolysis, which occurs in the cytosol and is facilitated by the influx of glucose via rapid mobilization of intracellular GLUT glucose transporters to the cell surface (33, 34). The metabolic switch from oxidative phosphorylation to anaerobic glycolysis in spite of sufficient oxygen levels for glucose oxidation in the mitochondria is known as the Warburg effect, originally described in proliferating cancer cells (35). While anaerobic glycolysis is relatively inefficient from an energy supply perspective, yielding only two molecules of ATP for each glucose molecule, this process allows leukocytes to quickly meet metabolic demands required to carry out functions in an inflammatory state such as cell activation, signaling, and proliferation.

Notably, recent research has indicated that the classic Warburg effect is not universally present in myeloid cells exposed to pathogens and that depending on the stimulus and environment a much more complex metabolic rewiring takes place upon cell activation (33). Although the glycolytic rate appears to be uniformly enhanced after stimulation, oxidative phosphorylation is affected in a variable way and sometimes even enhanced (33). Monocytes rendered immunotolerant in vitro not only showed a strongly impaired capacity to produce proinflammatory cytokines upon restimulation but also produced less lactate compared with nontolerant cells, indicative of a defective ability to mount a Warburg effect (32). Immunotolerant monocytes also showed evidence of impaired oxidative phosphorylation and β-oxidation, indicating a failure of all major metabolic pathways, a condition referred to as immunometabolic paralysis. Similar metabolic anomalies were found in peripheral blood mononuclear cells harvested from patients with sepsis, which were restored after recovery (32). These defects were associated with a dysfunctional mTOR pathway (32).

Similarly, a separate study showed marked alterations in the mTOR pathway of T cells from septic patients leading to a catabolic state, with failure to induce glycolysis, oxidative phosphorylation, ATP production, GLUT1 expression, or glucose entry and ultimate impairment of cellular proliferation (36). In addition, metabolic switching of immune-tolerant cells from glucose to fatty acid oxidation in both human sepsis and a murine CLP model via NAD^+-dependent proteins sirtuin 1 (SIRT1) and SIRT6 has been observed (37).

Itaconate has been identified as a central regulatory node between innate immune tolerance and a phenomenon called trained immunity, which represents a functional reprogramming of innate immune cells evoked by stimulation leading to an increased response toward a second challenge (38). Activation of immune cells triggers itaconate synthesis through induction of the enzyme immune-responsive gene 1 (IRG1), which produces immune tolerance in monocytes at least in part via activation of the inflammation inhibitor NRF2. β-Glucan, a fungal cell wall component, can prevent immune tolerance and induce trained immunity by inhibiting the expression of IRG1 (thereby reducing itaconate production) and increasing the expression of succinate dehydrogenase, which contributes to the integrity of the tricarboxylic acid cycle, resulting in an enhanced rather than an attenuated innate immune response after secondary stimulation (38). Ex vivo β-glucan treatment of monocytes from healthy humans exposed to LPS in vivo reinstated their capacity for cytokine production, suggesting that induction of trained immunity can be a therapeutic target in sepsis-induced immunosuppression (39). The potential of this treatment was not confirmed in humans in vivo so far (40).

Collectively, these data indicate broad metabolic defects in immune cells of patients with sepsis-induced immunosuppression, representing an avenue that warrants further investigation to identify potential therapeutic targets.

Cellular Anergy

In response to PAMPs and DAMPs, leukocytes release cytokines or upregulate surface receptors for antigen presentation. Attenuation of this expected response during sepsis is known as anergy, endotoxin tolerance, or immune cell exhaustion (5, 8). An anergic state is characterized by changes in transcription dominated by decreased expression of genes encoding proinflammatory cytokines and chemokines.

Monocytes and macrophages have important roles in sepsis-induced immunosuppression. Endotoxin tolerance, or the diminished ability of cells to release proinflammatory cytokines to a secondary bacterial pathogen following an initial bacterial challenge, is a typical feature of how sepsis influences monocytes. It is postulated that this tolerance mechanism may protect against a lethal challenge of LPS and prevent infection and ischemia-reperfusion injury (41, 42). Monocytes of septic patients typically show a diminished ability to release proinflammatory cytokines such as TNF, IL-1α, IL-6, and IL-12 following a second challenge, whereas their ability to release anti-inflammatory mediators such as IL-1 receptor antagonist (IL-1RA) and IL-10 is either unimpaired or, in some cases, even enhanced (41, 42). Yet, it has also been observed that the in vivo anti-inflammatory response following repeated experimental human endotoxemia was attenuated following a second LPS challenge (43). Nevertheless, these observed shifts toward an anti-inflammatory phenotype after LPS exposure suggest a cellular reprogramming of monocytes under septic conditions (42). Interestingly, the anti-inflammatory phenotype observed in endotoxin tolerance has also been demonstrated in organ-specific monocytes, such as the lung in animal peritonitis and in postmortem samples from patients who died of sepsis (13, 44–46). It should be noted, however, that studies in mice have indicated that some cell types do not show this phenotype or even become primed, including alveolar macrophages, Kupffer cells, microglial cells, and

lymphocytes, in the intestinal epithelium and skin (47). Similarly, human alveolar macrophages were primed after in vivo exposure to LPS (48), contrasting with the immunotolerant phenotype of blood monocytes after intravenous LPS administration (32). An additional consequence of endotoxin tolerance on monocytes and macrophages is diminished expression of human leukocyte antigen–DR isotype (HLA-DR), a cell surface receptor used for antigen presentation. Low levels of circulating $CD14^+$ $HLA\text{-}DR^+$ monocytes are well established as a surrogate for sepsis-induced immunosuppression and correlate with impaired outcomes, including a higher incidence of nosocomial infections and increased mortality (49, 50).

