Sepsis-induced immune dysfunction: can immune therapies reduce mortality?

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Sepsis is a systemic inflammatory response induced by an infection, leading to organ dysfunction and mortality. Historically, sepsis-induced organ dysfunction and lethality were attributed to the interplay between inflammatory and antiinflammatory responses. With advances in intensive care management and goal-directed interventions, early sepsis mortality has diminished, only to surge later after "recovery" from acute events, prompting a search for sepsis-induced alterations in immune function. Sepsis is well known to alter innate and adaptive immune responses for sustained periods after clinical "recovery," with immunosuppression being a prominent example of such alterations. Recent studies have centered on immune-modulatory therapy. These efforts are focused on defining and reversing the persistent immune cell dysfunction that is associated with mortality long after the acute events of sepsis have resolved.

Introduction

Sepsis is the constellation of symptoms occurring when an infection leads to a systemic inflammatory response (1), including fever, leukocytosis or leukopenia, decreased vascular resistance frequently leading to hypotension (septic shock), organ failure (severe sepsis), and death (2). Despite progress in antibiotic therapy, ventilator management, resuscitative strategies, and blood glucose maintenance, severe sepsis remains the leading cause of death in the intensive care unit (ICU) (3). Even more alarming is the escalating cost of sepsis-associated medical care, which is estimated at $17 billion annually in the United States (4). Given the expanding elderly population with immune senescence (5), overall sepsis mortality is expected to grow at an alarming rate during the next two decades (6).

Despite the more than 100 therapeutic clinical trials that have been conducted, no FDA-approved treatment options currently exist for sepsis. Even though substantial advances in our understanding of immune pathophysiology have resulted in improvements in survival, long-term sepsis mortality rates remain at 20% to 50% (7). Although there are sundry editorials, reviews, and commentaries opining a multitude of possible explanations, such as impaired cellular metabolism, tissue oxygenation, and myocardial dysfunction (8), one of the most accepted postulates describes a complex immune/inflammatory process that is still ill defined (8, 9). Historically, most research focused on ameliorating the hyperinflammatory response by attempting to block TNF and IL-1 (10); however, steady improvements in clinical treatment protocols (11) have resulted in more patients surviving the initial inflammatory and concomitant antiinflammatory response, only to manifest varying states of prolonged immune dysfunction, immune suppression (12), and persistent inflammation and catabolism (13), all of which are characterized by nosocomial infections, immune paralysis, and protracted events leading to death (14). Despite recent improvements in 30-day sepsis mortality rates, multiple reports confirm a dramatic and concerning increase in sepsis-associated mortality that escalates with time and particularly impacts the elderly population (7, 14). These data suggest that short-term gains in sepsis survival have been forfeited to escalating long-term sepsis mortality.

The sepsis death distribution has historically been biphasic, with an initial early peak at several days due to inadequate resuscitation, resulting in cardiac and pulmonary failure, and a late peak at several weeks due to persistent organ injury or failure (ref. 15 and Figure 1A). Considering the recent trend in mounting long-term mortality, a trimodal distribution is more indicative of the current death distribution (refs. 14, 15, and Figure 1B). The early peaks in mortality exist, albeit of much lower magnitude, and the third upswing occurs after 60 to 90 days and continues to soar over time (14, 16). These deaths are probably the consequence of more sophisticated ICU care that keeps elderly and comorbidly challenged patients alive longer in spite of ongoing immune, physiologic, and biochemical aberrations (17). Although the precise causes of long-term sepsis mortality are still uncertain, several reports suggest that advanced age, comorbidities, and persistent organ injury synergize to generate a deleterious state of chronic disease characterized by immune dysfunction, immune suppression, and persistent catabolism and inflammation (12, 13, 18). Furthermore, persistent inflammation, chronic immobility, catabolic drugs, and extended paralytics all contribute to a protracted state of immune dysregulation and chronic deterioration. Thus, investigators have been forced to refocus their efforts on the underlying innate and adaptive immune derangements that facilitate impaired sepsis recovery and survival, especially over the long term (19, 20). In this Review, we will highlight the multitude of sepsis-induced alterations in innate and adaptive immune cell function as well as the clinical implications and potential therapeutic interventions.

