

The immune system's role in sepsis progression, resolution, and long-term outcome

Matthew J. Delano¹ | Peter A. Ward²

¹Department of Surgery, Division of Acute Care Surgery, University of Michigan, Ann Arbor, MI, USA

²Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, USA

Correspondence

Peter A. Ward, Godfrey D. Stobbe Professor of Pathology, University of Michigan Medical School, Department of Pathology, Ann Arbor, MI, USA.

Email: pward@umich.edu

Funding information

National Institutes of Health, Grant/Award Number: GM-29507 and GM-61656; Godfrey D. Stobbe Endowment

Summary

Sepsis occurs when an infection exceeds local tissue containment and induces a series of dysregulated physiologic responses that result in organ dysfunction. A subset of patients with sepsis progress to septic shock, defined by profound circulatory, cellular, and metabolic abnormalities, and associated with a greater mortality. Historically, sepsis-induced organ dysfunction and lethality were attributed to the complex interplay between the initial inflammatory and later anti-inflammatory responses. With advances in intensive care medicine and goal-directed interventions, early 30-day sepsis mortality has diminished, only to steadily escalate long after “recovery” from acute events. As so many sepsis survivors succumb later to persistent, recurrent, nosocomial, and secondary infections, many investigators have turned their attention to the long-term sepsis-induced alterations in cellular immune function. Sepsis clearly alters the innate and adaptive immune responses for sustained periods of time after clinical recovery, with immune suppression, chronic inflammation, and persistence of bacterial representing such alterations. Understanding that sepsis-associated immune cell defects correlate with long-term mortality, more investigations have centered on the potential for immune modulatory therapy to improve long-term patient outcomes. These efforts are focused on more clearly defining and effectively reversing the persistent immune cell dysfunction associated with long-term sepsis mortality.

KEYWORDS

adaptive immune dysfunction, immune suppression sepsis, inflammation, innate immune dysfunction, sepsis

1 | INTRODUCTION

Until recently, sepsis was defined as the constellation of symptoms occurring when a bacterial, viral, or fungal infection leads to a systemic inflammatory response syndrome (SIRS), including fever, leukocytosis, or leukopenia, and decreased vascular resistance frequently leading to hypotension (septic shock), organ failure (severe sepsis), and death^{1,2} (Figure 1). However, vagueness in definitions and ineffective clinical strategies has led to discrepancies in the incidence of sepsis and the

observed mortality.³ In response, the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) were developed and address the limitations of previous definitions that were overfocused on SIRS and inflammation.⁴ In addition, the Consensus also dispelled the long-standing notion that SIRS criteria possess adequate specificity and sensitivity to define and diagnose sepsis. Lastly, the report debunked the misleading model that sepsis always follows a linear continuum from the SIRS through severe sepsis and septic shock, and declared the term “severe sepsis” redundant and unnecessary. Instead, the Consensus report recommends that sepsis be defined as a life-threatening organ dysfunction caused by a dysregulated host response to an infection (Figure 2). Organ dysfunction is now defined by an increase in the Sequential Organ

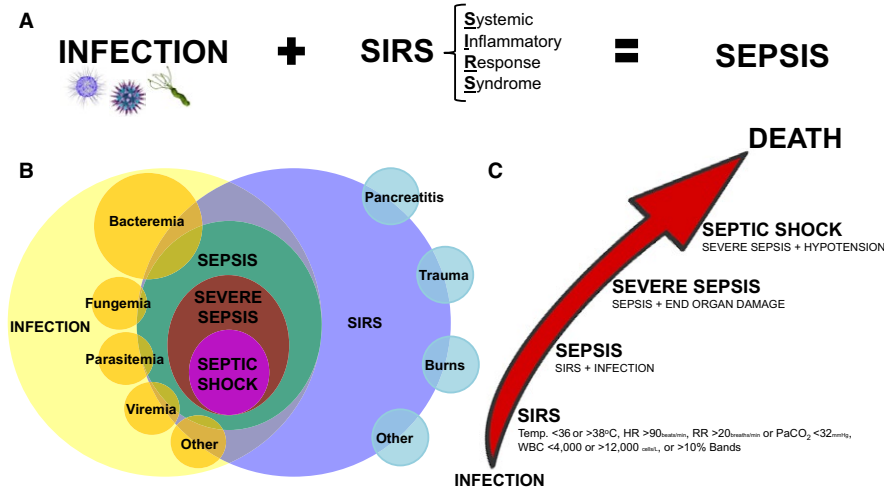


FIGURE 1 Earlier conceptual view and definition of systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic shock. (A) The concept of an infection exceeding local-regional control and inducing an inflammatory SIRS response has been the fundamental premise conceptualizing sepsis for over two decades. (B) Until recently, sepsis was defined as the constellation of symptoms occurring when a bacterial, viral, or fungal infection leads to a systemic inflammatory response syndrome, including fever, leukocytosis or leukopenia, and decreased vascular resistance frequently leading to hypotension (septic shock), organ failure (severe sepsis), and death. However, confounding the prior definition of sepsis is that other states of inflammation such as pancreatitis, trauma, and burns can also produce a SIRS response making the definition overly nebulous and misapplied in many instances. (C) In addition to the conceptual vagueness, the prior sepsis definition also implied that SIRS criteria possess adequate specificity and sensitivity to define and diagnose sepsis which is not always the case. Moreover, the prior sepsis model inferred that sepsis always follows a linear trajectory from SIRS through severe sepsis and septic shock, which is often times does not occur. Adapted from Bone RC et al.: *Chest*. 1992;101:1644-55

Failure Assessment (SOFA) score of 2 points or more, which is associated with an in-hospital mortality greater than 10% (Figure 3). Furthermore, septic shock is now defined as a subset of sepsis in which profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone. Clinically, patients with septic shock can be identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and serum lactate level greater than 2 mmol/L (>18 mg/dL) in the absence of hypovolemia with in-hospital mortality rates greater than 40% (Figure 4). In order to identify patients with the highest probability of poor outcome associated with sepsis, a new bedside clinical score named the quickSOFA (qSOFA) was created which consist of at least two of the following clinical criteria including, respiratory rate of 22/min or greater, altered mentation, or systolic blood pressure of 100 mm Hg or less⁴ (Figure 5).

Even though substantial advances in our clinical understanding, disease definition, and immune pathophysiology have improved overall sepsis survival, long-term sepsis mortality is abysmal, at 40%-80%.⁵ Despite progress in antibiotic therapy, ventilator management, resuscitative strategies, and blood glucose maintenance, sepsis remains the leading cause of death in the intensive care units (ICUs).⁶ Even more alarming is the escalating cost of sepsis-associated medical care, which is estimated at \$17 billion annually in the United States.⁷ Considering the rapidly expanding elderly population with large comorbidity burdens, physiologic frailty, and immune senescence,⁸ sepsis mortality is expected to rise at an alarming rate over the next two decades.⁹

Despite over 100 therapeutic clinical trials in sepsis, no FDA-approved treatment options currently exist that improve sepsis

survival.¹⁰ Although clinicians and investigators have opined in a sundry of editorials, reviews, and commentaries incriminating a multitude of possible explanations, such as impaired cellular metabolism, tissue oxygenation, and myocardial dysfunction,¹¹ the most accepted postulate describes a persistent and complex, immune/inflammatory interplay that is yet ill-defined.^{11,12} Traditionally, sepsis investigations attempted to improve 30-day survival by dampening the inflammatory response by way of IL-1 and TNF blockade¹³ with little success in reducing mortality. However, continued improvements in clinical treatment strategies over the past two decades¹⁴ have resulted in more patients surviving life-threatening sepsis and organ dysfunction, only to manifest prolonged states of immune dysfunction, immune suppression,¹⁵ persistent inflammation, and metabolic catabolism.¹⁶ These varying states of immune paralysis are characterized by impaired immune surveillance and the development of persistent, recurrent, secondary, and nosocomial infections which facilitate protracted events that often lead to death.¹⁷

Historically, the sepsis death distribution has been biphasic, with an initial early peak at several days due to inadequate fluid resuscitation, resulting in cardiac and pulmonary failure, and a late peak at several weeks due to persistent organ injury or failure.¹⁸ Considering the recent recognition in mounting long-term sepsis mortality, a trimodal pattern is more indicative of the current death distribution.¹⁷⁻¹⁹ The early peaks in mortality exist, albeit of much less magnitude, and the third upswing occurs after 60-90 days and continues to soar over the ensuing 3 years^{17,19,20} (Figure 6A, B). These deaths are speculated to be the consequence of more sophisticated ICU care that keeps elderly and comorbidly challenged⁹ patients alive longer in spite of ongoing immune, physiologic, biochemical, and metabolic aberrations.²¹ Although the

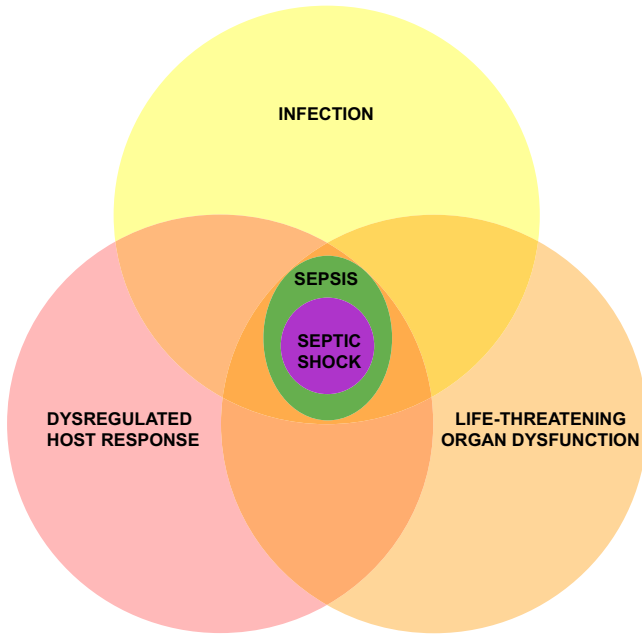


FIGURE 2 The Third International Consensus Definitions For Sepsis And Septic Shock. The current definitions for sepsis and septic shock were developed to address the limitations of previous definitions that were over focused on SIRS and inflammation. In addition, the Sepsis-3 also dispelled the long-standing notion that SIRS criteria possess adequate specificity and sensitivity to define and diagnose sepsis. Lastly, the report debunked the misleading model that sepsis always follows a linear continuum from the SIRS through severe sepsis and septic shock, and declared the term “severe sepsis” redundant and unnecessary. Instead, the Consensus report recommends that sepsis be defined as a life-threatening organ dysfunction caused by a dysregulated host response to an infection

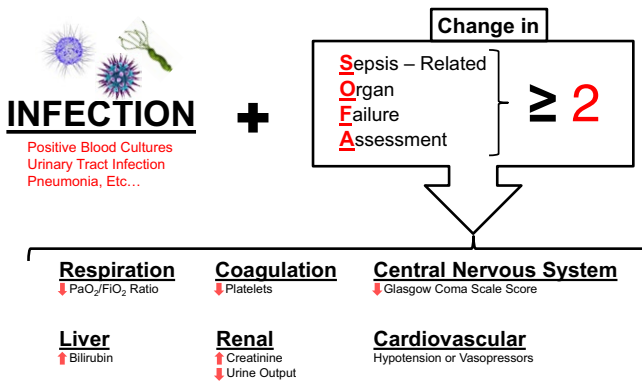


FIGURE 3 Organ dysfunction in sepsis and associated mortality. The Sepsis-3 consensus report defined organ dysfunction by an increase in the Sequential Organ Failure Assessment (SOFA) score of 2 points or more, which is associated with a in-hospital mortality greater than 10%

specific etiologies of long-term sepsis mortality are currently unclear, several reports suggest that advanced age, comorbidities, and persistent organ injury synergize to generate a damaging state of chronic and critically ill disease characterized by ongoing immune dysfunction, immune suppression, and catabolism and inflammation.^{15,16,22}

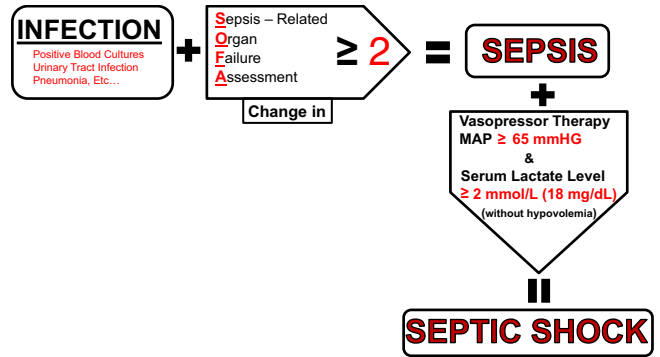


FIGURE 4 Definition of septic shock and associated mortality. Septic shock is now defined as a subset of sepsis in which profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone. Clinically, patients with septic shock can be identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mmHg or greater and serum lactate level greater than 2 mmol/L (>18 mg/dL) in the absence of hypovolemia with in-hospital mortality rates greater than 40%

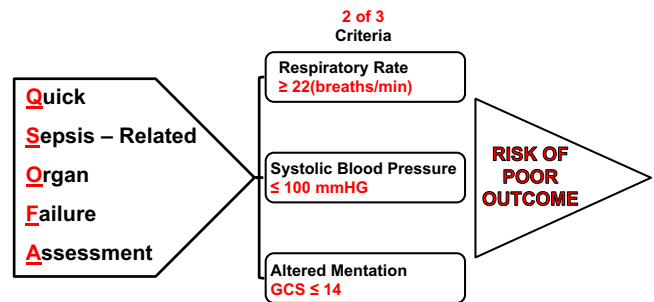


FIGURE 5 Bedside criteria defined to identify. To identify patients with the highest probability of poor outcome associated with sepsis, a new bedside clinical score named the quickSOFA (qSOFA) was created which consist of at least two of the following clinical criteria including, respiratory rate of 22/min or greater, altered mentation (GCS≤14), or systolic blood pressure of 100 mm Hg or less

Moreover, persistent inflammation combined with chronic immobility, catabolic drugs, and extended paralytic drugs all culminate to produce a state of immune dysregulation that facilitates infectious complications and terminates in chronic deterioration and death.²³ Thus, investigators have been forced to refocus their efforts on the underlying innate and adaptive immune system derangements that facilitate the development of infectious complications, impair sepsis recovery, and increase long-term mortality.^{24,25} In this review, we will outline the immune system's role in sepsis progression, resolution, and long-term outcome, and focus our attention on the clinical implications, and potential therapeutic interventions available to improve long-term survival.