In the adaptive immune response, $CD4^+$ T cells are the major effector cells and consist of four subpopulations: Th1, Th2, Th17, and Treg lymphocytes (5). Studies using both peripheral blood as well as postmortem spleen and lung tissue from deceased septic patients have shown evidence of impaired cytokine production by T cells (13, 51–53). The respective transcription factors for Th1 and Th2, T-bet and GATA3, have been shown to be significantly lower in nonsurvivors compared to healthy controls and septic survivors at various time points up to 28 days after diagnosis (54). In addition, Th17 cells play an important role in epithelial barrier infections as well as the clearance of extracellular fungal pathogens by chemotaxis and neutrophil activation (54, 55). In the aforementioned study (54), septic nonsurvivors were found to have the highest levels of Th17 transcription factor retinoic acid receptor–related orphan receptor-γt (ROR-γt) until day 7, after which these subsequently dropped to lower levels compared with septic survivors. This observation suggests a mechanism for increased susceptibility of patients with sepsis-induced immunosuppression to develop secondary fungal infections. Taken together, the decreased expression of these transcription factors provides evidence of an anergic state among effector T cells.

Exhaustion also manifests in $CD8^+$ T cell responses in the form of attenuated cell proliferation, impaired cytotoxic function, and attenuated IL-2 and IFN-γ production (5, 13, 56). Other pathologic T cell mechanisms include clonal deletion of pathogen-specific cells and increased expression of inhibitory receptors such as programmed cell death protein 1 (PD-1). Engagement of PD-1 with its ligands on exhausted T cells results in the release of immunosuppressive molecules and may culminate in apoptosis (57). An ex vivo study demonstrated that augmented peripheral blood T cell expression of PD-1 was associated with attenuated T cell proliferative capacity, increased incidence of nosocomial infections, and mortality in septic patients, lending further credence to a potential link between T cell exhaustion and impaired clinical outcomes (58). This concept is further reinforced by animal models of sepsis in which inhibition of PD-1 by antibodies or knockout mice improved survival (59–61).

Anti-Inflammatory State

The anti-inflammatory environment of sepsis is also a consequence of altered immune cell composition. For example, lymphopenia due to apoptotic loss is a common characteristic of septic patients (62). However, circulating Treg cell populations increase relative to other effector cells during sepsis, a trend that has been observed in the circulation as well as splenic tissue in animal and human studies (63, 64). The reasons for this shift in lymphocyte population toward Treg cells, which have broad regulatory and suppressive effects on other immune cells, may be explained by their resistance to apoptosis from increased expression of the antiapoptotic protein BCL-2 (5). In addition, the presence of DAMPs during sepsis, such as heat shock proteins, is known to induce Treg cells (5). An immunosuppressive lymphocytic phenotype predominates, characterized by increased expression of IL-10 by Treg and Th2 cells. The suppression of proinflammatory Th1 and monocyte responses further augments an anti-inflammatory environment, with decreased expression of proinflammatory cytokines such as TNF, IL-1β and IL-6 (65). Myeloid-derived suppressor

cells (MDSCs) represent a mixed population of predominantly immature myeloid cells that suppress effector immune cells, most notably T cells (66). MDSCs can inhibit immune functions through several mechanisms, including deprivation of L-arginine (essential for T cell functions), stimulation of expansion of Treg cells and suppression of macrophage and dendritic cell functions (66). Expansion of MDSCs is also associated with an increased risk of secondary infections in critically ill patients with sepsis (67, 68).

Neuro-Immune Interactions

It is now recognized the central nervous system (CNS) plays a role in regulating the immune response to inflammation and infection. Sepsis can be complicated by encephalopathy, which is associated with a disturbance of the physiological interactions between the brain and the immune system. Local neuroinflammation (caused by resident immune cells in the CNS such as microglia and astrocytes and infiltration of immune cells from outside the CNS) and ischemic injury (caused by impaired cerebrovascular autoregulation and a reduced cerebrovascular flow) have been implicated as the major pathophysiological mechanisms contributing to sepsis-associated encephalopathy (69). Brain dysfunction may cause a variety of immunosuppressive effects in the periphery, involving monocytes/macrophages (e.g., increased IL-10 production and M2 type polarization), dendritic cells (reduced responsiveness to TLR stimulation), neutrophils [impaired phagocytosis and reactive oxygen species (ROS) production], and T lymphocytes (an imbalance between Treg and proinflammatory lymphocyte subsets). Notably, most of these findings have been observed in animal studies with different types of brain injury (69). As such, the role of sepsis-associated encephalopathy in immunosuppression is an important topic for future investigations.