Immune cell dysfunction in sepsis

Sepsis affects the immune system by directly altering the lifespan, production, and function of the effector cells responsible for
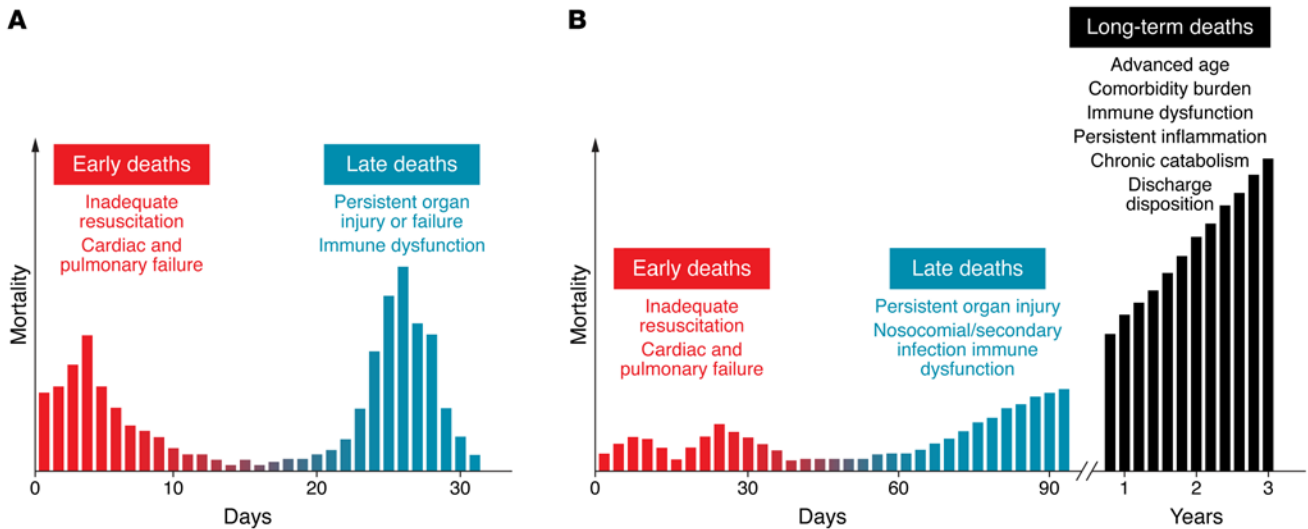

Figure 1. Historical and current sepsis mortality distribution. (**A**) Historically, sepsis deaths have occurred in a biphasic distribution, with an initial early peak at several days due to inadequate resuscitation, resulting in cardiac and pulmonary failure, and a late peak at several weeks due to persistent organ injury and failure. Considering the recent trend in sepsis outcomes, the elderly population, and mounting long-term mortality, a trimodal distribution may be more indicative of the current sepsis-associated death distribution. (**B**) The two early peaks in mortality exist, albeit with much lower magnitude than in the past. The third upswing occurs approximately 60 to 90 days after sepsis and continues to soar as time progresses. This delay in sepsis mortality is thought to be the consequence of the more sophisticated ICU care that keeps elderly and comorbidly challenged patients alive longer in spite of ongoing immune, physiologic, and biochemical aberrations.

homeostasis (21, 22). The hematopoietic and effector cells responsible for the maintenance of immune surveillance against offending pathogens are also involved in tissue regeneration and wound healing. Over the past two decades, a debate has persisted as to whether innate and adaptive immunity or inflammatory and antiinflammatory processes are more detrimental to sepsis survival (23). The inflammatory response was formerly thought to drive early mortality in the initial days of sepsis, and the compensatory antiinflammatory response was thought to induce mortality several days to weeks later through immune suppression and organ failure (24). However, new insights garnered using genomic analysis of tissue samples from septic patients (12) and severely injured trauma patients have identified an enduring inflammatory state driven by dysfunctional innate and suppressed adaptive immunity that culminates in persistent organ injury (25) and death of the patient (refs. 26, 27, and Figure 2). Although drawbacks in each of these studies exist, when the collective results are juxtaposed with contemporary patient outcomes, it is clear that a new paradigm is necessary to explain the long-term mortality surge following sepsis. It may be that inflammatory and antiinflammatory responses and innate and adaptive immune systems are each equally important and represent potential targets for immune therapy to improve sepsis outcomes (20–22, 28). The following discussion provides an overview of the sepsis-induced immune alterations in innate and adaptive cell types, along with the most promising immune modifiers being considered for future therapy in human sepsis. Although this discussion will focus on human sepsis, many of the current insights are gleaned from murine sepsis models. The authors recognize the ongoing debate about the efficacy of murine models to accurately reflect human disease processes (29). The authors' opinion is that both human and animal models are necessary for continued progress in the science of sepsis.