2 | IMMUNE DYSFUNCTION IN SEPSIS

Sepsis impacts the immune system by directly altering the life span, production, and function of effector cells responsible for homeostasis.^{26,27} The hematopoietic compartment constitutently replenishes

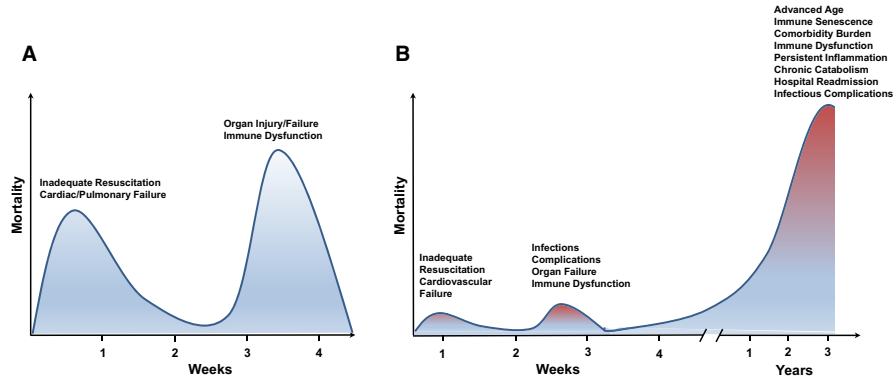


FIGURE 6 Past and present mortality distribution sepsis. (A) Classically, the mortality distribution from sepsis occurred in a biphasic pattern, with an initial peak due to inadequate resuscitation resulting in cardiac and pulmonary failure and a second peak at several weeks from persistent organ dysfunction. Considering the recent trends in physiologic frailty, the growing elderly population, and mounting long-term mortality, a trimodal distribution is more indicative of the current sepsis-associated mortality. (B) The two early peaks in mortality still do exist but with much less magnitude than in the past. The third and largest upswing occurs beginning at 2-3 months after sepsis and continues to steeply climb as time progresses. This delay in sepsis mortality is attributed to significant advances in ICU care that keeps the elderly and comorbidly challenged patients alive longer despite ongoing immune, physiologic, metabolomics, and biochemical aberrations

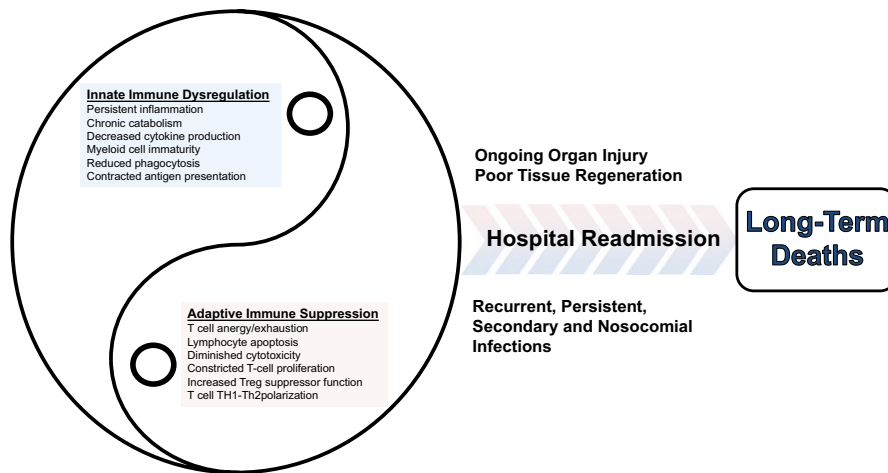


FIGURE 7 Inflammatory vs anti-inflammatory responses. An ongoing debate persists as to whether innate and adaptive immune dysfunction or inflammatory and anti-inflammatory processes are more detrimental to overall sepsis survival. In the past, the inflammatory response was thought to drive early mortality in the initial days of sepsis, and the compensatory anti-inflammatory response was thought to induce mortality weeks later through immune suppression and organ failure. However, new insights gathered from septic patient tissue samples and severely injured trauma patients have identified an enduring and simultaneous inflammatory and anti-inflammatory state of affairs driven by dysfunctional innate and suppressed adaptive immunity that together culminate in persistent organ injury, infectious complications requiring hospital readmission, and ultimately patient death. It is evident that the inflammatory and anti-inflammatory responses and innate and adaptive immune systems are each equally important, continually in a state of fluctuation, and ever at odds one another, as sepsis recovery progresses. This perpetual state of immunologic yin and yang is thought to drive ongoing inflammation, facilitate organ injury, and enable infectious complications that all preclude durable sepsis survival

terminally differentiated innate and adaptive cells which are intrinsically responsible for immune surveillance against offending pathogens, and concomitantly prerequisite for successful tissue regeneration and wound healing. Over the last two decades, a debate has persisted as to whether innate and adaptive immune dysfunction or inflammatory and anti-inflammatory processes are more detrimental to sepsis survival.²⁸ Formerly, the inflammatory response was thought to drive early mortality in the first several days of sepsis, and the compensatory anti-inflammatory response was thought to induce organ failure, immune suppression, and mortality weeks later.²⁹ However, new

insights gathered using genomic analysis of septic patient tissue samples¹⁵ and severely injured trauma patients have identified an enduring and simultaneous inflammatory and anti-inflammatory state driven by dysfunctional innate and suppressed adaptive immunity that together culminate in persistent organ injury³⁰ and patient death^{31,32} (Figure 7). Although shortcomings in each of these studies exist, when the collective results are compared with patient outcomes, it is clear that a paradigm shift is necessary to explain the long-term mortality surge after sepsis. It is evident that inflammatory and anti-inflammatory responses and innate and adaptive immune systems are each equally

important, and likely present targets for future immune therapies to improve long-term sepsis outcomes.^{25–27,33} The following discussion provides an overview of the sepsis-induced alterations in inflammation, innate and adaptive immune cell function, and the most promising immune response modifiers being considered for future human sepsis therapy.

3 | HYPERINFLAMMATION

Once the host loses local-regional containment of an infection, the body is systemically exposed to microbes, microbial components, and products of damaged tissue. This induces an inflammatory response and initiates sepsis-like responses through the recognition of pathogens and damaged tissue by way of pattern recognition receptors (PRRs) which are ubiquitous on immune cell surfaces. PRRs are expressed primarily on immune and phagocytic cells and on many types of somatic tissues. Microbial infections are recognized by pathogen-associated molecular patterns (PAMPs) that are expressed by both pathogenic and harmless microbes. PAMPs are recognized by PRRs such as Toll-like receptors (TLR), C-type and mannan-binding lectin receptors, NOD-like receptors, and RIG-I-like receptors. Proteins and cellular products released by tissue damage are similarly recognized as damage-associated molecular patterns (DAMPs).³⁴ During sepsis, systemic activation of the innate immune system by PAMPs and DAMPs results in a severe and persistent inflammatory response characterized by an excessive release of inflammatory cytokines such as IL-1, TNF, and IL-17, collectively known as the “cytokine storm”.³⁰ The exorbitant release of inflammatory cytokines occurs over a relatively short period of time (several days). In addition, intense complement activation and innate immune stimulation potentiate what should be a normal physiological response to infection, instead into an excessive inflammatory response resulting in tissue damage, cellular compromise, and molecular dysregulation that initiate organ dysfunction and even multi-organ failure.³⁰

Although some patients recover from this inflammatory state, for unknown reasons elderly patients with heavy comorbidity burdens fail to resolve this initial condition and progress to a state of persistent ongoing inflammation, immune cell dysfunction, and catabolic metabolism, all of which degrade the immune system's ability to clear infections and heal injured tissues.³⁵ Recently, investigators have suggested that therapeutic interventions that curb hyperinflammation, shift catabolism toward anabolism, and bolster immune function maybe beneficial in combination, once the initial episode of sepsis has subsided.^{24,36,37} Although in other disease states such as severe burns,³⁸ advanced cancers,^{24,25,39,40} and autoimmune diseases,⁴¹ combination therapies that reduce inflammation, optimize metabolism, and decrease infections are commonplace, there is as of yet no clear plan for the routine use of these or similar strategies to improve long-term outcome in sepsis.¹⁹ Until we embrace and adapt strategies for sepsis therapy that have been demonstrated to improve outcome in other inflammatory disease states, we may not be able to meaningfully improve sepsis survival.

4 | IMMUNE RESOLUTION

Resolution of the hyperinflammatory cytokine cascade was thought to begin days after the initial sepsis episode had passed. However recently, it has been discovered that compensatory anti-inflammatory pathways are activated shortly after sepsis initiation.²⁸ The hallmark cytokine is IL-10, which is produced by a variety of leukocytes, suppressing the production of IL-6 and interferon- γ (IFN γ), and stimulating the production of soluble TNF receptor and IL-1 receptor antagonist. These products neutralize pro-inflammatory TNF α and IL-1 signaling.⁴² At the subcellular level, autophagy provides a way to eliminate DAMPs and PAMPs by packaging pathogen components, damaged organelles, and cellular proteins into vesicles targeted for lysosomal degradation, resulting in reduced inflammation and cellular activation.⁴³

Resolution of inflammation after severe infection is not simply a passive process of curbing cytokine production and easing inflammatory pathways. Instead, dampening of inflammation involves an interdigitating, complex, and coordinated array of cellular processes and recently recognized molecular signals. Soon after pathologic bacteria are eliminated from the host, damaged tissues, cells, and leukocytes must be removed from the infection site. Under favorable circumstances, the defunctionalized tissue cells and leukocytes undergo apoptosis, becoming engulfed by macrophages and removed from the inflamed field, triggering the production of anti-inflammatory IL-10 and transforming growth factor β . Furthermore, recently discovered bioactive lipids termed lipoxins, resolvins, protectins, and maresins have been shown to reduce ROS, endothelial permeability, and leukocyte recruitment, and further enhance macrophage phagocytosis.^{44,45} In addition to anti-inflammatory cytokines, inflammation resolution is also governed by multiple subsets of regulatory immune cells such as regulatory T cells (Tregs)^{46,47} and myeloid-derived suppressor cells (MDSCs) that orchestrate inhibition over cytotoxic effectors and curb inflammatory cytokine production.⁴⁸

Historically, therapeutic interventions aimed at inhibiting the acute inflammatory response to infection and subsequent sepsis using glucocorticoids, non-steroidal anti-inflammatory agents, and anti-TNF α antibodies have repeatedly failed to improve outcomes in patients with sepsis and septic shock.¹⁰ Conversely, strategies that stimulate endogenous mediators that actively resolve inflammation have not been thoroughly explored because the critical signaling factors that regulate these native processes have remained elusive. Serhan et al.⁴⁴ demonstrated that eicosapentaenoic acid- and docosahexaenoic acid-derived lipids (coined pro-resolving mediators [SPMs], including resolvins, protectins, and maresins) increase early after states of infection, regulate inflammation resolution, and enhance bacterial clearance. Recently, Dalli et al.⁴⁹ reported a novel cohort of host-protective lipids termed 13-series resolvins (RvTs). These host-protective lipids are formed early during inflammation, promote bacterial phagocytosis, increase production of reactive oxygen species, and augment host recovery from systemic infection by accelerating the resolution of the acute inflammation.⁴⁹ Furthermore, the systemic administration of atorvastatin can increase RvT biosynthesis through activation of

COX-2 and accelerate infection resolution which can be reversed by COX-2 inhibition using celecoxib.⁴⁹ Together these findings shed light on a new group of endogenous mediators that resolve host inflammation without interfering with phagocytosis and identify a new mechanism for the pleiotropic effects of statins that mitigate inflammation and promote endothelial function.⁵⁰ Although statin therapy in sepsis has yielded little benefit in clinical trials,⁵¹ the discovery of a new class of bioactive lipids that resolve inflammation could provide a biomarker to identify subgroups with impaired RvT biosynthesis who may benefit from statin therapy.

5 | IMMUNE SUPPRESSION

Generally, therapeutic strategies to treat sepsis have focused on inhibiting the early hyperinflammatory phase. However, it is evident that a state of immune suppression exists concomitantly with persistent inflammation and enables the development of persistent, recurrent, secondary, and nosocomial infections which lead to poorer outcomes and increased long-term mortality.²⁵ Sepsis-induced immune suppression impacts both cellular effectors of the innate and adaptive immune systems.

Neutrophils, essential for bacterial eradication, display defects in chemotaxis and recruitment to sites of infection, in the setting of sepsis.^{52,53} Furthermore, the production and release of essential effector molecules, such as reactive oxygen species (ROS) and cytokines, are significantly impaired,⁵³⁻⁵⁵ leading to bacterial persistence and the development of infectious complications. Even though myeloid cell production of pro-inflammatory cytokines is reduced, overall myeloid cell production and release of anti-inflammatory cytokines (e.g. IL-10) is elevated.⁵⁴ Defects in antigen-presenting cell (APC) function, including reduced HLA-DR expression, endotoxin tolerance, and impaired cytokine production, all reduce the capabilities of APCs to stimulate lymphocyte-driven immune functions following sepsis.⁵⁶⁻⁵⁹ Apoptosis of lymphocytes and APCs (dendritic cells, T cells, and B cells) is considered a hallmark of septic immune suppression.^{60,61}

In addition to diminished innate function, adaptive immunity is similarly impaired. Splenocytes harvested from deceased sepsis patients demonstrate reduced numbers of CD4⁺ and CD8⁺ lymphocytes that emanate from substantial apoptosis.¹⁵ Moreover, CD4⁺ cell loss is associated with a reduced ability to mount appropriate immune responses to viral infections including cytomegalovirus, Epstein-Barr virus, herpes simplex virus, and human herpes virus reactivate after septic insults.⁶² However, reduced lymphocyte numbers are not just reflective of the risk for viral reactivation following sepsis. Lymphopenia, 4 days after the onset of sepsis, is associated with the development of secondary infection and is predictive of long-term mortality at 1 year after sepsis.⁶³

Overall these specific cellular alterations coalesce into a chronic state of immune suppression, characterized by persistent, recurrent, secondary, and nosocomial infectious complications⁶⁴ often resulting in hospital readmissions,⁶⁵⁻⁶⁷ and poor long-term survival.⁶⁸ Over the last 5 years it has become abundantly clear that in the weeks and

months following hospital discharge, sepsis survivors have increased readmission rates⁶⁹ due to infectious complications,⁷⁰ requiring more antibiotics, ICU days, and hospital resources, when compared to patients not experiencing sepsis.⁷¹ With the ever increasing, comorbidly challenged elderly population, demonstrating persistent inflammation, immune suppression, and immune senescence, the number of sepsis survivors that develop subsequent infections is predicted to rise substantially in the next decades.^{68,72} It is evident that sepsis induces a pathologic state of immune suppression that prompts the development of secondary infections while still in the ICU setting.⁷³ Several reports over the past 5 years demonstrate that sepsis survivors experience dramatically higher rates of subsequent infections long after the initial episode of sepsis has resolved.^{65,69,70,74-76} The increased hospital readmission rates due to infectious complications among sepsis survivors is a sign of ongoing immune suppression and dysregulation that if not corrected, diminishes life quality and durable survival.