Under physiological conditions, the CNS can influence peripheral immune functions through the hypothalamic-pituitary-adrenal (HPA) axis and the neuro-immune regulatory reflex. Activation of the HPA axis is a vital regulatory mechanism to maintain homeostasis, and ample evidence indicates that disruption of this pathway sensitizes rodents and humans for sepsis, at least in part through the lack of anti-inflammatory effects mediated by endogenously released glucocorticoids (70). A role for HPA axis activation in sepsis-induced immunosuppression seems unlikely, however, because in many patients with sepsis this axis is disturbed, which includes a reduced release of glucocorticoids from the adrenals and resistance of target tissues to this hormone (70). The neuro-immune regulatory reflex consists of peripheral sensory input via the afferent vagus nerve to the CNS, followed by stimulation of the efferent vagus nerve and subsequent activation of the splenic nerve in the celiac plexus (6, 71). The presence of PAMPs and DAMPs causes release of cytokines by monocytes, macrophages, T cells, and other immune cells. These signals are then detected by vagus nerve afferent neurons and sensory neurons in the dorsal root ganglia, which are transmitted to the spinal cord and brain. Descending signals from the CNS via the efferent vagus nerve and sympathetic nervous system culminate in the release of acetylcholine and catecholamines (such as norepinephrine), respectively, in the spleen (72). The splenic nerve secretes norepinephrine, which stimulates the release of acetylcholine from choline acetyl transferase–positive $CD4^+$ T cells. The anti-inflammatory effect of acetylcholine on macrophages has been shown to be mediated by the α7 nicotinic acetylcholine receptor (72). Stimulation of cholinergic nuclei in the brain or the vagus nerve, or administration of α7 receptor agonists, exerted anti-inflammatory effects and was protective against lethality in animal models of sepsis (72). However, trials investigating transvenous nerve stimulation and an α7 nicotinic acetylcholine receptor agonist did not modulate the immune response during experimental human endotoxemia (73, 74). Nonetheless, an implantable vagus nerve–stimulating device in epilepsy patients inhibited LPS-induced production of TNF, IL-1β, and IL-6 by blood leukocytes, and vagal nerve stimulation in patients with rheumatoid arthritis

inhibited TNF production and reduced disease severity (75). The role of this neuro-immune regulatory reflex in sepsis-induced immunosuppression remains to be established. Similarly, enhanced activity of the sympathetic nervous system has been associated with profound immunosuppression after stroke, at least in part through stimulation of β-adrenergic receptors on immune cells (69). Stimulation of β-adrenergic receptors in healthy humans through infusion of epinephrine was associated with a marked anti-inflammatory effect during experimental endotoxemia, as reflected by inhibited TNF and enhanced IL-10 release (76). Nonetheless, the role of the sympathetic nervous system in patients with sepsis-induced immunosuppression warrants further investigation.

Therapies That May Negatively Influence Immune Function

Several interventions applied as part of the treatment of patients with sepsis may alter immune function, including vasopressors and steroids.

Norepinephrine is the first-line vasopressor administered as therapy for septic shock. However, its potential for causing profound anti-inflammatory effects was recognized after evidence from multiple in vitro studies (77, 78). Subsequent experiments using in vivo murine models of sepsis showed that norepinephrine infusion attenuated LPS-induced TNF-α and chemokine responses, whereas plasma concentrations of IL-10 were increased, suggesting a net anti-inflammatory effect (79). Further, norepinephrine was found to functionally impair the host immune response in mice subjected to CLP, as indicated by increased bacterial loads in multiple body sites as compared to vehicle-infused CLP mice (79). Low-dose norepinephrine infusion augmented the IL-10 response and diminished plasma CXCL10 levels compared to a placebo during experimental endotoxemia in healthy volunteers (79). Finally, in a cohort of patients with septic shock who received norepinephrine therapy, patients with pre-existing use of β-blocker therapy for cardiovascular reasons had significantly higher plasma TNF-α/IL-10 ratios (reflecting the proinflammatory/anti-inflammatory balance) than subjects who did not (79). These and other data provide evidence that the underlying mechanism of norepinephrine-induced inhibition of proinflammatory cytokine release involves activation of the β2-adrenoreceptor (77, 79). Phenylephrine, another vasoactive agent that is generally regarded as a selective α-adrenergic agonist, may also compromise immune defense mechanisms. Phenylephrine increased IL-10 and reduced proinflammatory cytokine production induced by LPS through an effect on β-adrenergic receptors, and phenylephrine infusion promoted bacterial growth in mice with abdominal sepsis (80).

Treatment of patients in refractory septic shock with steroids, such as hydrocortisone, has been the focus of research for decades, with the primary aim of treating possible adrenal insufficiency (10). Glucocorticoids bind to cytosolic glucocorticoid receptors, which are ligand-dependent transcription factors that regulate gene transcription through mechanisms involving DNA binding, protein–protein interactions with other transcription factors, or composite binding to DNA and protein substrates. While these genomic effects of glucocorticoids manifest after several hours, glucocorticoids also exert biological effects within seconds to minutes of exposure. For example, glucocorticoid intercalation into membranes has been described as a glucocorticoid receptor–independent mechanism for altering cation transport through plasma membranes and promoting proton leak from mitochondria (10). The ligand-bound glucocorticoid receptor has also been observed to translocate to mitochondria, leading to subsequent apoptosis (10). Glucocorticoids further potentiate an anti-inflammatory environment through various means (10). On one hand, they reduce leukocyte trafficking through the endothelium via glucocorticoid-mediated down-regulation of intracellular adhesion molecule 1 and vascular adhesion molecule 1, and they reduce populations of monocytes, macrophages, and dendritic cells. On the other hand, glucocorticoids increase the number of circulating neutrophils due to their release from bone marrow and

demargination; these neutrophils may be functionally less efficient due to decreased expression of L-selectin, a receptor important for the early attachment to vascular endothelium during initiation of recruitment into sites of inflammation (10). Glucocorticoids have also been shown to stabilize lysosomal membranes, leading to a reduction in the number of proteolytic enzymes released by lysosomes. Similarly, glucocorticoids repress cells of the adaptive immune system via apoptosis of thymocytes and T cells, population shifts from a Th1 to Th2 phenotype, and reduced B cell lymphocyte activation, proliferation, and production of immunoglobulins (10). In healthy humans injected with LPS, prednisolone dose-dependently attenuated neutrophil activation and reduced the circulating levels of proinflammatory cytokines, while enhancing the release of IL-10 (81). Collectively, these data clearly indicate that hydrocortisone treatment in patients with septic shock is expected to impair immune defense mechanisms.