Neutrophils. Neutrophils are a fundamental component of innate immunity and essential for microbial containment and eradication and for sepsis survival (30). They comprise the majority of the cells in the BM and are the first responders to foreign invaders (31). Sepsis induces a state of delayed neutrophil apoptosis (32), leading to persistent neutrophil dysfunction, compounded by the release of immature neutrophils from BM that culminates in neutrophil deficits in oxidative burst (33), cell migration (34, 35), complement activation, and bacterial clearance (36), all of which contribute to ongoing immune dysfunction and inflammation persistence. These findings, coupled with insufficiencies in TLR signaling (37), chemokine-induced chemotaxis (38), altered apoptotic pathways, and neutrophil senescence (39), result in functional deficiencies that persist even after sepsis symptoms have disappeared. Accumulating evidence also indicates that neutrophils function as antigen-presenting cells (APCs) in a broad array of infections and control innate and adaptive responses through activation of CD4+ and CD8+ T cells (40, 41). More important, multiple studies in humans have implicated the complex array of neutrophil deficits in the development of nosocomial and secondary infections (42). Sepsis patients with the most pronounced derangements in neutrophil function are the most predisposed to develop ventilator-associated pneumonia and other nosocomial infections (43). The majority of patients who succumb to sepsis have ongoing infections (12), suggesting that defects in neutrophil-mediated bacterial clearance could serve as therapeutic targets to stimulate improved neutrophil production, maturation, and function.

Monocytes and macrophages. The impact of sepsis on monocyte subpopulations has been the subject of intense investigation over the past four decades. It has long been known that diminished mononuclear cell HLA-DR expression correlates with sepsis
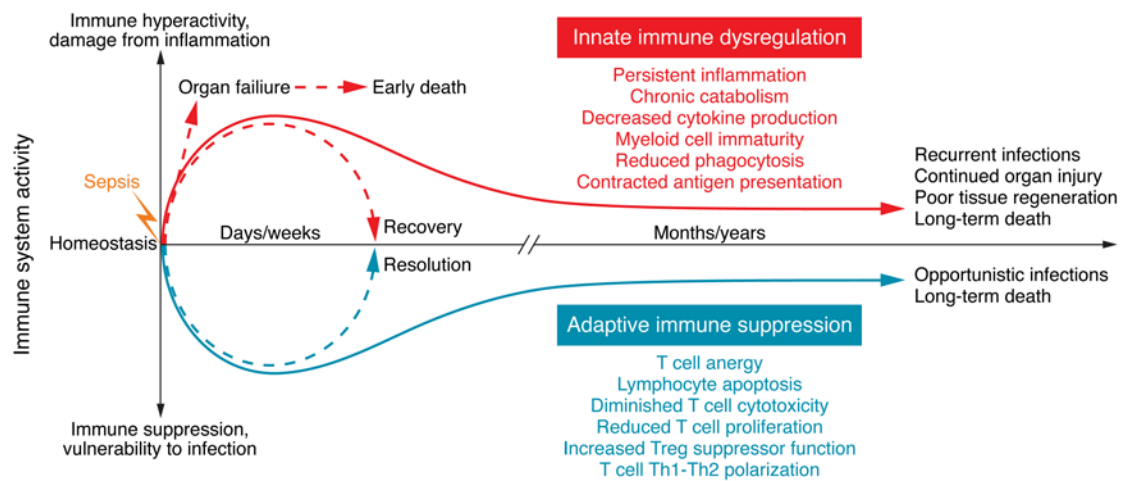
Figure 2. Immune dysregulation in sepsis. New insights into immune dysregulation have been gained using samples from deceased septic patients as well as from severely injured trauma patients. These studies demonstrate an enduring inflammatory state driven by dysfunctional innate and suppressed adaptive immunity that culminates in persistent organ injury and death of the patient. Although the initial inflammatory process, if unabated, contributes to organ failure and early mortality, this process is largely ameliorated by improvements in patient management protocols. However, considering that the vast majority of sepsis survivors are elderly with highly comorbid conditions, the short-term gains in survival have merely been pushed back by several months to a year. Although theories about the processes underlying this observation are numerous, the widespread consensus is that persistent derangements in innate and adaptive immune system cellular function are the main culprits driving long-term mortality.

lethality in humans (44). Furthermore, the diminished ability of blood monocytes from septic patients to release proinflammatory cytokines after endotoxin (LPS) challenge has been described as "endotoxin tolerance," which contributes to poor outcomes (45, 46). The major impact of endotoxin tolerance on monocytes and macrophages translates to decreased antigen presentation related to decreased HLA-DR expression (47). Moreover, blood monocytes from septic patients exhibit a diminished capacity to release the proinflammatory cytokines TNF, IL-1, IL-6, and IL-12 after LPS challenge, indicating that intracellular signaling has shifted toward production of the antiinflammatory mediators associated with nosocomial infections and increased mortality.