6 | MOLECULAR ALTERATIONS IN SEPSIS

Current sepsis observations suggest that multiple organ failure occurs even in the context of preserved cell morphology and in the absence of significant cell injury. In addition, organ dysfunction is often reversible, even in organs that regenerate poorly (heart, lung, central nervous system, kidneys). Therefore, it is apparent that sepsis-induced organ dysfunction occurs primarily through cellular and molecular dysregulation, as opposed to gross tissue damage. By this principle, immune dysfunction in sepsis is also associated with molecular alterations that alter cellular phenotype and function. Below, we outline several important pathways of cellular dysfunction that impact immune function during and after episodes of sepsis.

7 | COMPLEMENT ACTIVATION

It has long been known in human and rodent sepsis models that a robust and consumptive depletion of complement occurs, resulting in a sharp drop in hemolytic plasma complement and in the appearance in plasma of complement activation products.⁷⁷ There is also evidence that sepsis in humans causes shedding of the C5a receptor into plasma, likely due to release of microparticles from neutrophils.⁷⁸ Similar events occur in rats and mice with experimental polymicrobial sepsis.⁷⁹ In addition to complement activation, there is also a robust stimulation of both the fibrinolytic and clotting systems.⁸⁰ There is also evidence for activation of several clotting factors, some of which have C3 and C5 convertase activities, generating C3a as well as activation of thrombin which has C5 convertase activity and generates C5a and the terminal membrane attack complex.⁸¹ There is well-established evidence that activation of the complement system is often linked to activation of both the clotting and the fibrinolytic systems. Development of neutralizing C5a antibodies in murine models dramatically attenuated the intensity of sepsis, including greatly

improved 7-day survival, reduced levels of plasma cytokines, and decreased multiple organ failure.^{79,80,82} The steady progress achieved in understanding complement and how its production and deposition increase systemic inflammation, organ failure, and mortality, have resulted in the development and randomized phase 2 trial of a C5a inhibitor, CaCP29 (EudraCT Number: 2013-001037-40) which has shown great promise despite a historically large field of other failed antibody inhibitors.⁸³

8 | REDOX IMBALANCE

Clinical and experimental evidence has been well published that demonstrates septic patients exhibit overwhelming oxidative stress^{84–86} which results from uncontrolled production of ROS and reactive nitrogen species (RNS). This severe state of oxidative stress is produced by activated immune and epithelial cells that overexpress oxide synthases including inducible nitric oxide synthase (iNOS), or via mitochondrial production of ROS.^{86–88} ROS and RNS play crucial roles in sepsis progression by interfering with nitric oxide signaling cascades and oxidation/nitrosylation of proteins or nucleic acid substrates that results in harmful molecular function.^{87,89} Although antioxidant therapies have shown benefit during animal models of experimental sepsis, the same advantage has not translated into successful human clinical trials.⁹⁰

9 | CA²⁺ HOMEOSTASIS

Hypocalcemia is common in sepsis and correlates with disease-specific scores during critical illness.^{91–94} Altered intracellular calcium handling is hypothesized to be responsible for the observed systemic hypocalcemia.⁹⁴ Although systemic Ca²⁺ levels are reduced during sepsis, there are increased Ca²⁺ cytosolic levels which may stem from increased uptake. The same intracellular calcium fluctuations are elevated in a variety of tissues and cell types during sepsis,^{95–97} although the specific mechanisms underlying altered Ca²⁺ handling remain unclear. Heightened intracellular calcium leads to elevated inflammatory responses, cellular dysfunction, and can even be cytotoxic. In addition, the accumulation of Ca²⁺ in organs during sepsis is also associated with significant organ dysfunction.⁹⁸

10 | PARP1 AND PARP2 ACTIVATION

Poly(ADP-Ribose) Polymerase 1 (PARP1) and PARP2 are enzymes that catalyze poly(ADP-ribose)ylation of proteins. Catalytic activity of these enzymes is stimulated by DNA strand breaks. PARP activity is viewed as a sensor of DNA damage. PARP1 activation and initiation of the inflammatory response occur simultaneously.⁹⁹ PARP1 activity upregulates pro-inflammatory gene expression,¹⁰⁰ which is attributed to PARP1-induced alterations in chromatin structure and in transcriptional regulation.^{99,101} Because PARP1 also directly contributes to cell

death in affected tissues,⁹⁹ it is hypothesized that PARP1 also plays a role in sepsis-associated immune cell death. PARP1 genetic deficiency is protective during murine models of experimental sepsis, and is associated with significantly lowered plasma cytokine levels and reduced tissue/organ dysfunction.¹⁰² Inhibitors of PARP1 have been studied in clinical trials as potential cancer therapeutics, but trials for sepsis have not been initiated. Therefore, it is not clear whether inhibitors of PARP1 will be beneficial during the treatment of human sepsis.

11 | MITOCHONDRIAL DYSFUNCTION

Mitochondria are critical for maintaining an adequate supply of adenosine triphosphate (ATP) for cellular processes. In addition, damaged mitochondria can trigger cell death pathways through the release of mitochondrial cytochrome c.¹⁰³ Mitochondria are affected in several ways during sepsis. The generation of excessive amounts of ROS and RNS can directly inhibit respiration and damage respiratory chain components in mitochondria.^{104–106} In addition, impaired tissue perfusion (due to fluid loss, both intrinsic and extrinsic, as well as reduced vascular tone) leads to tissue hypoxia. Loss of tissue oxygenation significantly impairs oxidative phosphorylation and may trigger cell death pathways.¹⁰⁷ Mitochondrial dysfunction, or direct damage of mitochondria, can directly affect the generation of ATP. Not only will the drop in ATP negatively affect cellular processes but also severe lack of ATP can trigger cellular anergy, whereby the cell will not necessarily die, but instead acquire a hibernation-like state resulting in tissue dysfunction and organ failure.¹⁰⁸ The importance of mitochondrial dysfunction during sepsis is highlighted by the observations that cellular ATP levels are correlated with survival in both human and animal sepsis models.^{106,109}

12 | CELLULAR DEFECTS

The following discussion provides a summary of the sepsis-induced immune alterations in the majority of innate and adaptive cell types, along with the most promising candidates under consideration to be employed as immune response modifiers for future human sepsis therapy. Although we have attempted to focus this discussion on human sepsis, many of the current insights have been gleaned from animal sepsis model recapitulating aspects of human sepsis. The authors recognize the recent and ongoing debate about the efficacy of murine and other animal models to accurately reflect human disease processes.¹¹⁰ The authors' opinion is that both human and animal models are necessary if continued scientific progress and improved patient outcome is to continue. Even though we clearly realize that animals do not immunologically, metabolically, or genomically equal the state of human responses, it is not sustainable to merely test all of the proposed hypotheses in human disease systems without the benefit of animal correlation to explore scientific avenues not otherwise ethically amenable to the human condition.

13 | INNATE IMMUNITY

13.1 | Endothelium

Endothelial cells (ECs) form a single cell layer called the endothelium, which line all of the vasculature and lymphatic systems in the body and comprise a semi-permeable barrier between blood and lymph within vessels and the surrounding tissue. ECs are a heterogeneous population of cells that fulfill many physiological processes and participate in innate and adaptive immune responses. ECs function as danger signal sensors, therefore are one of the first cell types to detect invading microbes via endogenous metabolite-related danger signals in the bloodstream.¹¹¹ Lipopolysaccharide (LPS) exposure activates ECs, causing the production of pro-inflammatory cytokines and chemokines, which amplify the immune response by recruiting immune cells.¹¹² Therefore, ECs function as innate force multipliers, cell mobilizers, and immune regulators by modulating cellular function.¹¹³ In special circumstances, ECs can serve as antigen-presenting cells expressing both MHC I and II molecules and present endothelial antigens to T cells. ECs also express TLR-2 and TLR-4 enabling them to respond to LPS in states of bacterial infection.¹¹²

During sepsis a significant amount of EC dysfunction is present and manifests as several pathological processes including capillary leak, altered vasomotor tone, and microvascular thrombosis.¹¹⁴ A further consequence of damage to the endothelium is the release of pathological quantities of von Willebrand factor, which promotes platelet aggregation and adhesion to the subendothelial layer and the formation of pathologic thrombi. In sepsis, there may be direct destruction of the endothelial barrier, and an increased number of circulating endothelial cells that have been observed in patients with septic shock. Angiopoietin-2 (Ang-2) mediates endothelial microvascular leak and is an independent predictor of death and organ dysfunction during sepsis.¹¹⁵ As the early outcome of sepsis is mainly determined by the degree of organ failure, macro- and microvascular endothelial dysfunction has been proposed as an early biomarker of immune function and outcome. In patients with severe sepsis, *in vivo* measured endothelial dysfunction coincides with lower numbers and reduced function of circulating progenitor cells implicated in endothelial repair.¹¹⁶ The results suggest that cellular markers of endothelial repair might be valuable in the assessment and evolution of organ dysfunction and even outcome following episodes of sepsis. ECs also release microparticles which are protective against vasomotor hyporeactivity, which accounts for hypotension in patients with septic shock.¹¹⁷ Taken together, there is a large amount of data to suggest that ECs are key regulators of the physiologic and immunologic dysfunction during and after sepsis, and that EC modulation is possibly beneficial to improve long-term human sepsis survival.

13.2 | Neutrophils

Neutrophils are the most prevalent and integral cell type of innate function, essential for microbial containment and eradication, and prerequisite for long-term sepsis survival.¹¹⁸ They comprise the majority of the cellularity in the bone marrow (BM) and are the very

first responders to sites of microbial infections.¹¹⁹ One of the most pronounced innate immune alterations in sepsis is a delayed state of neutrophil apoptosis,¹²⁰ leading to ongoing neutrophil dysfunction. This delayed state of neutrophil apoptosis is further compounded by the release of immature band-like neutrophils from the BM that demonstrate clear deficits in oxidative burst,¹²¹ cellular migration patterns,^{122,123} complement activation ability, and bacterial eradication,⁵⁴ which all combine and contribute to persistent immune dysfunction and inflammation. These findings combined with TLR signaling deficits,¹²⁴ chemokine-induced chemotaxis reductions,¹²⁵ altered apoptosis signaling pathways, and neutrophil immune senescence,¹²⁶ result in a sundry of functional deficiencies that endure long after sepsis symptoms have subsided. A host of mounting scientific evidence also suggests that neutrophils may function as APCs in a broad array of pathological infections and act as cross-talkers between innate and adaptive responses through activation of CD4⁺ and CD8⁺ effector cells.^{127,128} More importantly, multiple human studies have implicated the complex array of persistent neutrophil dysfunction in the development of hospital acquired infections.¹²⁹ Furthermore, patients with the most pronounced derangements in neutrophil function following sepsis are the most susceptible to develop intensive care unit complications such as ventilator-associated pneumonia and other nosocomial infections.¹³⁰ The vast majority of patients who die from sepsis have ongoing infections,¹⁵ suggesting that defects in innate immunity in general and neutrophil-mediated bacterial clearance in particular, could serve as potential therapeutic targets to regulate neutrophil apoptosis, production, maturation, and function.

In addition to the ability to eliminate pathogens by phagocytosis, oxidative burst, and/or degranulation, it has recently been shown that neutrophils can eradicate a wide range of microorganisms by forming neutrophil extracellular traps (NETs).¹³¹ This novel mechanism entails the release of antimicrobial proteins anchored to a chromatin network of activated neutrophils. A rapidly expanding body of evidence demonstrates that NET release is integral in the pathogenesis of diseases such as sepsis, atherosclerosis, and autoimmunity.^{132,133} DNA is the major structural component of NETs. In addition, granule and cytoplasmic proteins, including neutrophil elastase, myeloperoxidase, cathepsin G, proteinase 3, gelatinase, LL-37, lactoferrin, and calprotectin as well as histones H1, H2A, H2B, H3, and H4, are all embedded in the DNA backbone comprising the NET. The DNA fibers promote physical containment of the microbes, whereas the histones and granular proteins confer an antimicrobial function to the NETs. Neutrophil NET production requires platelet-neutrophil interactions and can be inhibited by platelet depletion or disruption of integrin-mediated platelet-neutrophil binding. Released into the vasculature to ensnare bacteria from the bloodstream, NETs prevent further bacterial dissemination.¹³⁴ During sepsis, NET release increases bacterial trapping by fourfold in liver sinusoids. Furthermore, the correlation between the presence of NETs in peripheral blood and organ dysfunction was evaluated in 31 septic patients. Elevated NET concentrations were observed in septic patients in contrast with the healthy controls, and increased NET levels were associated with sepsis severity and organ dysfunction.¹³⁵ Collectively, these insights provide a foundation from

which to explore the potential of immune modulatory therapy to exploit the benefits of NET formation in states of bacterial infection while minimizing the hazards of worsening organ dysfunction.

13.3 | Monocytes and macrophages

The impact of an episode of sepsis on human monocyte subpopulations has long been the subject of intense investigation over past half century. For decades, it has been apparent that reduced mononuclear cell HLA-DR expression clearly correlates with human sepsis mortality.¹³⁶ Moreover, the reduced capacity of blood monocytes from septic patients to release pro-inflammatory cytokines after endotoxin (LPS) challenge has been described as “endotoxin tolerance”, which has been suggested to facilitate poor short- and long-term sepsis outcomes.^{137,138} Although a sundry of complex mononuclear cell signaling pathways are altered and contribute to the establishment of endotoxin tolerance, the major implication on monocytes, and to a lesser extent macrophages, is reduced antigen presentation related to diminished HLA-DR cell surface expression.¹³⁹ In addition to the clear and persistent reductions in HLA-DR cell surface expression, monocytes from septic patients also demonstrate a reduced ability to secrete the pro-inflammatory cytokines TNF, IL-1, IL-6, and IL-12 after LPS challenge. The reduced monocyte capacity to secrete pro-inflammatory cytokines suggests that intracellular signaling has shifted toward the production of anti-inflammatory mediators which are associated with hospital acquired, ongoing, and secondary infections which ultimately increase sepsis-associated mortality.