BIOMARKERS FOR IDENTIFICATION OF SEPSIS-INDUCED IMMUNOSUPPRESSION

Various biomarkers to identify, and perhaps prognosticate, sepsis-induced immune dysfunction have been utilized based on our evolving knowledge of immune cell receptors, their ligands, and signaling pathways. An earlier study using splenic and lung tissue demonstrated that, compared to patients who die of nonsepsis etiologies, those who die in the ICU following sepsis have characteristics consistent with immunosuppression (13). Harvested immune cells were assessed for effector or suppressor populations, receptor expression (with differentiation between immune enhancement versus suppression versus antigen presentation), and cytokine secretion (13). Multiple inhibitory mechanisms such as a dominance of inhibitory overactivating receptors, expansion of suppressive cell types, and induction of inhibitory ligands on both antigen-presenting cells and tissue parenchymal cells were identified (13). Although differences existed between cells of the spleen and lung, they both shared common immunosuppressive mechanisms, including decreased HLA-DR and CD86 expression; this and other findings helped begin to address questions on immunologic changes in sepsis that extend beyond organ-specific pathology (13). Evidence from this study also further reinforced the concept that immunosuppression due to sepsis impacts both innate and adaptive immune pathways.

The use of biomarkers of immunosuppression in clinical practice requires relatively easy-to-perform and reproducible analyses in biosamples that can be readily obtained. The immunosuppressed patient can be identified by several potential approaches using blood samples. The first is a demonstrable reduction in ex vivo production of TNF from leukocytes stimulated with LPS, as measured by enzyme-linked immunosorbent assay (ELISA) or other types of immune assay (82). However, this approach is limited by a lack of standardization across investigators. For example, the volume of blood used, the strain of bacteria from which LPS originates, the method of LPS purification used, the incubation time, and the method of TNF measurement are all variables that must be held constant to define thresholds for diagnosis (83). Another approach utilizes flow cytometric analysis with fluorochrome-tagged antibodies to determine whether HLA-DR expression of $CD14^+$ blood monocytes is diminished beyond a threshold of <30% (84). This method was also plagued by a lack of standardization inherent in varying lots of antibodies and differences in cytometer calibration across centers. To address the lack of standardization, standard beads that express a known quantity of fluorescent antibodies on their surfaces were developed. This technology allows each investigator to calculate the number of HLA-DR antibodies bound per cell (AB/C) in a way that is reproducible across centers, with 10,000–15,000 AB/C suggested to represent moderate immunosuppression, and values <8,000 (85) and <5,000 AB/C proposed to define severe immunosuppression (86). Consensus now exists for considering low-monocyte HLA-DR

as a surrogate for sepsis-induced immunosuppression, and it has become the most-studied and validated biomarker in the field (87).

Alternative methods for identifying sepsis-induced immunosuppression are under study. One approach to examine HLA-DR expression involves measuring MHC II–related genes by quantitative reverse transcription polymerase chain reaction (PCR), which may be less vulnerable to analytical variation and can be incorporated in bedside tests (88). Other potential biomarkers may lie in the PD-1/PD-L1 pathway. The interaction between PD-1 and its ligands PD-L1 and PD-L2 generates inhibitory signals that mitigate T cell responses (89). Patients with sepsis demonstrated increased PD-1 expression on circulating monocytes, granulocytes, and lymphocytes proportional to disease severity (90). The aforementioned postmortem study of sepsis patients found increased PD-1 expression on T cells, together with increased PD-L1 and PD-L2 expression on dendritic cells (13). This study (13) and other investigations (58, 91) reported associations of increased PD-1 expression on T cells and PD-L1 expression of antigen-presenting cells with lymphopenia, T cell apoptosis, and mortality in patients with sepsis. A recent study reported two common phenotypes of sepsis survivors based on circulating C-reactive protein and soluble PD-L1 levels, one of which had persistent elevation of both biomarkers and a higher risk of readmission and mortality, particularly due to cardiovascular disease and cancer (92). This study proposed circulating soluble PD-L1 as a marker of immunosuppression, but this association has yet to be established using cellular and functional assays. More recently, increased expression of monocyte PD-L1 and $CD4^+$ lymphocyte PD-1 on circulating cells of patients with sepsis and septic shock has been explored in sepsis pathophysiology; these markers were associated with increased mortality and development of secondary infections (58, 93). Thus, patterns of PD-1 and/or PD-L1 expression may represent a promising avenue toward future use as prognostic biomarkers. As discussed previously, gene expression profiling of blood leukocytes has revealed molecular subtypes (SRS1 and Mars1) among patients with sepsis with a more immunosuppressed phenotype (17, 19); PCR-based molecular biomarkers could be derived from these signatures. Lymphopenia has been used as a marker of immunosuppression in patients with sepsis, and several studies reported an association between lymphopenia and poor outcomes in sepsis patients, including an increased risk of subsequent nosocomial infections and increased mortality (94, 95).