Low monocyte HLA-DR expression levels serve as a surrogate marker of monocyte "anergy," development of nosocomial infections, and death (48–50). Several studies correlate low monocyte HLA-DR expression levels with diminished monocyte function and reduced antigen-specific lymphocyte proliferation (51, 52), suggesting that monocyte anergy and immune suppression independently contribute to the increased risk of adverse events in sepsis. Although the mechanisms underpinning LPS tolerance are not fully understood, sepsis-induced monocyte epigenetic reprogramming may play a pivotal role in the suppressive monocyte phenotype (53). Monocyte mRNA analysis demonstrates an increased expression of inhibitory cytokine genes and reduced expression of proinflammatory chemokine genes (54). Recent reports of studies done in humans make a convincing case for epigenetic reprogramming as the epicenter of monocyte anergy, although the functional impact of these epigenetic alterations is unknown (55).

NK cells. NK cells were formerly viewed as rudimentary killers of cells that either lacked self-identification or were infected by viruses. However, we now understand that NK cells act as immune regulators. NK cells are divided into different subpopulations on the basis of CD16 and CD56 expression (56). Human sepsis data indicate that both CD56^{hi} and CD56^{lo} NK cell subpopulations are altered during

sepsis. These alterations appear to be associated with increased mortality in septic humans (57–59). Additionally, NK cell cytotoxic function is decreased (60). Similar to monocyte LPS tolerance, NK cell ex vivo production of IFN-γ in response to TLR agonists is diminished. This observation suggests that NK cell tolerance may be responsible for the reactivation of latent viruses such as CMV, which is frequently described in critically ill patient populations and may serve as a potential target for therapeutic intervention (61).

DCs. DCs are classified as either conventional DCs (cDCs) or plasmacytoid DCs (pDCs). The former are similar to monocytes and secrete IL-12, while the latter are similar to plasma cells and secrete IFN-α. cDCs and pDCs are of particular interest because of their enhanced apoptosis in sepsis patients (62) and in those who develop nosocomial infections (63). Similar to the reduction in HLA-DR expression observed on monocytes, DCs also show reduced HLA-DR expression and produce increased amounts of immune-suppressive IL-10 (64). Furthermore, coculture of DCs with T cells facilitates T cell anergy or Treg proliferation, both of which are associated with immune dysfunction. Multiple reports have shown that prevention of sepsis-induced DC apoptosis or augmentation of DC function enhances sepsis survival (65, 66). One report demonstrated that immune suppression can be ameliorated by DC treatment with growth factor FMS-like tyrosine kinase 3 ligand (FLT3L) (67). FLT3L treatment in models of burn sepsis enhances DC cytokine secretion (IL-12, IL-15, and IFN-γ) and augments CD4$^+$ T cell, NK cell, and neutrophil function (67). Further investigations suggest that the improvements in sepsis survival gained from DC therapy occur through TLR signaling and increased expression of MHC class II antigens and the costimulatory molecules CD80 and CD86 (68). These observations have led researchers to postulate that DC numbers and functional improvements may be primary targets for therapeutic interventions in sepsis (65, 66).

Myeloid-derived suppressor cells, stem cells, and myelopoiesis. Myeloid-derived suppressor cells (MDSCs) are a population

of immature myeloid cells that expand dramatically in sepsis, suppress adaptive immune responses, and signal through TLR-mediated pathways (40, 69). Sepsis-associated MDSCs are phenotypically similar to the MDSCs described in advanced cancer (40, 70). Although MDSCs can inhibit CD8+ T cell function, the actual impact of MDSCs in human sepsis is unknown. The collective reports suggest a beneficial role of MDSCs centered on replenishing innate cell function and immune surveillance through emergency granulopoiesis (35). We found that, before MDSC expansion occurs, there is a window of susceptibility to the secondary infections and subsequent mortality that are associated with reduced BM cell numbers and reduced blood and tissue neutrophil numbers and function (33). Optimal MDSC expansion through enhanced granulopoiesis confers long-term immunity to secondary infections in sepsis (71). Because of the inherent difficulty of immune phenotyping immature myeloid cells in humans versus the relative ease of doing so in mice, few clinical studies have investigated the roles of MDSCs in septic patients (72). Nonetheless, there is considerable interest in myelopoiesis, MDSC expansion, and hematopoietic cell function (33, 40, 71, 73, 74). Given the importance of an efficient regeneration of functioning neutrophils, monocytes, and DCs, it is no surprise that MDSCs expand to meet the continual need for functional innate immune cells. And given that it takes the BM five to seven days to produce a mature neutrophil, an expansive pool (~18 × 10^{11}) of immature myeloid precursors in BM and secondary lymphoid organs (75) is a necessity. In humans, approximately 16 × 10^{10} neutrophils are produced daily, and this cell population can be rapidly increased by 5- to 10-fold in response to infection. Our work has shown that myeloid expansion involving hematopoietic stem cells (HSCs) occurs through c-KIT–, type I IFN– (IFN-I), and CXCL10-dependent mechanisms that involve IFN-I–secreting B cells (73, 74). Moreover, impaired HSC proliferation and development in human BM transplant models is associated with mortality due to secondary infections (76). Humans with suppressed granulopoiesis clearly experience infections, demonstrating the essential requirement for effective neutrophil production. Conversely, overzealous MDSC proliferation may facilitate a physiologic syndrome of persistent inflammation, such as that seen in adult respiratory distress syndrome (ARDS) or persistent inflammation, immunosuppression, and catabolism syndrome (PICS), causing poor outcomes in patients with sepsis (13). The specific contributions of myelopoiesis and MDSCs to sepsis recovery versus those of persistent inflammation and catabolism remain poorly understood.