Currently, many investigators agree that reduced monocyte HLA-DR expression is a surrogate marker of monocyte “anergy”, development of ongoing infections, and patient death.¹⁴⁰⁻¹⁴² In addition to diminished pro-inflammatory cytokine secretion, several reports associate low monocyte HLA-DR expression with reduced antigen-specific lymphocyte proliferation.^{143,144} These findings suggest that sepsis-induced monocyte anergy and immune suppression separately contribute to the increased risk of complications and adverse outcomes in sepsis. Although the mechanisms accounting for monocyte LPS tolerance are not clear at this moment, sepsis-induced monocyte epigenetic reprogramming may play a pivotal role in the establishment of LPS tolerance, myeloid anergy, and the overall immune-suppressive monocyte phenotype.¹⁴⁵ Analysis of human monocyte mRNA clearly shows increased levels of inhibitory cytokine genes and reduced levels of pro-inflammatory chemokine genes.¹⁴⁶ Recent reports on human monocytes make a substantial and convincing argument for the important impact of epigenetic reprogramming on the establishment of monocyte anergy; however, the true functional impact of these epigenetic alterations on long-term outcomes in human sepsis is still unknown.¹⁴⁷

13.4 | Natural killer cells (NK)

Historically, NK cells were thought of as undeveloped assassins of host cells that either lacked self-identification or were infected by viruses. However, thankfully the field on innate immunity has moved forward and we now clearly know that NK cells act as regulators immune

complex immune functions. NK cells are divided into various different subgroups based on CD16 and CD56 cell surface expression.¹⁴⁸ Human sepsis evidence indicates that both CD56^{hi} and CD56^{low} NK cell subgroups are significantly altered during episodes of sepsis. These observed alterations have recently been associated in several reports with increased lethality in human sepsis.¹⁴⁹⁻¹⁵¹ Additionally, NK cell cytotoxic function in human sepsis is greatly decreased.¹⁵² Similarly, to LPS tolerance in monocytes, NK cell ex vivo production of IFN γ in response to TLR agonists is also gravely diminished. This evidence indicates that NK cell tolerance may be responsible for the reactivation of latent viruses such as CMV, which is frequently encountered in intensive care unit populations and more importantly may serve as a future target for therapeutic intervention.¹⁵³ Despite the lack of published data, it is apparent that NK cells are major effectors of the final outcome in sepsis through modulation of INF- γ production.¹⁵⁴ Contradictory results in clinical studies may be explained by sampling time variability and apparent patient heterogeneity. It is evident that NK cell activation provides protection from pneumonia through excess production of INF- γ which inhibits bacterial growth and has a major impact on sepsis outcome.¹⁵⁵

13.5 | Dendritic cells (DCs)

DCs are traditionally characterized as either conventional DCs (cDCs) or plasmacytoid DCs (pDCs). cDCs are similar to monocytes and secrete IL-12, while pDCs are similar to plasma cells and secrete large amounts of IFN α . cDCs and pDCs are of particular interest due to their enhanced apoptosis during sepsis⁶⁰ and in patients who developed nosocomial infections.¹⁵⁶ Although DCs have varying immune functions compared with monocytes, like monocytes, DCs also exhibit reduced HLA-DR expression and produce increased amounts of immune-suppressive IL-10.¹⁵⁷ Furthermore, co-culture of DCs with T effectors induces T-cell anergy and Treg proliferation, which both correlate with sepsis-induced immune dysfunction. A couple of recent investigations have demonstrated that prevention of sepsis-induced DC apoptosis or augmentation of DC function enhances sepsis long-term survival.^{158,159} Several reports demonstrate that immune suppression can be ameliorated by DC treatment with growth factor FMS-like tyrosine kinase 3 ligand (FLT3L). FLT3L therapy models of burn-wound sepsis enhances DC cytokine secretion (IL-12, IL-15, and IFN γ), and augments CD4⁺ T cell, NK, and neutrophil function.¹⁶⁰ Still further investigations indicate that the DC-associated gains in sepsis survival occur through TLR signaling pathways, increased MHC class II antigen, and costimulatory molecules CD80 and CD86 expression.⁵⁷ These observations have led researchers and clinicians together to surmise that improvements in DC number and function may be high yield targets for future therapeutic interventions in sepsis.^{158,159}

13.6 | Myeloid-derived suppressor cells (MDSCs) and myelopoiesis

MDSCs are a heterogeneous population of immature myeloid cells that expand dramatically in sepsis, impede immune responses, and

signal through TLR-mediated pathways.^{127,161} MDSCs associated with sepsis are phenotypically similar to the MDSCs described in states of advanced cancer.^{127,162} Although MDSCs have been demonstrated to inhibit CD8⁺ cell function, the actual and functional impact of MDSCs in human sepsis is still uncertain. A summation of the current literature implies a beneficial role centered on replenishing innate cell function and immune surveillance through emergency granulopoiesis.¹²³ Prior to MDSC expansion, we have identified a window of susceptibility to secondary infections and subsequent mortality associated with reduced BM cells, and reduced blood and tissue neutrophil numbers and function.¹²¹ We have also demonstrated that robust MDSC expansion through enhanced granulopoiesis imparts lasting immunity to secondary and nosocomial infections in sepsis.¹⁶³ Due to the inherent difficulty in immune phenotyping immature myeloid cells between mice and humans, very limited investigations and clinical studies have closely examined the roles of MDSCs in human sepsis.¹⁶⁴ Nonetheless, there is mounting interest being paid to myelopoiesis, MDSC expansion, emergency granulopoiesis, and hematopoietic stem cell production and function.^{121,127,163,165,166} Due to the importance of efficiently regenerating functioning neutrophils, monocytes, and DCs, it is no surprise that MDSCs expand to meet the continual need for functional innate immune cells. Considering that 5-7 days are required for the BM to adequately produce a functioning neutrophil, an expansive immature pool (approximately 18×10^{11}) of myeloid lineage precursors in the BM and secondary lymphoid organs¹⁶⁷ is required to maintain a functioning cohort of innate immune cells. In humans, approximately 16×10^{10} neutrophils are produced daily which can be rapidly increased 5- to 10-fold in response to foreign microbial invaders and pathologic states of infection. Our prior work has clearly demonstrated 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.^{165,166} Moreover, impaired HSC proliferation, development, and function in human BM transplant models is clearly associated with increased mortality to secondary, chronic, and nosocomial infections.¹⁶⁸ Humans with reduced granulopoiesis ability undoubtedly experience more frequent, severe, and anomalous infections, demonstrating the essential requirement for effective neutrophil production. Recently, patients with sepsis have been shown to have MDSCs persistently increased, functionally immune-suppressive, and associated with adverse outcomes including increased nosocomial infections, prolonged intensive care unit stays, and poor functional status at discharge.¹⁶⁹ Conversely, overzealous MDSC proliferation may facilitate a physiologic syndrome of persistent inflammation, such as in adult respiratory distress syndrome or persistent inflammation immunosuppression, and catabolism syndrome, causing patients with sepsis to experience a poor outcome.¹⁶ Recent work by Terashima et al. demonstrated that acute inflammation causes the reduction of peripheral lymphocytes and common lymphoid progenitors in the bone marrow, which is also associated with a dramatic decrease in the osteoblast number. Moreover, osteoblast-specific IL-7 production during myelopoiesis was shown to be pivotal in the regulation of lymphopoiesis during systemic inflammation and may serve as a target for improved

lymphocyte production.¹⁷⁰ The specific contributions of lymphopoiesis, myelopoiesis, and MDSCs to sepsis recovery vs persistent inflammation and catabolism remain poorly understood. However, new insights into these processes and their roles in sepsis resolution and recovery will hopefully present new targets for immune modulatory therapy to improve sepsis outcomes.

14 | ADAPTIVE IMMUNITY

14.1 | Lymphoid apoptosis and immune suppression

In the majority of septic patients, circulating lymphocytes and gastrointestinal epithelial cells undergo significant apoptosis, while apoptosis/necrosis in the heart, kidneys, and lungs is not apparent.¹⁷¹ Lymphocyte apoptosis has now been accepted as an important step in the pathogenesis of sepsis and contributes to septic immunosuppression.¹⁷² Lymphocyte apoptosis has been described in both septic patients and animal sepsis models.^{173,174} Importantly, lymphocyte apoptosis has been shown to occur through both the intrinsic (Fas, FasL) and extrinsic (TNF) pathways.¹⁷⁴ Although the defining factors for this phenomenon are still not clear, recent evidence suggest that the release of extracellular histones during sepsis may drive lymphocyte apoptosis.^{175,176} Therapeutic blockade of lymphocyte apoptosis and/or restoring lymphocyte function have generated promising preclinical data that may lead to new treatments after episodes of sepsis in humans.^{171,177,178}

14.2 | Gamma delta T cells ($\gamma\delta$ T cells)

$\gamma\delta$ T cells are a diminutive subset of T cells that possess a rather distinct T-cell receptor (TCR) on their cell surface. The majority of T cells have a TCR composed of two α and β glycoprotein chains, while $\gamma\delta$ T cells have a TCR that is made up of one γ chain and one δ chain. This uniquely distinct group of T cells exists mainly in and around the gut mucosa within a population of intraepithelial lymphocytes.¹⁷⁹ Although the antigens to which $\gamma\delta$ T cells respond are still unknown, it is suspected that these cells recognize lipid antigens from pathogens present on mucosal surfaces within the intestine.¹⁸⁰ What is clear is that upon activation, $\gamma\delta$ T cells release IFN γ , IL-17, and other inflammatory chemokines. In humans with sepsis, the number of circulating $\gamma\delta$ T cells is significantly diminished, and these reductions correlate clearly with the highest rates of sepsis lethality.¹⁸¹ The observed reduction in $\gamma\delta$ T cells in the gut mucosa may serve to potentiate typically non-invasive intestinal bacteria types to become more invasive and translocate into the host systemic circulation, causing pathological infections following episodes of sepsis.¹⁸² In patients with acute sepsis, circulating neutrophils display a similar APC-like phenotype and readily process soluble proteins for cross-presentation of antigenic peptides to CD8⁺ T cells, at a time when peripheral $\gamma\delta$ T cells are highly activated.¹²⁸ From their findings, the authors conclude that unconventional T cells represent key controllers of neutrophil-driven innate and adaptive responses to a broad range of pathogens and may serve as targets for additional immune enhancement. In addition, others have also postulated that tremendous potential exists for the

therapeutic potential of NKT cellular targeting and immune enhancement in sepsis.¹⁸³

14.3 | T_H cell subpopulations

T-helper cells (Th cells) assist other cell types, including B-cell differentiation, cytotoxic T-cell activation, and monocyte stimulation with immunological processes. When confronted with peptide antigens by MHC class II molecules expressed on APCs, CD4⁺ cells are quickly activated, rapidly proliferate, and efficiently secrete cytokines that regulate adaptive and innate responses. Upon activation, CD4⁺ cells have the capability to differentiate into one of several T-cell subsets including Th1, Th2, Th3, Th17, Th22, Th9, or T follicular helper (T_{FH}), which facilitate various immune responses through differing cytokine generation and secretion.¹⁸⁴ Although numerous reports relate the effects of sepsis on circulating and peripheral CD4⁺ T-cell subsets,⁴⁶ the following section will highlight only the most relevant investigations to convey the important themes and potential areas of therapeutic interest.

Similar to the phenomenon observed in neutrophils and monocytes, one of the most deleterious T-cell defects induced by sepsis is development of apoptosis that decimates CD4⁺ populations.^{15,185} In humans who die from sepsis, there was a much greater magnitude of lymphocyte (specifically CD4⁺) apoptosis than in T cell from sepsis survivors.¹⁵ Of the CD4⁺ cells that manage to persist, multiple investigations demonstrate that both Th1- and Th2-associated cytokine production is reduced during and long after sepsis subsides.¹⁸⁶ Marked reductions in the transcription factors T-bet and GATA3, which modulate the Th1 and Th2 response, respectively, support the notion that CD4⁺ subsets are suppressed during sepsis.¹⁸⁷ There are a sundry of factors and influences that regulate CD4⁺ Th subpopulation differentiation, including histone methylation and chromatin remodeling, which together are postulated to suppress Th1 and Th2 CD4⁺ T-cell functions.¹⁸⁸ However, the sepsis-induced immune impact is not only relegated to Th1 and Th2 CD4⁺ T cells but also to Th17 subsets and the other Th subsets as well. Th17 cytokine production is reduced in sepsis and probably negatively impacts long-term mortality.¹⁸⁹ Given the fundamental role of Th17 in eradication of pathologic fungal infections, reduced Th17 cytokine production in sepsis is probably responsible for the increased susceptibility to fungal infections frequently observed in critically ill populations.¹⁹⁰ Circulating CD4⁺ and CD8⁺ cells from patients with candidemia display an immune phenotype consistent with immune suppression, T-cell exhaustion, and downregulation of positive co-stimulatory molecules.¹⁹¹ 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.¹⁷⁷ These findings may help explain why patients with fungal sepsis have a high mortality despite appropriate antifungal therapy.

14.4 | Regulatory T cells (Tregs)

Tregs are master regulators of adaptive immunity that suppress responses of other effector T-cell subsets, helping to maintain

self-tolerance and suppress autoimmune disease. In states of sepsis, critical illness, and states of inflammation, Tregs potentiate deleterious effector T-cell (Teff) suppression that prolongs recovery and may dispose to increased complications. Increased Treg ratios are present early after episodes of sepsis and remain elevated in those patients who died from sepsis while hospitalized, placing a high level of attention on Treg function. Other reports relate that Treg number increases are due to effector Th cell loss from apoptosis rather than an absolute increase in Treg numbers.⁴⁷ This observation suggests that Tregs are resistant to sepsis-induced apoptosis, thereby preventing the recovering immune system from mounting excessive autoimmune responses during the heightened initial inflammatory phase. Moreover, heat shock proteins and histones that induce mononuclear cell epigenetic changes also play a role as inducers of Tregs in sepsis.¹⁹² Recent murine reports demonstrate that Tregs are detrimental to Teff proliferation and immune function.¹⁹³ This effect is ameliorated by the administration of therapeutic siRNAs that inhibit Treg differentiation.⁴⁷ In other investigations, glucocorticoid-induced TNF receptor-related protein (GITR) inhibitory antibodies were used to block Treg function, resulting in improved immune function and microbial killing.¹⁹⁴ Treg-associated immune dysfunction in sepsis has also been linked to more rapid cancer and solid tumor cell growth, which probably stems from a reduced overall cytotoxic T cell (CTL) and mononuclear cell immune surveillance functions.¹⁹⁵ In conclusion, a strong notion exist that Tregs are resistant to apoptosis in sepsis, augment ongoing Teff cell dysregulation, contribute to infection development, and potentially serve as targets for immune modulation.