Sepsis biomarkers, including those of immunosuppression, have almost exclusively been evaluated in blood or blood cells. Such analyses are hampered by the fact that they do not necessarily provide insight into the immune state at the tissue level. Moreover, further studies are warranted to obtain insight into the dynamics of immune alterations relative to the inciting infection. Ideally, biomarkers should be measurable at the bedside and provide real-time information about the pathophysiological state and immune dysfunction in an individual patient. Biomarkers should also provide information about the function and/or activity of an immunological pathway targeted by a therapeutic intervention. If adequate, these biomarkers can be used for selection of patients for a certain therapy and to monitor the specific effect of that intervention on the immune system.

CONSEQUENCES AND COMPLICATIONS ASSOCIATED WITH SEPSIS-INDUCED IMMUNOSUPPRESSION

There is growing recognition of the potential for sepsis to exert consequential and long-term effects aside from the events surrounding the primary infection. These include increased susceptibility to secondary and opportunistic infections, as well as persistent immune disturbances that may culminate in low-grade inflammation and precipitate immunosenescence.

Many pathogens typically associated with secondary or nosocomial infections in the ICU are weakly virulent and/or opportunistic, including *Acinetobacter* spp., *Enterococcus* spp., *Pseudomonas*

Table 1 Consequences and complications of sepsis-induced immunosuppression[a]

Complication	Examples	Risk factors	Time course or outcome
Secondary infection			
Bacterial	*Acinetobacter* spp. *Enterococcus* spp. *Pseudomonas* spp. *Stenotrophomonas* spp.	Mechanical ventilation Antibiotic therapy	Days–weeks
Fungal	*Candida* spp. *Aspergillus* spp.	Central venous catheters Mechanical ventilation Immunocompromised	Days–weeks
Viral reactivation	Herpes viruses (CMV, EBV, HSV) Polyomaviruses (BK, JC) Anelloviruses (TTV, TTMDV, TTMV)	Seropositivity, glucocorticoid use Organ transplant recipient	Days–weeks
PICS			
Inflammation Immunosuppression Catabolism	High CRP, neutrophilia Lymphopenia Malnutrition, low protein state	Prolonged hospitalization Chronic critical illness Organ dysfunction	Exacerbation of chronic disease Poor wound healing Decreased functional status
Recurrent infection			
	Hospital readmission	Sepsis survivor	Weeks–months

[a]Low virulence bacterial organisms are frequent causes of nosocomial infections in the ICU, most commonly in the form of pneumonia associated with use of mechanical ventilation and in the setting of antibiotic therapy. Invasive fungal infections are typically caused by *Candida* species such as *C. albicans* and associated with presence of central venous catheters or (mucosal) barrier breakdown. Immunocompromised patients are at increased risk of invasive pulmonary aspergillosis. Viral reactivation of dormant viruses is another feature of sepsis-induced immunosuppression and thought to reflect an impaired immune surveillance as well as immune dysfunction. Patients with prolonged ICU stays are at risk for PICS, characterized by features of inflammation, immunosuppression, and catabolism. PICS predisposes to poor outcomes, including poor wound healing, functional decline, and worsening of chronic disease. Sepsis-induced immunosuppression predisposes to recurrent infection and hospital readmission due to persistent immune dysfunction.
Abbreviations: CMV, cytomegalovirus; CRP, C-reactive protein; EBV, Epstein-Barr virus; HSV, herpes simplex virus; ICU, intensive care unit; JC, John Cunningham; NA, not applicable; PICS, persistent inflammation, immunosuppression, and catabolism syndrome; TTMDV, torque tino midi viruses; TTMV, torque tino mini viruses; TTV, torque tino virus.

spp., and *Stenotrophomonas* spp. (96), and are thus informative of an impaired host response to critical illness and sepsis (97). Likewise, patients with sepsis are more vulnerable to developing systemic fungal infections, most prominently candidiasis (98), and they relatively often show evidence of reactivation of dormant viruses (**Table 1**). Among patients with septic shock, 68% had documented herpesvirus reactivations without prior immunodeficiency, with concurrent cytomegalovirus (CMV) and Epstein-Barr virus reactivations independently associated with mortality (99). Another study showed that 40% of septic patients had viremia with herpesviruses, polyomaviruses, and anelloviruses, with subjects positive for CMV at higher risk for fungal infection and death (100). CMV reactivation in sepsis has now been linked in multiple studies with critical illness especially due to sepsis, risk of poor outcomes, and mortality (101). The increased susceptibility of critically ill patients to secondary infections is not restricted to those with sepsis; patients admitted to the ICU with noninfectious conditions are also affected (102). Patients with a prolonged stay in the ICU frequently suffer from an illness that has been referred to as persistent inflammation, immunosuppression, and catabolism syndrome (PICS) (103). This syndrome is characterized by an overall disturbance of homeostasis, with features of both sustained inflammation and immunosuppression. This prolonged complex immune dysregulation, together with invasive supportive treatments such as mechanical ventilation and intravascular catheters, serve as risk factors for secondary infections by opportunistic pathogens in critically ill patients. Additional evidence that complex immune dysregulation rather than immunosuppression per se may

be associated with the occurrence of secondary ICU-acquired infections in patients with sepsis comes from a study showing that patients who later developed secondary infections had a more dysregulated host response during the first four days after ICU admission. This was reflected by enhanced inflammation and coagulation activation, stronger endothelial cell activation, and a more disturbed vascular integrity (104). In general, owing to the lack of data from controlled clinical trials, it is difficult to causally link specific immune aberrations, including immunosuppression, to specific clinical outcomes.