γδ T cells. γδ T cells comprise a small subset of T cells that possess a distinct T cell receptor (TCR) on their cell surface. Most T cells have a TCR composed of two α and β glycoprotein chains, while γδ T cells have a TCR that is made up of one γ chain and one δ chain. This group of T cells exists chiefly in the gut mucosa within a population of intraepithelial lymphocytes (77). Although the antigens to which γδ T cells respond are unknown, it is suspected that these cells recognize lipid antigens from pathogens present on mucosal surfaces within the intestine (78). Upon activation, γδ T cells release IFN-γ, IL-17, and other chemokines. In septic patients, the number of circulating γδ T cells is significantly reduced, and the reductions correlate with the highest rates of mortality (79).

The reduction in γδ T cells in the intestinal mucosa may serve to potentiate otherwise noninvasive intestinal bacteria to become invasive and translocate into the systemic circulation, causing infections following sepsis (80).

CD4+ T cells and Th cell subpopulations. Th cells assist other cells with immunologic processes, including B cell differentiation, cytotoxic T cell activation, and monocyte stimulation. When presented with peptide antigens by MHC class II molecules expressed on APCs, CD4+ T cells become activated, rapidly divide, and secrete cytokines that regulate immune responses. Once activated, CD4+ T cells can differentiate into one of several subsets including Th1, Th2, Th3, Th17, Th22, Th9, or T follicular helper (Tfh) cells, which facilitate differing immune responses through cytokine production (81). Although numerous reports detail the effects of sepsis on circulating and peripheral CD4+ T cell subsets (82), we will highlight only selected reports to convey to the reader the common themes and potential areas of therapeutic interest.

One of the most notable T cell defects induced by sepsis is the development of apoptosis, which decimates CD4+ cell populations (12, 83). In humans who succumbed to sepsis, there was a much greater magnitude of lymphocyte (specifically CD4+) apoptosis than was found in cells from sepsis survivors (12). Of the CD4+ cells that persist, multiple reports demonstrate that both Th1- and Th2-associated cytokine production is diminished during sepsis (84). Marked reductions in the transcription factors T-bet and GATA3, which modulate the Th1 and Th2 responses, respectively, support the notion that CD4+ subsets are suppressed during sepsis (85). There are many factors that regulate CD4+ Th cell subpopulation differentiation, including histone methylation and chromatin remodeling, which, together, are postulated to suppress Th1 and Th2 CD4+ T cell functions (86). However, the sepsis-induced immune impact is not only relegated to Th1 and Th2 CD4+ T cells, but also to Th17 cell subsets and probably other Th cell subsets as well. The Th17 cytokine response is diminished in sepsis and may negatively impact mortality (87). Given the fundamental role of Th17 in the eradication of fungal infections, reduced Th17 cytokine production in sepsis may be responsible for the increased susceptibility to fungal infections frequently encountered in critically ill patient populations (88). Moreover, IL-7 treatment has been demonstrated to increase Th17 cell responsiveness and reduce mortality from secondary fungal infections, making IL-17 a potential therapeutic agent (89).

Tregs. Tregs are a component of adaptive immunity that suppresses responses of other effector T cell subsets, helping to maintain tolerance to self-antigens and suppress autoimmune disease (90). In states of sepsis and critical illness, Tregs may potentiate undesirable effector T cell (Teff) suppression that prolongs recovery. Increased Treg ratios have been described early after sepsis and remained elevated in those patients who died. Further studies revealed that the increase in Tregs was due to the loss of effector Th cells rather than an absolute increase in Treg numbers (91). This observation suggests that Tregs are more resistant to sepsis-induced apoptosis, thereby preventing the recovering immune system from mounting excessive autoimmune responses. Moreover, heat shock proteins and histones that induce mononuclear cell epigenetic changes have also been implicated as inducers of Tregs in sepsis (92). Recent mouse studies demonstrated that

Tregs are detrimental to Teff proliferation and function (93). This effect was mitigated by siRNAs that inhibited Treg differentiation (91). In other reports, glucocorticoid-induced TNF receptor–related protein (GITR) inhibitory Abs were used to block Treg function, resulting in improved immune function and microbial killing (94). Treg-associated immune dysfunction in sepsis has also been linked to increased solid tumor growth, probably from diminished cytotoxic T lymphocyte (CTL) and mononuclear cell effects (95). Therefore, the notion exists that Tregs persist in sepsis, augment immune dysregulation, contribute to poor outcomes, and may serve as targets for immune modulation.