14.5 | B cells

B cells are a very diverse immune cell population with varying functional and phenotypical attributes. Historically, B-cell function was understood to only encompass producing antibodies and plasma cells for long-term antibody responses.¹⁹⁶ Conversely, a rapidly growing body of knowledge and collection of recent reports demonstrate that B cells play a much more pivotal role in sepsis immune biology than previously suspected. Clearly, humans with septic shock have overall reductions in B-cell numbers; however, the most significant deficit in B-cell number is in CD5⁺ B1a-type cells, which correlate and are predictive of survivors and non-survivors following episodes of sepsis.¹⁹⁷ In mouse models of human sepsis, B cells are necessary to improve cytokine production, reduce bacterial load, and improve survival through type I IFN signaling.¹⁹⁸ A recent investigation identified an innate response activator (IRA) B-cell population, which is phenotypically and functionally distinct from B1a cells and depends on PRRs, which produces granulocyte-macrophage-CSF (GM-CSF). Inhibition of IRA B cells impairs bacterial eradication, enhances a cytokine storm, and perpetuates the symptoms of septic shock. These recent clarifications position IRA B cells as immunological gatekeepers of bacterial infection elimination and simultaneously recognize IRA B cells as a new therapeutic target to improve survival in human sepsis.¹⁹⁹ Lastly, IRA B-cell generation of IL-3 has been revealed to greatly enhance sepsis-associated inflammation, induce myeloid production of Ly-6C^{hi}

mononuclear cells, and potentiate cytokine production, while elevated plasma IL-3 levels correlate with increased human sepsis mortality.²⁰⁰ B cells stimulated *ex vivo* in both aging and sepsis patients demonstrate significant reductions in supernatant IgM production.⁷⁶ This finding is clinically relevant and interesting and may explain why elderly patients with decreased IgM production are more susceptible to Gram-negative bacteria and fungal infection. Reduced immunocompetent B cells may be related to increased secondary infection after sepsis, especially in the elderly. All things considered, the recent and vast advancement in our understanding of B-cell biology has provided a great insight into the B-cell role in immune modulation, emergency myelopoiesis, and IL-3 production which is also new potential therapeutic focus in sepsis.²⁰¹

15 | IMMUNE MODULATORY THERAPIES IN SEPSIS

15.1 | G-CSF and GM-CSF

Granulocyte-colony stimulating factor (G-CSF) is a glycoprotein that stimulates the production of stem cells, progenitors, and granulocytes of all maturity ranges.²⁰² G-CSF is very efficacious in reducing the incidence of sepsis in patients with low absolute neutrophil counts, such as those undergoing BM transplant, cancer chemotherapy, or autoimmune radiation.²⁰³ Two randomized controlled human trials with recombinant G-CSF have been conducted in a goal-directed effort to bolster neutrophil production, maturity, and overall function. Clinicians and research investigators alike hypothesized G-CSF that administration would improve granulocyte function and microbial elimination in such circumstances. Although an increase in blood leukocyte counts was realized, there was no improvement in 28-day patient mortality.^{204,205} Although the initial conclusions of these two clinical studies were disheartening, the fact remains as the premise of this report that most of the sepsis-induced mortality occurs in a protracted process beyond 90 days.¹⁷ This makes one wonder if a longer study therapy or observation time would have changes in the investigation outcomes. However as of this time, the impact of G-CSF on mortality beyond 28 days is unknown. Given the ongoing and continuous alterations observed in granulocyte production, myelopoiesis, and neutrophil function especially in comorbidly challenged patients such as those with diabetes and physiologic frailty, prolonged G-CSF administration may be efficacious for improved immune surveillance, infection eradication, tissue regeneration, and sepsis survival in future clinical trials.

GM-CSF is another cytokine that enhances stem cells and progenitors to produce neutrophils, monocytes, and macrophages.²⁰⁶ In the immune-suppressive phase of sepsis, patients who were ventilator-dependent and treated with recombinant GM-CSF had fewer ventilator and ICU days.^{207,208} Furthermore, recombinant GM-CSF treatment in immune-suppressed pediatric populations with severe sepsis restored TNF production in lymphocytes and significantly reduced hospital-associated infections.²⁰⁹ Even further evidence for GM-CSF therapy is gleaned from a meta-analysis of over 12 clinical studies

involving either G-CSF or GM-CSF that demonstrated that either therapy significantly reduced the rate of infectious complications.²¹⁰ In light of the fact that 70%-80% patients who succumb to sepsis harbor persistent, chronic, ongoing, or secondary infections,¹⁵ GM-CSF and/or G-CSF in combination with other immune modulatory agents may prove invaluable for enhancing infection eradication during and after sepsis and improved long-term survival after sepsis.^{204,211}

15.2 | Interferon gamma (IFN γ)

IFN γ is unique and the sole member of the type II IFN family. Adequate IFN γ production and signaling is critical for appropriate immune function against viral, bacterial, and protozoal invaders. Moreover, IFN γ is a central inducer of macrophage activation, inducing stimulating class I MHC expression.¹³⁹ When treated with recombinant IFN γ , patients with severe sepsis and decreased monocyte HLA-DR levels demonstrate reversal of sepsis-induced monocyte dysfunction and overall improved sepsis survival.²¹² Although the majority of the interventional therapeutic trials with IFN γ were done in burn and mixed severely injured trauma cohorts, the largest of these reports clearly demonstrated a decrease in infection-related mortality among patients treated with IFN γ .²¹³ Moreover, a new study of severely injured trauma patients revealed that 42 of 63 genes identified as being differentially expressed in patients with uncomplicated vs complicated outcomes were specifically associated with IFN signaling. The investigators discovered that IFN-associated genes were suppressed in trauma patients with complicated outcomes,^{31,32} implying that this set of genes may be useful for identifying patients at risk for complications after trauma. In addition, these patients, prone to develop complications, may preferentially respond to therapies utilizing IL-7, IL-15, IFN γ , and GITR agonists. IFN γ is very promising if it is targeted to the patient populations who may benefit the greatest such as those who demonstrate or are at risk for immune suppression, decreased monocyte HLA-DR expression, adaptive immune dysfunction, or chronic inflammation in prolonged hospital stays. In a recent report, recombinant IFN γ treatment was able to partially restore immune metabolic defects associated with immune paralysis in humans after sepsis further suggesting that IFN γ therapy after sepsis may benefit a multitude of cellular immune functions.²¹⁴ Though IFN γ offers promise as a potential immune modulatory therapy given its ability to rejuvenate monocyte/macrophage function and adaptive immunity, IFN γ may be more efficacious if administered in a time-phased approach coupled with the likes of GM-CSF and G-CSF or even IL-7 and/or PD-1 inhibitors to bolster specific immune function, reduce secondary and nosocomial infections, and improve long-term survival as sepsis recovery evolves.

15.3 | Programmed cell death protein-1 and ligand (PD-1 and PD-L1)

Steady progress in cancer biology has generated a new class of drugs that inhibit programmed cell death. Programmed cell death protein-1 (PD-1) is a protein that under homeostasis generates an inhibitory

signal that reduces CD8⁺ T-cell proliferation and accumulation in secondary lymphoid organs such as lymph nodes. PD-1 is expressed on most T and B lymphocytes and myeloid cells. The ligand for PD-1 (PD-L1) is ubiquitously expressed on epithelial and endothelial cells, monocytes, macrophages, and DCs.²¹⁵ Considering that PD-1 is up-regulated on both CD4⁺ and CD8⁺ cells during viral infections and cancer states, it is often associated with the phenomenon of "T-cell exhaustion", which is thought to stem from prolonged periods of exposure to self-antigens.²¹⁶ Both anti-PD-1 and anti-PD-L1 therapies have demonstrated promising results in human trials involving cancer and viral infection. Therefore, sepsis biologists have postulated that anti-PD-1 and anti-PD-L1 therapy could also have similar beneficial results with reducing sepsis-induced immune dysfunction that drives ongoing infectious complications.²¹⁷ Patients with septic shock demonstrate increased levels of PD-1 and PD-L1 on their monocyte and T-lymphocyte cell types.²¹⁸ Recent studies demonstrate granulocyte PD-L1 upregulation results in potentiation of lymphocyte apoptosis through contact inhibition, which correlates with outcome.²¹⁹ Moreover, in clinically relevant animal models of experimental sepsis, inhibition of PD-1 and PD-L1 signaling pathways improved survival and reduced the number of fungal infections.²²⁰ Considering the beneficial impact on adaptive immunity and tumor eradication strategies, it makes sense that PD-1 and PD-L1 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.

15.4 | Recombinant human IL-3, IL-7, and IL-15

Considering the well documented and significant loss of lymphocytes in sepsis, IL-7 administration has been suggested as possible treatment strategy. IL-7 is a hematopoietic cytokine produced by BM stromal cells and is prerequisite for B- and T-cell production, maturation, homeostasis, and maintenance.²²¹ IL-7 is an attractive therapy due to its ability to upregulate the anti-apoptotic protein BCL-2, which in turn causes increased numbers of peripheral blood CD4⁺ and CD8⁺ cells. IL-7 also augments TCR diversity, which is lost in septic patients and is associated with increased development of nosocomial infections and hospital complications.^{177,222} Low doses of recombinant human IL-7 preferentially activate Tregs from patients with sepsis.²²³ Although recent evidence exists that IL-7 can improve lymphocyte PD-1 expression, cytokine-driven peripheral T-cell expansion and survival remain intact.²²⁴ However, when IL-7 was administered to patients with HIV, lymphocyte expression of PD-1 was decreased.²²⁵ Although IL-7 has the potential to work synergistically with other therapies targeting PD-1 or PD-L1 to improve aspects of T-cell function lost in sepsis, the capability of PD-1 or PD-L1 to reduce hospital acquired infections or improve survival in human sepsis is currently unknown. Emerging evidence suggest that sepsis reduces bone osteoblast number, which induces lymphopenia through IL-7 downregulation, demonstrating a reciprocal interaction between the immune and bone systems and identifying bone cells as potential therapeutic targets in sepsis.¹⁷⁰ Although clinical studies do support IL-7 therapy in HIV-infected

patients receiving antiretroviral therapy, no human sepsis trials currently exist. Given the promise that IL-7 has demonstrated in other inflammatory disease states, sepsis clinical trials with IL-7 either alone or in combination with other immune modulators such as anti-PD-1 should be considered.

Although still in preclinical scientific and experimental phases, it is worth mentioning IL-3 and IL-15 as potential sepsis therapies that have gained considerable attention. Considering the central role of IL-15 in the development and activation of effector and memory T, NK, and NKT cells and neutrophils, it has become a hopeful therapeutic candidate for future sepsis trials.²²⁶ In murine models of experimental sepsis, IL-15 treatment diminished overall immune dysfunction and reduced mortality.²²⁷ The synergistic impact that IL-3 plays in stem cell and progenitor maturation and development along with IL-7 makes IL-3 an appealing therapy to augment the potential impact of IL-7 therapy.^{200,201} However, there is very little evidence relating to the impact of either IL-3 or IL-15 in states of human sepsis and further discourse is purely speculative.

15.5 | Vagal nerve modulation

A growing body of evidence over the last two decades has revealed that systemic cytokine production and release is controlled by the 10th cranial nerve (vagus nerve). The scientific thrust of this discovery was done by the Tracey laboratory which elucidated the efferent arm of the inflammatory reflex^{228,229} as the cholinergic anti-inflammatory pathway.²³⁰ Accumulating experimental evidence establishes that vagus nerve activation operated via cholinergic anti-inflammatory signaling via $\alpha 7$ nicotinic acetylcholine receptors (7nAChR) expressed on non-neuronal cytokine-producing cells. Therefore, 7nAChR receptor agonists inhibit sepsis-associated cytokine release and protect animals in experimental models of sepsis and in a variety of other experimental inflammatory models.²³¹ Vagus nerve stimulation has an anti-inflammatory effect in sepsis and downregulates pro-inflammatory cytokine production in sepsis, decreasing the plasma protein levels of high mobility group box 1 (HMGB1) and improving survival in septic peritonitis models.²³² Surgical vagotomy increases the plasma levels of pro-inflammatory cytokines, tissue damage, and mortality in sepsis.²³³ Conversely, in rat models of sepsis, vagus nerve electrical stimulation attenuates and prevents hypotension,²³⁴ modulates coagulation, and fibrinolysis activation which all decrease organ dysfunction.²³⁵ Hence, the therapeutic potential of vagal efferent fiber modulation to treat disorders characterized by cytokine dysregulation is of great interest.²³⁶ However, until very recently, a paucity of real human data existed indicating that vagus nerve modulation was achievable let alone beneficial to outcomes in diseases of inflammation.²³⁷ Recently, clinical trial data demonstrate that stimulating the vagus nerve with a tiny implantable bioelectronic device significantly improves measures of disease activity in patients with rheumatoid arthritis, specifically reducing TNF production.²³⁸ Given the positive results of vagus nerve modulation in experimental models of sepsis, this latest report offers the real potential for systemic cytokine modulation over the long term.