About 40% of sepsis patients are rehospitalized within 90 days of discharge, which may be for multiple reasons, including hastened progression of preexisting chronic conditions, remaining organ damage, and impaired immune function (105). Mechanisms that might contribute to long-lasting immune dysfunction include reprogramming of immune cells by epigenetic and metabolic alterations. In this context, considering the relatively rapid turnover of leukocytes, studies on the effect of sepsis on the bone marrow would be of interest. Indeed, knowledge on the extent and specifics of possible sustained immunosuppression after sepsis is limited, and small studies have suggested that the main cellular features of sepsis-induced immunosuppression as described above are barely detectable or undetectable at or after hospital discharge (106, 107).

POTENTIAL THERAPIES FOR SEPSIS-INDUCED IMMUNOSUPPRESSION

The aim of immune stimulatory therapy is to restore immune cell function in order to clear the infection that caused sepsis or to prevent or eradicate secondary infections, thereby potentially reducing late mortality in sepsis (5). Several immune-enhancing strategies have been evaluated in patients with sepsis, including cytokines, immune checkpoint inhibitors, and intravenous immunoglobulin (**Figure 2**).

Granulocyte-Macrophage Colony-Stimulating Factor

Granulocyte-macrophage colony-stimulating factor (GM-CSF) accelerates the production of neutrophils, monocytes, and macrophages and stimulates monocyte survival and functions (108). GM-CSF was tested as an adjunctive immune-enhancing therapy in several small trials in adult patients with sepsis. In a phase II study of sepsis patients, GM-CSF administration was associated with increased blood granulocyte superoxide production and improved blood and alveolar leukocyte phagocytic function without affecting clinical outcomes (109). In another trial, GM-CSF infusion resulted in the activation of circulating leukocytes, as indicated by increased CD11b expression on neutrophils and monocytes, and enhanced HLA-DR expression on monocytes. Infections also resolved more often in patients receiving GM-CSF (110). In a controlled trial in patients with abdominal sepsis, GM-CSF treatment reduced the number of infectious complications and the length of hospitalization (111). GM-CSF treatment of sepsis patients with reduced monocyte HLA-DR expression led to restoration of HLA-DR levels and reduced length of ICU stays (85). In children with sepsis, GM-CSF improved the capacity of blood leukocytes to release TNF upon stimulation and lessened the incidence of secondary infections (112). A meta-analysis of trials that tested the effect of GM-CSF in sepsis patients reported an increased resolution from infection, but no survival benefit (113). Of note, however, is that the studies used a variety of GM-CSF treatment regimens, and a large clinical trial is needed to obtain a definite answer regarding the effect of GM-CSF in treating sepsis. A large study evaluating the efficacy of GM-CSF to prevent secondary infections in patients with sepsis and reduced monocyte HLA-DR expression was announced several years ago (NCT02361528; **https://www.clinicaltrials.gov/**), but results have not been published up to now.