B cells. B cells represent a heterogeneous cell population with varying functional and phenotypical properties. Until recently, the function of B cells in sepsis was relegated to the production of Abs and the development of memory plasma B cells (96). However, many recent reports demonstrated that B cells play a more important role in sepsis than previously thought. Although patients with septic shock demonstrate diminished overall B cell numbers, the largest deficits in B cell number is in CD5^{+} B1a-type cells, which can be predictive of survivors and nonsurvivors (97). In murine sepsis models, B cells are needed to improve cytokine production, reduce bacteremia, and improve outcomes through IFN-I (98). Others have recently described an innate response activator (IRA) B cell population that is phenotypically and functionally distinct from B1a cells, depends on pattern recognition receptors (PRRs), and produces granulocyte-macrophage CSF (GM-CSF). Deletion of IRA B cells impairs bacterial clearance, causes a cytokine storm, and precipitates septic shock. These observations position IRA B cells as gatekeepers of bacterial infection and identify new treatment targets for patients with sepsis (99). Additionally, IRA B cell production of IL-3 has been shown to potentiate inflammation in sepsis, induce myelopoiesis of Ly-6C^{hi} mononuclear cells, and augment the cytokine storm, while high plasma IL-3 levels are independently associated with increased mortality in patients with sepsis (100). Taken together, these reports not only further our understanding of the role of B cells in immune activation and emergency myelopoiesis, but also identify IL-3 as a potential therapeutic target in sepsis (101).

Potential immune-modulatory therapies

Granulocyte CSF and GM-CSF in sepsis. Granulocyte CSF (G-CSF) is a glycoprotein that stimulates the production of stem cells and granulocytes (102). G-CSF is also highly efficacious in reducing the incidence of sepsis in patients with low absolute neutrophil counts, such as those undergoing BM transplantation, chemotherapy, or radiation (103). Two randomized clinical trials involving recombinant G-CSF have been conducted in an attempt to increase neutrophil production and function. Investigators hypothesized that administration of G-CSF would improve neutrophil function and microbial eradication in such circumstances. While there was an increase in total leukocyte numbers in blood, there was no improvement in 28-day mortality rates (104, 105). Although the initial conclusion of these two clinical studies was discouraging, the fact remains that most of the sepsis-induced mortality occurs in a protracted process beyond 90 days (14). The impact of G-CSF on mortality beyond 28 days is unknown. Given that persistent derangements in myelopoiesis, granulopoiesis, and

neutrophil function are hallmarks of long-term sepsis mortality, especially in diabetic and elderly populations, prolonged G-CSF administration might prove efficacious for infection eradication and survival in future clinical trials.

GM-CSF is a cytokine that stimulates stem cells to produce neutrophils, monocytes, and macrophages (106). In the immunosuppressive phase of sepsis, patients who were ventilator dependent were treated with recombinant GM-CSF and had fewer ventilator and ICU days (107, 108). Recombinant GM-CSF therapy in immunosuppressed pediatric patients with sepsis restored TNF production in lymphocytes and reduced nosocomial infections (109). Moreover, a meta-analysis of more than 12 clinical studies involving either G-CSF or GM-CSF demonstrated that either therapy significantly reduced the rate of infection (110). Considering that 70%–80% of patients who die from sepsis harbored persistent infections (12), GM-CSF and/or G-CSF, in combination with other immune-modulatory agents, may prove valuable for infection eradication during sepsis and improved long-term survival after sepsis.