In addition, the potential also exists for cellular modulation at the immune phenotype level as demonstrated by a recent report from Gabanyi et al.²³⁹ Incorporating technologically advanced imaging and transcriptional profiling techniques, the authors demonstrate that lamina propria zone macrophages, residing close to fecal contents, are primarily pro-inflammatory, poised to mount robust inflammatory responses should the epithelial barrier be breached. Conversely, muscularis macrophages, residing deeper in the gut wall, are primarily anti-inflammatory, expressing a tissue protective phenotype. Their search for the mechanism of this phenotypic switch revealed a breakthrough discovery that adrenergic neural signals regulate the tissue protective switch by norepinephrine signaling through $\beta 2$ AR.²³⁹ This is the first direct evidence of a closed loop neural reflex that regulates gut immunity. Moreover, considering the newest insights into gut barrier function, host-pathogen interaction, and microbiota contribution to gastrointestinal dysfunction in sepsis, the novel finding that neuronal reflexes control macrophage phenotypic regulation adds importantly to the growing list of neural reflexes implicated in modulating innate and adaptive cellular immune function.²⁴⁰

15.6 | Metabolic regulation of immunity

The immune system protects against foreign invaders, maintains optimal tissue homeostasis, and facilitates wound healing throughout the life of the organism. These diverse and integral functions require precise control of cellular, metabolic, and bioenergetics pathways. While these initial observations were described at the turn of the century,²⁴¹ more recent investigations have better defined the molecular basis for how extracellular signals control the uptake, anabolism, and catabolism of nutrients in quiescent, activated, and inflammatory immune cells. These reports collectively reveal that oxidative metabolism, glycolysis, and glutaminolysis are preferentially utilized by immune cells and decide cell fate and effector functions.²⁴²⁻²⁴⁴ For more than a century, we have known that propagation of a successful innate

effector response is dependent on glucose metabolism.²⁴⁵ In addition, we have also long understood that mitogen-driven proliferation of adaptive immune cells is predicated on the utilization of extracellular glutamine.^{246,247}

Under homeostatic conditions, immune cells rely on oxidative phosphorylation and β -oxidation as energy sources for ATP production to maintain cellular equipoise.²⁴⁸ However, after stimulation, leukocytes shift their metabolism toward aerobic glycolysis in a process known as the Warburg effect.²⁴⁹ In this metabolic shift, cellular energy is predominantly manufactured by an increase in glycolysis followed by lactic acid fermentation (lactate production) in the cytosol, rather than a low rate of glycolysis followed by oxidation of pyruvate in mitochondria.²⁵⁰ Hypoxia-inducible factor-1a (HIF-1a) and the mammalian target of rapamycin (mTOR) are major drivers of this metabolic switch and hence cellular fate^{251,252} (Figure 8). When innate immune cells are deficient in myeloid-specific HIF1a, mice are not protected against *S. aureus* sepsis, indicating that this HIF1a pathway and glycolytic flux are integral for septic immune responses.²⁵³ However, the HIF-1a is not the only metabolic intermediate that drives immune responses in sepsis. Upon exposure to LPS, macrophages demonstrate a shift from oxidative phosphorylation to glycolysis and succinate and induce IL-1 β production.^{254,255} The glucose transporter 1 (Glut1) mediates an increase in glycolysis that facilitates a macrophage pro-inflammatory phenotype.²⁵⁶ In a recent report, human leukocytes rendered tolerant by exposure to LPS after isolation from patients with sepsis and immune paralysis demonstrated a generalized metabolic defect at the level of glycolysis and oxidative metabolism, which was restored after patient recovery.²¹⁴ Compared with baseline, blood gene expression in patients who developed ICU-acquired infections post sepsis revealed reduced expression of genes involved in gluconeogenesis and glycolysis.⁷³ Furthermore, the genomic response of patients with sepsis was consistent with immune suppression at the onset of secondary infection in the ICU setting. Another report on sepsis patients identified regulatory genetic variants involving key mediators of gene networks

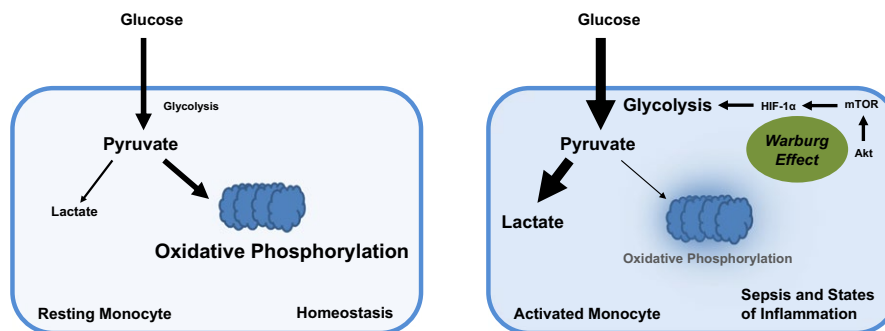


FIGURE 8 Alterations in metabolic function determine immune phenotype. Immune cells rely on oxidative phosphorylation and β -oxidation as energy sources for ATP production to maintain cellular equipoise at homeostasis. However, after stimulation, leukocytes shift their metabolism toward aerobic glycolysis in a process known as the Warburg effect. In this metabolic shift, cellular energy is predominantly manufactured by an increase in glycolysis followed by lactic acid fermentation (lactate production) in the cytosol, rather than a low rate of glycolysis followed by oxidation of pyruvate in mitochondria. Hypoxia-inducible factor-1a (HIF-1a) and the mammalian target of rapamycin (mTOR) are major drivers of this metabolic switch and hence determines cellular fate. These metabolic shifts have been incriminated in immune suppression and secondary infection progression in humans. A clearer understanding of the metabolic checkpoints that control immune cell function, transition, and maturation will provide new insights for modulating systemic inflammation, cellular immunity, and sepsis recovery

implicated in the hypoxic response and the switch to glycolysis that occurs in sepsis, including HIF1 α and mTOR, and mediators of endotoxin tolerance, T-cell activation, and viral defense.²⁵⁷ A clearer understanding of the metabolic checkpoints that control immune cell function, transition, and maturation will provide new insights for modulating systemic inflammation, cellular immunity, and sepsis recovery. The realization that oncogenesis and immune responses have a common mechanism for metabolic switching after stimulation indicates fundamental cellular processes that can serve as a potential therapeutic targets.

15.7 | Propranolol, oxandrolone, and dronabinol

The adrenergic system is a powerful modulator of the immune system.²⁵⁸ Hematopoietic and lymphopoietic tissues such as the spleen, thymus, lymph nodes, and bone marrow are all predominantly innervated by the sympathetic neurons. Except for CD4⁺ Th2 cells, the majority of lymphoid cells express beta-adrenergic receptors on their cell surface. Bone marrow production and differentiation of monocytes is influenced by the adrenergic system and immune cell apoptosis is at least partly mediated by catecholamines, via alpha-adrenergic and beta-adrenergic pathways.^{259,260} The adrenergic system also modulates cell death, mitochondrial function, and inflammatory signaling.²⁶¹ Although a great deal of focus has centered on the cardiovascular benefits of beta blockade in sepsis,²⁶² the ubiquitous nature of the adrenergic system begs the question whether there are additional mechanisms, whereby beta blockers may exert beneficial influences on immune process.

It has been recognized for over 50 years that epinephrine enhances bacterial infections and reduces the absolute number of bacteria necessary for a lethal dose in both *Clostridia* species and pathogenic aerobic organisms.²⁶³ Catecholamines also enhance biofilm formation and stimulate bacterial growth in *Staphylococcus epidermidis* infections.²⁶⁴ *Escherichia coli* O157:H7, *Salmonella enterica*, and *Yersinia enterocolitica* proliferation are all greatly enhanced by dopamine and norepinephrine through the catechol moiety and its subsequent acquisition by the bacteria.^{265,266}

Although there is a clear connection between the adrenergic and immune systems, further investigation is still required to elucidate the fundamental mechanisms. For instance, beta blockade reduces pro-inflammatory cytokines in heart failure,²⁶⁷ critically ill trauma patients,²⁶⁸ and appears to have a beneficial effect on Th1 to Th2 helper T-cell ratio.²⁶⁹ In a clinical trial including 55 severely injured patients at increased risk for heart disease, administration of metoprolol or esmolol decreased serum interleukin- (IL-) 6 levels.²⁷⁰ Christensen et al.²⁷¹ conducted a retrospective study on 8087 ICU patients over 6 years. In this case-matched study of 3112 patients, the 30-day mortality was 25.7% among beta blocker users and 31.4% among non-users (OR 0.74 [95% CI: 0.63-0.87]). Herndon and colleagues have successfully shown that propranolol reduces heart rate by 20% in burned septic children, decreases systemic hypermetabolism, and diminishes muscle-protein catabolism over the ensuing 12 months.²⁷² A retrospective study in trauma patients suggests that pretreatment with

beta blockade is associated with a significant decrease in fatal outcome and healing time.²⁷³ Given the multiple studies demonstrating a benefit from beta blockade in general and propranolol in particular, the administration of propranolol in burn wounds greater than 20% is considered the standard of care. However, the propranolol-induced benefit of reducing hypermetabolism and muscle-protein catabolism in the recovery and long-term survival of sepsis patients requires further investigation to prove efficacy.

Although oxandrolone (testosterone derivative) has not been shown to impact immune function in patients with severe burns or sepsis per se, it has become a mainstay of therapy in severely burned populations to improve weight loss, protect muscle mass, and minimize long-term cachectic metabolism.²⁷⁴ In addition, oxandrolone is also associated with improved donor site wound healing.²⁷⁵ In one prospective study, treatment with 10 mg of oxandrolone every 12 hours decreased hospital length of stay.²⁷⁶ Lastly in a prospective, double-blind, randomized single-center trial, oxandrolone administered at a dose of 0.1 mg/kg every 12 hours decreased hospital length of stay, preserved lean body mass, improved overall body composition, and enhanced liver protein synthesis.³⁸ Taken together, there is ample evidence that oxandrolone therapy ameliorates the protein wasting, hypermetabolic state, and long-term cachectic milieu facilitated by severe burn injury. Although direct evidence establishing oxandrolone as a modulator of immune cell function is lacking, insurmountable data exist indicating that protein wasting, cachexia, weight loss, and hypermetabolism are all associated with poor immune function and abysmal outcome in severely injured trauma and critically ill sepsis cohorts.²⁷⁷ This observation has led some to garner optimism for the use of oxandrolone to counteract the well-documented catabolism syndrome observed after sepsis.¹⁶

Dronabinol is the International Nonproprietary Name for the pure isomer of THC (-)-trans- Δ^9 -tetrahydrocannabinol, which is the main isomer found in cannabis. It is used to treat anorexia in people with HIV/AIDS as well as for refractory nausea and vomiting in people undergoing chemotherapy.²⁷⁸ Antagonists at the cannabinoid receptors 1 or 2 (CB1 or CB2) prevent the delay of GI transit and thus may be powerful tools in the future treatment of septic ileus.²⁷⁹ Dexanabinol, a synthetic cannabinoid devoid of psychotropic effects, improves neurological outcome in models of brain trauma, ischemia, and meningitis. In addition, when given 2-3 min before LPS-induced rat endotoxemia, completely abolishes the typical hypotensive response. Furthermore, the drug also markedly suppressed in vitro TNF α production and nitric oxide generation in murine peritoneal macrophages and rat alveolar macrophage cell line exposed to LPS. Dexanabinol may, therefore, have therapeutic implications in the treatment of TNF α -mediated pathologies such as sepsis.²⁸⁰

16 | BIOMARKERS

Over 180 biomarkers have been unsuccessfully evaluated for use in sepsis over the past five decades.²⁸¹ Most of the biomarkers had been tested clinically, primarily as prognostic markers in sepsis; relatively few

have been used for sepsis diagnosis and prognosis. None of the markers have demonstrated sufficient specificity or sensitivity for reasonable utility in clinical practice. In the past, procalcitonin and C-reactive protein have been most widely used but are limited in their ability to distinguish sepsis from other inflammatory conditions or to predict outcome. More recently, the use of serum lactate levels greater than 2 mmol/L (>18 mg/dL) in the absence of hypovolemia is now a biomarker used in the diagnosis of septic shock.⁴ The identification of precise biomarkers to detect and quantitate immune suppression in septic patients will be key to successful interdiction with immune modulatory therapies in sepsis. At the present time, reduced HLA-DR expression on monocytes is the most reliable biomarker to assess the immune status of critically ill patients. Reduced monocyte HLA-DR expression and failure of HLA-DR expression restoration are indicative of immune paralysis, the susceptibility to opportunistic infections, and overall sepsis outcome.²⁸² In addition, the decreased expression of monocyte HLA-DR, the most robust biomarker of sepsis-induced immune suppression, is easily measured by flow cytometry analysis which is quick, easy, and affordable.²⁸³ In a recent report, investigators were able to demonstrate that monocyte PD-L1 expression after 3-4 days of sepsis was associated with risk stratification and mortality. Moreover, monocyte PD-L1 expression was a promising independent prognostic marker for septic shock patients.²⁸⁴ As our knowledge of cellular phenotype and prognosis grows, we will hopefully be able to capitalize on our newfound knowledge of immune suppression and intervene prior to adverse outcomes.

Considering our discussion of cell-specific immune dysregulation and targeted approaches to restore or enhance cellular function also implies that sensitive and specific tests exist for measuring cellular function and dysfunction that easily identify sepsis survivors who may potentially benefit from immune therapy. For instance, the ability to determine that neutrophil phagocytosis and oxidative burst capability is reduced, CD4⁺ cell immune exhaustion is present, and that Th17 cell cytokine production is diminished are all valuable functional read-outs that may serve as new markers of immune dysfunction that can specifically be augmented by targeted therapy. Lastly, genomic prediction modeling can be implemented to identify the inter-individual variation between transcriptomes of patients with sepsis and used to predict long-term outcome.²⁵⁷ As we more clearly begin to understand which aspects of immune dysfunction and suppression are the most crucial for successful outcome, we will be able to develop comprehensive panels of biomarkers comprised of immune phenotypes, cellular functions, genomic alterations, and signaling intermediates to more precisely guide specific immune therapy interventions.

17 | IMMUNE MODULATORY INTERVENTION

The sepsis landscape is littered with failed therapeutic interventions to block particular pathways or processes in humans.¹⁰ Although there are as many explanations as failures, continued attempts to augment immune processes at early time points with the expectation that sepsis mortality will be halved at 2 years are doomed to fail.²⁶ However, if the

sepsis-induced immune derangements are analyzed alongside other immune-mediated disease processes such as cancer, autoimmune disease, or HIV, where immune modulatory therapy has improved patient survival, it is obvious that single-agent therapy is ineffective. Rather, it is preferable to use multiple agents in combination that are introduced at the correct moment and synergistically altered over time, according to disease-specific progression, patient immune responses, and defined host/pathogen genomic interactions.²⁸⁵ Cancer chemotherapy strategies already utilize a personalized combination of therapies to induce, maintain, and prolong cancer disease-free survival based on patient disease progression and tumor-specific genetic patterns. We propose a similar strategy be applied in 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 postsepsis recovery evolves.²² Considering the ease of modern genomic determination and patient screening for specific genetic variations, therapies that enhance microbial eradication could be used to develop a personalized treatment plan. In addition, the widespread and routine use of flow cytometry technology for immune cell phenotyping in blood borne and other cancers types already provides a solid platform for immune cell profiling during cancer, sepsis, and other inflammatory states. Although no FDA-approved sepsis biomarkers exist at present, using a thoughtful approach to plan patient follow-up, evaluate nutritional status, determine immune cell function, and assess inflammation status, will serve as a foundation to gain the necessary insights to identify the appropriate times and immune therapies to provide to the patient.

The collective willingness of sepsis investigators and patient care providers to replace their favorite molecules and interesting mechanistic pathways with a transparent and unified approach to streamline research and patient outcomes to learn meaningful lessons from prior negative intervention trials is paramount. The adoption of the adaptive trial design that has been successfully employed in other disease states should be employed to quickly adjust interventional sepsis trials based on interim analysis and quality reassessment instead of the classic and dogmatic trial design that is methodical and slow natured.²⁸⁶

Finally, the recognition that an episode of sepsis is associated with long-term debilitation, deterioration, and demise in very definable patient populations begs the question why we have such poor follow-up and care after hospital discharge? Patients with cancer follow up with their oncologist and receive chemotherapy for years. Patients with HIV and hepatitis C routinely follow up with their infectious disease specialist for ongoing disease evaluation and microbial therapy alterations over the long term to achieve remission. Until the greater body of inflammation investigators and sepsis clinicians alike coalesce to provide the same long-term care and follow-up, as done in other specialties in other disease states, the long-term sepsis mortality will continue to climb with time.