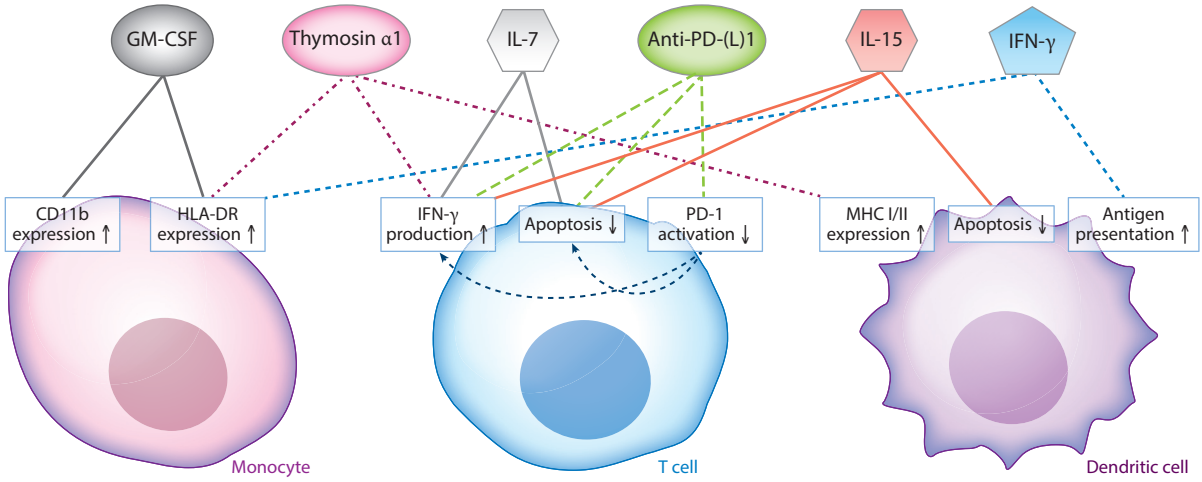

Figure 2

Immune stimulatory agents for the treatment of sepsis-induced immunosuppression. The aim of immune stimulatory therapy is to restore immune cell function to treat either the infection that caused sepsis or conditions that predisposed to a secondary infection, thereby potentially reducing late mortality in sepsis. Several immune-enhancing strategies have been evaluated in animal models or in patients with sepsis. Apart from restoring the suppressed expression of monocyte HLA-DR that most therapies show, other effects are observed. GM-CSF infusion also results in increased expression of CD11b and the ex vivo LPS-stimulated proinflammatory cytokine response. Thymosin α1 therapy increases production of IFN-γ from lymphocytes and enhances expression of MHC class I/II on dendritic cells. IFN-γ increases antigen presentation on dendritic cells, ultimately increasing their pathogen-killing abilities. IL-7 treatment has been shown to diminish T cell apoptosis and increase their IFN-γ production. Preclinical use of IL-15 therapy leads to a reduction in apoptosis of dendritic cells and T cells, while increasing T cell IFN-γ production. Ex vivo treatment of $CD8^+$ T cells from sepsis patients with the checkpoint inhibitor anti-PD-L1 shows diminished PD-1 activation and apoptosis, with improved IFN-γ production. Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; HLA-DR, human leukocyte antigen–DR isotype; MHC, major histocompatibility complex; IFN-γ, interferon gamma; IL, interleukin; LPS, lipopolysaccharide; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1.

Interferon-γ

IFN-γ mainly acts on antigen-presenting cells, increasing their antigen-presenting, phagocytosis, and killing capacities (114). Peripheral blood mononuclear cells from patients with sepsis showed a reduced capacity to release IFN-γ upon stimulation relative to controls (13). Administration of recombinant IFN-γ to humans increased HLA-DR expression on monocytes during experimental endotoxemia (115) and clinical sepsis (84, 116), and case series suggest this treatment can restore monocyte functions and improve outcomes in sepsis (84, 117, 118). In patients with fungal sepsis, subcutaneous treatment with recombinant IFN-γ partially restored the metabolic defects of immune tolerant peripheral blood mononuclear cells by promoting glycolysis (32). Recombinant IFN-γ was evaluated in a recently completed trial (PROMISE; NCT03332225) in which patients were allocated to this therapy based on low monocyte HLA-DR expression; the results of this study await publication.

Interleukin-7

IL-7 is crucial for the growth, differentiation, and effector functions of T cells, whereas it negatively regulates Treg cells. Results from preclinical studies have generated enthusiasm about the potential use of recombinant IL-7 as an adjunctive therapy in sepsis (119). In a murine two-hit model of sepsis, IL-7 administration diminished T cell apoptosis, enhanced IFN-γ production,

improved leukocyte migration to sites of infection, facilitated pathogen elimination, and increased survival (119). Ex vivo treatment with IL-7 of cells from patients with sepsis increased T lymphocyte functions such as proliferation and IFN-γ production (120) and restored immunometabolic failure in lymphocytes by amending changes in mTOR activation (36). In a recent prospective, randomized, double-blind, placebo-controlled trial involving 27 patients with septic shock and severe lymphopenia, recombinant human IL-7 induced a three- to fourfold increase in absolute lymphocyte counts and circulating $CD4^+$ and $CD8^+$ T cells that persisted for weeks; IL-7 treatment also increased T cell proliferation and activation (121). IL-7 treatment is generally well tolerated, as documented in a variety of patient groups. These results warrant further investigation of the effects of IL-7 treatment on clinical outcomes.

Interleukin-15

IL-15 treatment was associated with immune-enhancing effects in preclinical sepsis models of pneumonia and peritonitis as reflected by a reduction in apoptosis of NK cells, dendritic cells, $CD8^+$ T cells, and intestinal epithelial cells (122). In addition, IL-15 increased plasma IFN-γ levels and the percentage of NK cells that produced IFN-γ. These effects were accompanied by a reduced lethality in these models (122). Thus far, IL-15 has not been tested in patients with sepsis. IL-15 administration to cancer patients was associated with toxicity, including hypotension, thrombocytopenia, and liver injury, which may impede its use in sepsis patients (123).

Checkpoint Regulators

Checkpoint regulators are membrane-bound proteins important for maintaining a balanced immune response (124, 125). Following the presentation of an antigen by MHC class I/II receptors expressed by antigen-presenting cells, checkpoint regulators provide a second signal that instructs T cells how to respond to this antigen. Cell surface inhibitory immune checkpoints implicated in the pathogenesis of sepsis include PD-1, PD-L1, PD-L2, cytotoxic T lymphocyte antigen-4 (CTLA-4), B and T lymphocyte attenuator (BTLA), lymphocyte activation gene 3 (*LAG-3*), and T cell membrane protein-3 (TIM-3) (124). Sepsis is associated with increased expression of these inhibitory checkpoint regulators on several cell types, which may facilitate immunosuppression, and several therapeutics targeting checkpoint inhibitors improved host defense and survival in preclinical sepsis models (124, 125). The PD-1 receptor system represents a key checkpoint-regulating mechanism that has been tested as a potential therapeutic target in patients with sepsis. Ex vivo treatment of $CD8^+$ T cells from sepsis patients with an anti-PD-1 antibody showed diminished apoptosis and improved IFN-γ production (91). The interaction between PD-1 and PD-L1 may also abate myeloid cell function during sepsis, as indicated by increased PD-L1 expression on neutrophils and monocytes correlating with a decreased phagocytic capacity of these cells in sepsis patients (126). In agreement, ex vivo treatment with an anti-PD-1 antibody improved myeloid cell phagocytic function of blood leukocytes harvested from sepsis patients (126). Antibodies that inhibit the PD-1/PD-L1 pathway are used in cancer patients (127), and several phase I/II clinical trials of an anti-PD-L1 antibody in sepsis patients have been conducted. These show that this treatment is well tolerated and does not fuel systemic inflammation or cytokine release (128–130); absolute lymphocyte counts and monocyte HLA-DR expression levels increased during anti-PD-L1 therapy (128). At present, it remains to be established whether inhibition of the PD-1/PD-L1 pathway affects major clinical outcomes such as the occurrence of secondary infections and mortality in patients with sepsis. Unfortunately, plans to conduct a large phase III trial were prematurely terminated.