IFN-γ. IFN-γ is the only member of the type II IFN family and is crucial for immune function against viral, bacterial, and protozoal infections. Moreover, IFN-γ is a key activator of macrophages, inducing class I MHC expression (47). When treated with recombinant IFN-γ, patients with sepsis and decreased monocyte HLA-DR expression showed reversal of monocyte dysfunction and improved sepsis survival (111). Although the majority of the interventional studies with IFN-γ were done in burn and severely injured trauma cohorts, the largest of these trials reported a decrease in the number of infection-related deaths among patients treated with IFN-γ (112). Furthermore, a recent study of severely injured trauma patients revealed that 42 of 63 genes identified as being differentially expressed in uncomplicated and complicated trauma patients were specifically associated with IFN signaling. The authors found that IFN-associated genes were suppressed in trauma patients with complicated outcomes (26, 27), implying that this set of genes may be useful for identifying patients at risk for complications after trauma and that these patients might respond positively to therapies utilizing IL-7, IL-15, IFN-γ, and GITR agonists. IFN-γ may be more effective in sepsis populations if targeted to patients who demonstrate or are at risk for immune suppression, decreased monocyte HLA-DR expression, adaptive immune dysfunction, or chronic inflammation during prolonged hospital stays. Though IFN-γ offers promise as a potential immune therapy due to its ability to rejuvenate monocyte function and adaptive immunity, IFN-γ may be more efficacious if administered in a time-phased approach in conjunction with GM-CSF and G-CSF, or even IL-7 and/or IL-15, in order to bolster specific immune function, reduce secondary and nosocomial infections, and improve long-term survival as sepsis recovery evolves.

Programmed cell death protein 1 and ligand. As a result of steady progress in tumor biology, a new class of drugs has been developed that inhibit programmed cell death protein 1 (PD-1), a protein that under normal circumstances sends an inhibitory signal that reduces CD8^{+} T cell proliferation and accumulation in lymph nodes. PD-1 is expressed on T and B lymphocytes and myeloid cells. The PD-1 ligand PD-L1 is expressed on epithelial and endothelial cells, monocytes, macrophages, and DCs (113). Given that PD-1 is upregulated

Table 1. Immune modulators and proposed benefits for sepsis therapy

Immune modulator	G-CSF	GM-CSF	IFN-γ	PD-1 and PD-L1	IL-3	IL-7	IL-15
Proposed benefit	Improves neutrophil and monocyte production and release	Improves neutrophil and monocyte production and function	Improves monocyte HLA-DR expression and function	Biomarkers to identify candidates for immune-modulatory therapy	Promotes stem cell and progenitor development	Increases T cell proliferation and recruitment	Decreases NK cell, T cell, and NKT cell apoptosis
	Improves myelopoiesis and granulopoiesis	Enhances monocytic and lymphocytic cytotoxicity	Reduces infection and related complications	Reverse T cell exhaustion	Enhances lymphopoiesis in combination with IL-7	Decreases lymphocyte apoptosis	Increases NK cell, T cell, and NKT cell proliferation and activation
		Augments T cell responses	Improves immunity against fungal infections	Promote lymphocyte proliferation		Increases T cell IFN-γ secretion	
		Reduces nosocomial infection acquisition		Augment neutrophilic and monocytic cytotoxicity		Improves T cell homing and pathogen clearance	
		Reduces ventilator days		Reduce opportunistic infections		Increases T cell receptor diversity	

on CD4+ and CD8+ T cells in states of viral infection and cancer, it is often associated with the phenomenon of "T cell exhaustion," which is thought to result from prolonged periods of antigen exposure (114). Both anti–PD-1 and anti–PD-L1 treatments have shown great promise in trials against cancer and viral infection. Hence, it has been postulated that anti–PD-1 and anti–PD-L1 therapies could have similar beneficial effects in patients suffering from sepsis-induced immune dysfunction (115). Patients with severe sepsis show increased levels of PD-1 and PD-L1 on their monocyte and T lymphocyte populations (116). Recent studies demonstrated neutrophil PD-L1 upregulation on neutrophils from septic mice and humans, resulting in the potentiation of lymphocyte apoptosis through contact inhibition, which correlated with outcome (117). Moreover, in clinically relevant animal models of bacterial sepsis, inhibition of PD-1 and PD-L1 signaling improved survival and reduced the incidence of fungal infections (118). Considering the beneficial impact on adaptive immunity and tumor eradication strategies, it makes sense that PD-1 and PD-1L could concomitantly serve as biomarkers of sepsis-initiated immune suppression as well as prospective therapeutic targets to reverse adaptive immune dysfunction and improve long-term survival.

Recombinant human IL-3, IL-7, and IL-15. Bearing in mind the significant loss of lymphocytes in severe sepsis, several investigators have postulated that IL-7 administration is a potential therapeutic strategy. IL-7 is a hematopoietic cytokine produced by stromal cells and is required for B and T cell production, development, homeostasis, and maintenance (119). IL-7 is an attractive molecule due to its ability to upregulate expression of the antiapoptotic protein BCL2, which causes increased numbers of blood CD4+ and CD8+ lymphocytes. Moreover, IL-7 enhances TCR diversity, which is lost in septic patients and is associated with increased nosocomial infections and mortality (89, 120). Low doses of recombinant human IL-7 preferentially activate Teffs from patients with sepsis (121). Although evidence exists that IL-7 can increase PD-1 expression in lymphocytes, which is a hallmark of lymphocyte exhaustion, cytokine-driven peripheral T cell expansion and survival remain

intact (122). However, when IL-7 was administered to patients with HIV, lymphocyte expression of PD-1 was decreased (123). Although IL-7 has the potential to work synergistically with therapies targeting PD-1 or PD-L1 to improve the functional aspects or T cell functions that are presumed to be lost in sepsis, the impact in human sepsis is still unclear. Although clinical trials support the use of IL-7 in HIV-infected patients receiving antiretroviral therapy, no sepsis trials exist to date. Given the promise that IL-7 has shown in other disease states of lymphopenia and immune suppression, sepsis trials with IL-7 alone or in combination with other immune modulators such as anti–PD-1 should be seriously considered.