Although the specific combinations of immune modulation therapy are numerous and all associated with select pros and cons, the following combinations are to serve as an example, to illustrate how long-term sepsis treatment strategies could be employed. These are by no means the only options or even the most correct for all or any

TABLE 1 Potential immune modulatory therapies and their proposed benefits to improve long-term outcomes following sepsis

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

patients, but hopefully will convey to the reader how a successful strategy to curb long-term mortality based on the observed immune defects could be employed.

For example, GM-CSF treatment with IL-3 may enhance 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). Lastly, a low-dose combination of oxandrolone, propranolol, and even dronabinol may ameliorate protein catabolism and persistent inflammation and promote anabolism, which has already been implemented in promoting recovery of severely burned patients (Table 2). Moreover, with improvements in biomarkers, cellular function determination, and host/pathogen genomic prediction models, strategically engineered combinations of immune modulators could be employed in a goal-directed manner based on patient comorbidity profiles, 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. Employing 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 prior sepsis clinical trials.

TABLE 2 Immune modulators used in other disease states with dysregulated inflammation and metabolism and their proposed benefits to improve long-term outcomes following sepsis

Immune modulator	Propranolol	Oxandrolone	Dronabinol
Benefit	Reduce inflammatory cytokine production	Improve weight loss	Improve gut transit, Increase appetite
	Diminish muscle protein catabolism	Protect muscle mass, Reduce length of stay	Reduce TNF α production
	Improve 30-day survival	Minimize cachectic metabolism	Reduce nitric oxide generation

18 | SEPSIS TRIAL DESIGNS

In clinical studies, the enrollment criteria are typically very broad, and the agent is administered on the basis of a standard formula for only a short period (a few days). There is little information on how an agent changes the host response and host-pathogen interactions, and the primary end point is death from any cause. Such a research strategy is probably overly simplistic in that it does not select patients who are most likely to benefit, cannot adjust therapy on the basis of the evolving host response and clinical course, and does not capture potentially important effects except for effects on 28-day mortality. A more advanced strategy would be to implement an adaptive clinical trial design

that evaluates a sepsis treatment by observing clinical outcomes and side effects on a routine schedule.²⁸⁷ This information would then be used to modify the study protocols in accord with those newly acquired observations. The “adaptation process” would continue throughout the trial as described in the trial protocol. Modifications may include drug dosage, sample size, patient selection criteria, and drug cocktail administration. In some instances, trials have become an ongoing process that routinely adds and drops therapies and patient cohorts as more insight is gained.²⁸⁸ Most importantly, the aim of an adaptive trial is to more quickly identify therapies that have a beneficial effect and the patient populations that are appropriate for such treatment.²⁸⁹

19 | CONCLUSIONS

Sepsis induces a multitude of defects in immunity that cause protracted inflammation, immune suppression, susceptibility to infections, and insurmountable death. Although there are new cell-based methodologies available to identify patients with postsepsis immune dysregulation, it is still unclear which interventions and at what time points targeting cell-specific deficits will be most beneficial for sepsis survival. Considering the overlapping, inter-related, and interdigitating complexity of immune cell 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 fail. Conversely, the notion of more thorough and rigorous patient stratification and selection, coupled with strategic and thoughtful long-term monitoring of immune function, combined with goal-directed immune modulatory therapy will, over time, provide optimal clinical benefit to those surviving initial sepsis.

ACKNOWLEDGEMENTS

M. J. D. acknowledges the Research and Education Foundation Scholarship that supported this work and was generously provided by the 2015 American Association for the Surgery of Trauma and the 2016 Shock Society Faculty Research Award for Early Career Faculty Investigators. Additional support was provided by National Institutes of Health grants, GM-29507 and GM-61656, and from the Godfrey D. Stobbe Endowment (P. A. W.).

CONFLICTS OF INTEREST

The authors declare there are no commercial or financial conflicts of interest related to the studies.

REFERENCES

- Leadership Members. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med.* 1992;20:864–874.
- Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med.* 2003;31:1250–1256.
- Kaukonen KM, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. *N Engl J Med.* 2015;372:1629–1638.
- Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA.* 2016;315:801–810.
- Gaieski DF, Edwards JM, Kallan MJ, Carr BG. Benchmarking the incidence and mortality of severe sepsis in the United States. *Crit Care Med.* 2013;41:1167–1174.
- Mayr FB, Yende S, Angus DC. Epidemiology of severe sepsis. *Virulence.* 2014;5:4–11.
- Coopersmith CM, Wunsch H, Fink MP, et al. A comparison of critical care research funding and the financial burden of critical illness in the United States. *Crit Care Med.* 2012;40:1072–1079.
- Martin GS, Mannino DM, Moss M. The effect of age on the development and outcome of adult sepsis. *Crit Care Med.* 2006;34:15–21.
- Kahn JM, Le T, Angus DC, et al. The epidemiology of chronic critical illness in the United States*. *Crit Care Med.* 2015;43:282–287.
- Marshall JC. Why have clinical trials in sepsis failed? *Trends Mol Med.* 2014;20:195–203.
- Deutschman CS, Tracey KJ. Sepsis: Current dogma and new perspectives. *Immunity.* 2014;40:463–475.
- Ward PA, Bosmann M. A historical perspective on sepsis. *Am J Pathol.* 2012;181:2–7.
- Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature.* 1987;330:662–664.
- Schorr CA, Dellinger RP. The Surviving Sepsis Campaign: Past, present and future. *Trends Mol Med.* 2014;20:192–194.
- Boomer JS, To K, Chang KC, et al. Immunosuppression in patients who die of sepsis and multiple organ failure. *JAMA.* 2011;306:2594–2605.
- Gentile LF, Cuenca AG, Efron PA, et al. Persistent inflammation and immunosuppression: A common syndrome and new horizon for surgical intensive care. *J Trauma Acute Care Surg.* 2012;72:1491–1501.
- Winters BD, Eberlein M, Leung J, Needham DM, Pronovost PJ, Sevransky JE. Long-term mortality and quality of life in sepsis: A systematic review. *Crit Care Med.* 2010;38:1276–1283.
- Moore FA, Moore EE. Evolving concepts in the pathogenesis of post-injury multiple organ failure. *Surg Clin North Am.* 1995;75:257–277.
- Delano MJ, Ward PA. Sepsis-induced immune dysfunction: Can immune therapies reduce mortality? *J Clin Invest.* 2016;126:23–31.
- Needham DM, Davidson J, Cohen H, et al. Improving long-term outcomes after discharge from intensive care unit: Report from a stakeholders' conference. *Crit Care Med.* 2012;40:502–509.
- Yende S, D'Angelo G, Kellum JA, et al. Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis. *Am J Respir Crit Care Med.* 2008;177:1242–1247.
- Elliott D, Davidson JE, Harvey MA, et al. Exploring the scope of post-intensive care syndrome therapy and care: Engagement of non-critical care providers and survivors in a second stakeholders meeting. *Crit Care Med.* 2014;42:2518–2526.
- Annane D. What is the evidence for harm of neuromuscular blockade and corticosteroid use in the intensive care unit? *Semin Respir Crit Care Med.* 2016;37:51–56.
- Hotchkiss RS, Moldawer LL. Parallels between cancer and infectious disease. *N Engl J Med.* 2014;371:380–383.
- Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: From cellular dysfunctions to immunotherapy. *Nat Rev Immunol.* 2013;13:862–874.
- Delano MJ, Moldawer LL. Magic bullets and surrogate biomarkers circa 2009. *Crit Care Med.* 2009;37:1796–1798.
- Bosmann M, Ward PA. The inflammatory response in sepsis. *Trends Immunol.* 2013;34:129–136.
- Hotchkiss RS, Coopersmith CM, McDunn JE, Ferguson TA. The sepsis seesaw: Tilting toward immunosuppression. *Nat Med.* 2009;15:496–497.

29. Bone RC, Grodzin CJ, Balk RA. Sepsis: A new hypothesis for pathogenesis of the disease process. *Chest*. 1997;112:235–243.
30. Rittirsch D, Flierl MA, Ward PA. Harmful molecular mechanisms in sepsis. *Nat Rev Immunol*. 2008;8:776–787.
31. Xiao W, Mindrinos MN, Seok J, et al. A genomic storm in critically injured humans. *J Exp Med*. 2011;208:2581–2590.
32. Cuenca AG, Gentile LF, Lopez MC, et al. Development of a genomic metric that can be rapidly used to predict clinical outcome in severely injured trauma patients. *Crit Care Med*. 2013;41:1175–1185.
33. Hutchins NA, Unsinger J, Hotchkiss RS, Ayala A. The new normal: Immunomodulatory agents against sepsis immune suppression. *Trends Mol Med*. 2014;20:224–233.
34. Medzhitov R, Janeway C Jr. Innate immunity. *N Engl J Med*. 2000;343:338–344.
35. Goodwin AJ, Rice DA, Simpson KN, Ford DW. Frequency, cost, and risk factors of readmissions among severe sepsis survivors. *Crit Care Med*. 2015;43:738–746.
36. Gauglitz GG, Williams FN, Herndon DN, Jeschke MG. Burns: Where are we standing with propranolol, oxandrolone, recombinant human growth hormone, and the new incretin analogs? *Curr Opin Clin Nutr Metab Care*. 2011;14:176–181.
37. Norbury WB, Jeschke MG, Herndon DN. Metabolism modulators in sepsis: Propranolol. *Crit Care Med*. 2007;35:S616–620.
38. Jeschke MG, Finnerty CC, Suman OE, Kulp G, Mlcak RP, Herndon DN. The effect of oxandrolone on the endocrinologic, inflammatory, and hypermetabolic responses during the acute phase postburn. *Ann Surg*. 2007;246:351–360; discussion 360–352.
39. Goldszmid RS, Dzutsev A, Trinchieri G. Host immune response to infection and cancer: Unexpected commonalities. *Cell Host Microbe*. 2014;15:295–305.
40. Bohlius J, Herbst C, Reiser M, Schwarzer G, Engert A. Granulopoiesis-stimulating factors to prevent adverse effects in the treatment of malignant lymphoma. *Cochrane Database Syst Rev*. 2008;8:CD003189.
41. Smilek DE, Ehlers MR, Nepom GT. Restoring the balance: Immunotherapeutic combinations for autoimmune disease. *Dis Model Mech*. 2014;7:503–513.
42. Gilroy DW, Vallance P. Resolution for sepsis? *Circulation*. 2005;111:2–4.
43. Fullerton JN, Gilroy DW. Resolution of inflammation: A new therapeutic frontier. *Nat Rev Drug Discov*. 2016;15:551–567.
44. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. *Nature*. 2014;510:92–101.
45. Buckley CD, Gilroy DW, Serhan CN. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. *Immunity*. 2014;40:315–327.
46. Venet F, Chung CS, Monneret G, et al. Regulatory T cell populations in sepsis and trauma. *J Leukoc Biol*. 2008;83:523–535.
47. Venet F, Chung CS, Kherouf H, et al. Increased circulating regulatory T cells (CD4⁺CD25⁺CD127⁻) contribute to lymphocyte anergy in septic shock patients. *Intensive Care Med*. 2009;35:678–686.
48. Brudecki L, Ferguson DA, McCall CE, El Gazzar M. Myeloid-derived suppressor cells evolve during sepsis and can enhance or attenuate the systemic inflammatory response. *Infect Immun*. 2012;80:2026–2034.
49. Dalli J, Chiang N, Serhan CN. Elucidation of novel 13-series resolvins that increase with atorvastatin and clear infections. *Nat Med*. 2015;21:1071–1075.
50. Lee CR, Zeldin DC. Resolving infectious inflammation by targeting the host response. *N Engl J Med*. 2015;373:2183–2185.
51. Deshpande A, Pasupuleti V, Rothberg MB. Statin therapy and mortality from sepsis: A meta-analysis of randomized trials. *Am J Med*. 2015;128(410–417):e411.
52. Cummings CJ, Martin TR, Frevert CW, et al. Expression and function of the chemokine receptors CXCR1 and CXCR2 in sepsis. *J Immunol*. 1999;162:2341–2346.
53. Kovach MA, Standiford TJ. The function of neutrophils in sepsis. *Curr Opin Infect Dis*. 2012;25:321–327.
54. Grailer JJ, Kalbitz M, Zetoune FS, Ward PA. Persistent neutrophil dysfunction and suppression of acute lung injury in mice following cecal ligation and puncture sepsis. *J Innate Immunity*. 2014;6:695–705.
55. Huber-Lang M, Sarma VJ, Lu KT, et al. Role of C5a in multiorgan failure during sepsis. *J Immunol*. 2001;166:1193–1199.
56. Cavaillon JM, Adib-Conquy M. Monocytes/macrophages and sepsis. *Crit Care Med*. 2005;33:S506–509.
57. Benjamim CF, Lundy SK, Lukacs NW, Hogaboam CM, Kunkel SL. Reversal of long-term sepsis-induced immunosuppression by dendritic cells. *Blood*. 2005;105:3588–3595.
58. Pene F, Zuber B, Courtine E, et al. Dendritic cells modulate lung response to *Pseudomonas aeruginosa* in a murine model of sepsis-induced immune dysfunction. *J Immunol*. 2008;181:8513–8520.
59. Grimaldi D, Litjós JF, Pene F. Post-infectious immune suppression: A new paradigm of severe infections. *Med Mal Infect*. 2014;44:455–463.
60. Hotchkiss RS, Tinsley KW, Swanson PE, et al. Depletion of dendritic cells, but not macrophages, in patients with sepsis. *J Immunol*. 2002;168:2493–2500.
61. Hotchkiss RS, Swanson PE, Freeman BD, et al. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. *Crit Care Med*. 1999;27:1230–1251.
62. Walton AH, Muenzer JT, Rasche D, et al. Reactivation of multiple viruses in patients with sepsis. *PLoS ONE*. 2014;9:e98819.
63. Drewry AM, Samra N, Skrupky LP, Fuller BM, Compton SM, Hotchkiss RS. Persistent lymphopenia after diagnosis of sepsis predicts mortality. *Shock*. 2014;42:383–391.
64. Otto GP, Sossdorf M, Claus RA, et al. The late phase of sepsis is characterized by an increased microbiological burden and death rate. *Crit Care*. 2011;15:R183.
65. Prescott HC, Langa KM, Iwashyna TJ. Readmission diagnoses after hospitalization for severe sepsis and other acute medical conditions. *JAMA*. 2015;313:1055–1057.
66. Jones TK, Fuchs BD, Small DS, et al. Post-acute care use and hospital readmission after sepsis. *Ann Am Thorac Soc*. 2015;12:904–913.
67. Chang DW, Tseng CH, Shapiro MF. Rehospitalizations following sepsis: Common and costly. *Crit Care Med*. 2015;43:2085–2093.
68. Jensen US, Knudsen JD, Wehberg S, Gregson DB, Laupland KB. Risk factors for recurrence and death after bacteraemia: A population-based study. *Clin Microbiol Infect*. 2011;17:1148–1154.
69. Prescott HC, Dickson RP, Rogers MA, Langa KM, Iwashyna TJ. Hospitalization type and subsequent severe sepsis. *Am J Respir Crit Care Med*. 2015;192:581–588.
70. Prescott HC. Toward a nuanced understanding of the role of infection in readmissions after sepsis. *Crit Care Med*. 2016;44:634–635.
71. Sun A, Netzer G, Small DS, et al. Association between index hospitalization and hospital readmission in sepsis survivors. *Crit Care Med*. 2016;44:478–487.
72. Leibovici L. Long-term consequences of severe infections. *Clin Microbiol Infect*. 2013;19:510–512.
73. van Vught LA, Klein Klouwenberg PM, Spitoni C, et al. Incidence, risk factors, and attributable mortality of secondary infections in the intensive care unit after admission for sepsis. *JAMA*. 2016;315:1469–1479.
74. Wang T, Derhovanessian A, De Cruz S, Belperio JA, Deng JC, Hoo GS. Subsequent infections in survivors of sepsis: Epidemiology and outcomes. *J Intensive Care Med*. 2014;29:87–95.
75. Sogaard M, Thomsen RW, Bang RB, Schonheyder HC, Norgaard M. Trends in length of stay, mortality and readmission among patients with community-acquired bacteraemia. *Clin Microbiol Infect*. 2015;21(789):e781–787.
76. Suzuki K, Inoue S, Kametani Y, et al. Reduced immunocompetent B cells and increased secondary infection in elderly patients with severe sepsis. *Shock*. 2016;46:270–278.