Thymosin α1

Thymosin α1 is an endogenous 28-amino acid peptide predominantly synthesized in the thymus. Synthetic thymosin α1 has been tested as an immune modulator in several research and clinical settings, including immune deficiencies and opportunistic infections caused by *Pseudomonas* and molds (131). Preclinical evidence indicates that thymosin α1 may assist in restoring immune responses in patients with sepsis (132). It can stimulate TLR2 and TLR9 on myeloid and dendritic cells, enhance the expression of MHC class I/II on dendritic cells, and induce the production of immune-stimulating cytokines like IL-2, IL-12, IFN-γ, and IFN-α. Furthermore, thymosin α1 can activate complement receptor–mediated phagocytosis, thereby assisting in pathogen elimination, and improve cytotoxic T cell and NK cell responses. In small trials of sepsis patients, thymosin α1 was reported to exert beneficial effects, both immunologically (reduced release of proinflammatory cytokines, and an increase in lymphocyte counts and monocyte HLA-DR expression) and clinically (decreased length of ICU stay and improved survival) (132). The largest trial published to date (ETASS; NCT00711620) was performed in China, involving 367 patients with sepsis (133). In this single-blind, randomized controlled trial, thymosin α1 increased monocyte HLA-DR expression and reduced 28-day mortality from 35% to 26%, an effect that almost reached statistical significance ($P = 0.06$) (133). A new randomized trial involving 1,106 sepsis patients is underway in China, with completion expected in 2021 (NCT02867267).

Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIg) is a preparation of pooled IgG that can opsonize and neutralize microorganisms and toxins. Potential beneficial effects of IVIg in sepsis include inhibition of apoptosis of immune cells and attenuation of inflammation (134). Low immunoglobulin levels have been associated with poorer outcomes in patients with sepsis, suggesting that replacement by IVIg may benefit this population (135). The effect of IVIg in sepsis has been evaluated in many randomized clinical trials (136). Meta-analyses have indicated that IVIg does not provide a survival benefit to patients with sepsis and, in the most recent Surviving Sepsis Guidelines, the use of IVIg is not recommended as a sepsis treatment (137). Two recent meta-analyses evaluated the effects of IgM-enriched IVIg in sepsis patients (138, 139), one of which indicated that these preparations may reduce mortality (139). At present, a large randomized controlled trial (PEPPER; NCT03334006) that examines the effect of IgM-enriched IVIg in sepsis patients is ongoing (140). It should be noted that the efficacy of IVIg in sepsis likely depends on infection characteristics and the causative pathogen. IVIg administration reduced mortality caused by streptococcal toxic shock syndrome in a meta-analysis of five trials (from 34% in the control group to 16% in the intervention group; $P = 0.01$) (141) and is now considered part of a standard of care in this severe infection. IVIg has also been recommended as an adjunctive therapy for source control in necrotizing soft tissues infections by *Streptococcus* group A (evidence level 2B) (142).

CONCLUSION AND FUTURE PERSPECTIVES

It is now recognized that sepsis may lead to profound and sustained immunosuppression. Many investigations have implicated immunosuppression as an important denominator of the increased susceptibility of patients with sepsis to secondary infections and late mortality. It should be noted, however, that all studies reported were observational in nature and, at present, definite proof for a causal relationship between immunosuppression and the occurrence of secondary infections in sepsis patients is lacking. This would require controlled clinical trials showing that immune-stimulating agents significantly reduce the number of secondary infections and, ideally, mortality.

Patients who require prolonged invasive and aggressive treatment in the ICU likely suffer from a profound dysregulation of immune, metabolic, and other regulatory systems that from an immunological perspective is associated with both exaggerated inflammation and immunosuppression depending on the cell type and organ site studied and that fails to return to physiological homeostasis. Despite the tremendous increase in our knowledge of immunosuppression in sepsis, it remains unclear which mechanism(s) initiate and drive the sustained deviation from homeostasis in sepsis and to what extent this influences clinical outcome. Likewise, it remains to be established whether the causes of the wide variety of hyperinflammatory and suppressive immune anomalies converge at the level of one or a limited number of elicitors. The challenge ahead lies not only in identifying targetable pathways of the immunopathology of sepsis that impact clinically meaningful outcomes but also in developing diagnostic tools that identify individual patients with characteristics most likely to benefit from an intervention based on a particular biological phenotype. This approach, which has been termed predictive enrichment of the treatment population, requires biomarker sets that can be measured in easily obtainable body fluids (e.g., blood and urine) and yet are indicative of pathophysiological events at a variety of body sites. Such biomarker sets should be easily and reproducibly measurable at or near the bedside, with little hands-on time and using fully automated equipment. With such tools, precision medicine—that is, treatment strategies taking individual patient characteristics into account—might increase the chances of demonstrating the beneficial therapeutic effects in subgroups of patients with sepsis.

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