Although still in preclinical studies, it is worth mentioning IL-3 and IL-15 as potential sepsis therapies. Considering the integral role of IL-15 in the development and activation of effector and memory T, NK, and NKT cells and neutrophils, it has become a promising candidate for immune therapy (124). In mouse models of sepsis, treatment with IL-15 diminished immune dysfunction and improved survival (125). The synergistic role that IL-3 plays in HSC and progenitor development along with IL-7 makes IL-3 an appealing therapy to augment the potential impact of IL-7 (100, 101). However, there is a paucity of data relating to the effects of either IL-3 or IL-15 in states of sepsis, and further discussion is speculative.

Immune-modulatory intervention. The sepsis landscape is riddled with failed single-agent therapeutic interventions to block particular pathways or processes in humans. Although there are as many explanations as failures, continued attempts to augment immune processes with single agents at early time points, with the expectation that sepsis mortality will be halved at 6 or 12 months, are doomed to fail. However, if the sepsis-induced immune derangements are juxtaposed with other immune-mediated disease processes, such as cancer, autoimmune disease, or HIV, in which immune-modulatory therapy has improved patient outcomes, it is clear that single-agent therapy is not ideal. Rather, it is preferable to use a combination of multiple agents that are introduced and altered over time, according to disease-specific

progression, patient immune responses, and defined host/pathogen genomic interactions (126). Cancer chemotherapy strategies use a personalized combination of agents to induce, maintain, and prolong cancer remission on the basis of patient disease progression and tumor-specific genetic patterns. We believe that the same strategy could be applied to sepsis therapy with a high probability of success if the interventions are tailored to specific host/pathogen genomic patterns and immune perturbations that occur in the elderly and in patients with comorbidities as post-sepsis recovery evolves (18). Given the ease of modern genomic determination, screening patients for specific genetic variations that influence microbial eradication could be used to develop a personalized treatment plan. For example, GM-CSF, in combination with IL-3, may bolster monocyte and neutrophil production and function early after sepsis when the mature pool of these cells is depleted. Next, a combination of anti–PD-1 or anti–PD-L1, coupled with IFN-γ, may prove beneficial for lymphocyte activation and augmentation of innate immune surveillance to prevent secondary and nosocomial infections (Table 1). Last, a low-dose combination of oxandrolone, testosterone, and propranolol, or even dronabinol, may ameliorate protein catabolism and persistent inflammation and promote anabolism, which has already been implemented to promote recovery in severely burned patients. Moreover, with improvements in biomarkers, cellular function determination, and host/pathogen genomic prediction models, strategically engineered combinations of immune modulators could be used in a goal-directed manner on the basis of the patient's comorbidity profile, immune function, and recovery course. For example, poorly maintained type 2 diabetic patients recovering from sepsis are predisposed to develop secondary and nosocomial infections associated with poor neutrophil and lymphocyte function. This group of patients may benefit from the concomitant administration of G-CSF, GM-CSF, and anti–PD-1 or anti–PD-1L early in the sepsis recovery phase to prevent ongoing infection and subsequent mortality, followed by IFN-γ therapy to facilitate an infection-free period and promote more durable recovery. Using this strategy in sepsis would allow for tailored and monitored interventions that vary over time with specific patient populations, minimizing the human physiologic heterogeneity that has plagued previous sepsis clinical trials.

Conclusions

Sepsis induces a multitude of defects in immunity that cause protracted inflammation, immune suppression, susceptibility to infections, and death. Although there are new cell-based methodologies available to identify patients with post-sepsis immune dysregulation, it is still unclear which interventions targeting cell-specific deficits will be most beneficial. Considering the interrelated and interdigitating complexity of immune derangements as well as the protracted and convoluted road to mortality, we believe that single-agent immune-modulatory intervention, as attempted in past sepsis trials, will probably fail. Conversely, the notion of more thorough and rigorous patient selection, coupled with frequent monitoring of immune function and goal-directed immune-modulatory therapy involving multiple agents, may, over time, provide optimal clinical benefit.

Acknowledgments

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