77. Martin GS. Sepsis, severe sepsis and septic shock: Changes in incidence, pathogens and outcomes. *Expert Rev Anti Infect Ther.* 2012;10:701–706.
78. Unnewehr H, Rittirsch D, Sarma JV, et al. Changes and regulation of the C5a receptor on neutrophils during septic shock in humans. *J Immunol.* 2013;190:4215–4225.
79. Czermak BJ, Sarma V, Pierson CL, et al. Protective effects of C5a blockade in sepsis. *Nat Med.* 1999;5:788–792.
80. Huber-Lang MS, Sarma JV, McGuire SR, et al. Protective effects of anti-C5a peptide antibodies in experimental sepsis. *FASEB J.* 2001;15:568–570.
81. Huber-Lang M, Sarma JV, Zetoune FS, et al. Generation of C5a in the absence of C3: A new complement activation pathway. *Nat Med.* 2006;12:682–687.
82. Ward PA. The harmful role of c5a on innate immunity in sepsis. *J Innate Immun.* 2010;2:439–445.
83. Melis JP, Strumane K, Ruuls SR, Beurskens FJ, Schuurman J, Parren PW. Complement in therapy and disease: Regulating the complement system with antibody-based therapeutics. *Mol Immunol.* 2015;67:117–130.
84. Ogilvie AC, Groeneveld AB, Straub JP, Thijs LG. Plasma lipid peroxides and antioxidants in human septic shock. *Intensive Care Med.* 1991;17:40–44.
85. Cowley HC, Bacon PJ, Goode HF, Webster NR, Jones JG, Menon DK. Plasma antioxidant potential in severe sepsis: A comparison of survivors and nonsurvivors. *Crit Care Med.* 1996;24:1179–1183.
86. Santos SS, Brunialti MK, Rigato O, Machado FR, Silva E, Salomao R. Generation of nitric oxide and reactive oxygen species by neutrophils and monocytes from septic patients and association with outcomes. *Shock.* 2012;38:18–23.
87. Andrades ME, Morina A, Spasic S, Spasojevic I. Bench-to-bedside review: Sepsis – From the redox point of view. *Crit Care.* 2011;15:230.
88. Ojeda Ojeda M, Larrondo Muguercia H, Magdariaga Figuerola A, et al. Temporal trends of circulating nitric oxide and pro-inflammatory cytokine responses ex vivo in intra-abdominal sepsis: Results from a cohort study. *Inflamm Res.* 2011;60:289–297.
89. Dare AJ, Phillips AR, Hickey AJ, et al. A systematic review of experimental treatments for mitochondrial dysfunction in sepsis and multiple organ dysfunction syndrome. *Free Radic Biol Med.* 2009;47:1517–1525.
90. Mishra V. Oxidative stress and role of antioxidant supplementation in critical illness. *Clin Lab.* 2007;53:199–209.
91. Muller B, Becker KL, Kranzlin M, et al. Disordered calcium homeostasis of sepsis: Association with calcitonin precursors. *Eur J Clin Invest.* 2000;30:823–831.
92. Zivin JR, Gooley T, Zager RA, Ryan MJ. Hypocalcemia: A pervasive metabolic abnormality in the critically ill. *Am J Kidney Dis.* 2001;37:689–698.
93. Desai TK, Carlson RW, Geheb MA. Prevalence and clinical implications of hypocalcemia in acutely ill patients in a medical intensive care setting. *Am J Med.* 1988;84:209–214.
94. Sayeed MM. Signaling mechanisms of altered cellular responses in trauma, burn, and sepsis: Role of Ca²⁺. *Arch Surg.* 2000;135:1432–1442.
95. Zaloga GP, Washburn D, Black KW, Prielipp R. Human sepsis increases lymphocyte intracellular calcium. *Crit Care Med.* 1993;21:196–202.
96. Thompson M, Kliewer A, Maass D, et al. Increased cardiomyocyte intracellular calcium during endotoxin-induced cardiac dysfunction in guinea pigs. *Pediatr Res.* 2000;47:669–676.
97. Song SK, Karl IE, Ackerman JJ, Hotchkiss RS. Increased intracellular Ca²⁺: A critical link in the pathophysiology of sepsis? *Proc Natl Acad Sci USA.* 1993;90:3933–3937.
98. Collage RD, Howell GM, Zhang X, et al. Calcium supplementation during sepsis exacerbates organ failure and mortality via calcium/calmodulin-dependent protein kinase signaling. *Crit Care Med.* 2013;41:e352–360.
99. Ba X, Garg NJ. Signaling mechanism of poly(ADP-ribose) polymerase-1 (PARP-1) in inflammatory diseases. *Am J Pathol.* 2011;178:946–955.
100. Liaudet L, Oddo M. Role of poly(adenosine diphosphate-ribose) polymerase 1 in septic peritonitis. *Curr Opin Crit Care.* 2003;9:152–158.
101. Wongsrichanalai C, Namsiripongpun V, Pornsilapatip J, Kyle DE, Wilde H. Sensitivity of QBC malaria test. *Lancet.* 1992;340:792–793.
102. Soriano FG, Liaudet L, Szabo E, et al. Resistance to acute septic peritonitis in poly(ADP-ribose) polymerase-1-deficient mice. *Shock.* 2002;17:286–292.
103. Hotchkiss RS, Strasser A, McDunn JE, Swanson PE. Cell death. *N Engl J Med.* 2009;361:1570–1583.
104. Larsen FJ, Schiffer TA, Weitzberg E, Lundberg JO. Regulation of mitochondrial function and energetics by reactive nitrogen oxides. *Free Radic Biol Med.* 2012;53:1919–1928.
105. Szabo C, Modis K. Pathophysiological roles of peroxynitrite in circulatory shock. *Shock.* 2010;34(Suppl 1):4–14.
106. Brealey D, Brand M, Hargreaves I, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. *Lancet.* 2002;360:219–223.
107. Srinivasan S, Avadhani NG. Cytochrome c oxidase dysfunction in oxidative stress. *Free Radic Biol Med.* 2012;53:1252–1263.
108. Singer M. The role of mitochondrial dysfunction in sepsis-induced multi-organ failure. *Virulence.* 2014;5:66–72.
109. Brealey D, Karyampudi S, Jacques TS, et al. Mitochondrial dysfunction in a long-term rodent model of sepsis and organ failure. *Am J Physiol Regul Integr Comp Physiol.* 2004;286:R491–497.
110. Gentile LF, Nacionales DC, Lopez MC, et al. A better understanding of why murine models of trauma do not recapitulate the human syndrome. *Crit Care Med.* 2014;42:1406–1413.
111. Opitz B, Eitel J, Meixenberger K, Suttrop N. Role of Toll-like receptors, NOD-like receptors and RIG-I-like receptors in endothelial cells and systemic infections. *Thromb Haemost.* 2009;102:1103–1109.
112. Faure E, Equils O, Sieling PA, et al. Bacterial lipopolysaccharide activates NF-kappaB through toll-like receptor 4 (TLR-4) in cultured human dermal endothelial cells. Differential expression of TLR-4 and TLR-2 in endothelial cells. *J Biol Chem.* 2000;275:11058–11063.
113. Ince C, Mayeux PR, Nguyen T, et al. The endothelium in sepsis. *Shock.* 2016;45:259–270.
114. Colbert JF, Schmidt EP. Endothelial and microcirculatory function and dysfunction in sepsis. *Clin Chest Med.* 2016;37:263–275.
115. Mikacenic C, Hahn WO, Price BL, et al. Biomarkers of endothelial activation are associated with poor outcome in critical illness. *PLoS ONE.* 2015;10:e0141251.
116. van Ierssel SH, Van Craenenbroeck EM, Hoymans VY, Vrints CJ, Conraads VM, Jorens PG. Endothelium dependent vasomotion and in vitro markers of endothelial repair in patients with severe sepsis: An observational study. *PLoS ONE.* 2013;8:e69499.
117. Mostefai HA, Meziani F, Mastronardi ML, et al. Circulating microparticles from patients with septic shock exert protective role in vascular function. *Am J Respir Crit Care Med.* 2008;178:1148–1155.
118. Nathan C. Neutrophils and immunity: Challenges and opportunities. *Nat Rev Immunol.* 2006;6:173–182.
119. Link DC. Neutrophil homeostasis: A new role for stromal cell-derived factor-1. *Immunol Res.* 2005;32:169–178.
120. Hotchkiss RS, Nicholson DW. Apoptosis and caspases regulate death and inflammation in sepsis. *Nat Rev Immunol.* 2006;6:813–822.
121. Delano MJ, Thayer T, Gabrilovich S, et al. Sepsis induces early alterations in innate immunity that impact mortality to secondary infection. *J Immunol.* 2011;186:195–202.
122. Eash KJ, Greenbaum AM, Gopalan PK, Link DC. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *J Clin Invest.* 2010;120:2423–2431.
123. Delano MJ, Kelly-Scumpia KM, Thayer TC, et al. Neutrophil mobilization from the bone marrow during polymicrobial sepsis is dependent on CXCL12 signaling. *J Immunol.* 2011;187:911–918.

124. Lerman YV, Lim K, Hyun YM, et al. Sepsis lethality via exacerbated tissue infiltration and TLR-induced cytokine production by neutrophils is integrin alpha3beta1-dependent. *Blood*. 2014;124:3515–3523.
125. Alves-Filho JC, Freitas A, Souto FO, et al. Regulation of chemokine receptor by Toll-like receptor 2 is critical to neutrophil migration and resistance to polymicrobial sepsis. *Proc Natl Acad Sci USA*. 2009;106:4018–4023.
126. Nacionales DC, Gentile LF, Vanzant E, et al. Aged mice are unable to mount an effective myeloid response to sepsis. *J Immunol*. 2014;192:612–622.
127. Delano MJ, Scumpia PO, Weinstein JS, et al. MyD88-dependent expansion of an immature GR-1(+)CD11b(+) population induces T cell suppression and Th2 polarization in sepsis. *J Exp Med*. 2007;204:1463–1474.
128. Davey MS, Morgan MP, Liuzzi AR, et al. Microbe-specific unconventional T cells induce human neutrophil differentiation into antigen cross-presenting cells. *J Immunol*. 2014;193:3704–3716.
129. Morris AC, Brittan M, Wilkinson TS, et al. C5a-mediated neutrophil dysfunction is RhoA-dependent and predicts infection in critically ill patients. *Blood*. 2011;117:5178–5188.
130. Wilkinson TS, Morris AC, Kefala K, et al. Ventilator-associated pneumonia is characterized by excessive release of neutrophil proteases in the lung. *Chest*. 2012;142:1425–1432.
131. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. *Science*. 2004;303:1532–1535.
132. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science*. 2015;349:316–320.
133. Sorensen OE, Borregaard N. Neutrophil extracellular traps – The dark side of neutrophils. *J Clin Invest*. 2016;126:1612–1620.
134. McDonald B, Urrutia R, Yipp BG, Jenne CN, Kubes P. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe*. 2012;12:324–333.
135. Czaikoski PG, Mota JM, Nascimento DC, et al. Neutrophil extracellular traps induce organ damage during experimental and clinical sepsis. *PLoS ONE*. 2016;11:e0148142.
136. Cheadle WG, Wilson M, Hershman MJ, Bergamini D, Richardson JD, Polk HC Jr. Comparison of trauma assessment scores and their use in prediction of infection and death. *Ann Surg*. 1989;209:541–545; discussion 545–546.
137. Livingston DH, Appel SH, Wellhausen SR, Sonnenfeld G, Polk HC Jr. Depressed interferon gamma production and monocyte HLA-DR expression after severe injury. *Arch Surg*. 1988;123:1309–1312.
138. Munoz C, Carlet J, Fitting C, Misset B, Bleriot JP, Cavaillon JM. Dysregulation of in vitro cytokine production by monocytes during sepsis. *J Clin Invest*. 1991;88:1747–1754.
139. Docke WD, Randow F, Syrbe U, et al. Monocyte deactivation in septic patients: Restoration by IFN-gamma treatment. *Nat Med*. 1997;3:678–681.
140. Cazalis MA, Friggeri A, Cave L, et al. Decreased HLA-DR antigen-associated invariant chain (CD74) mRNA expression predicts mortality after septic shock. *Crit Care*. 2013;17:R287.
141. Cheron A, Floccard B, Allaouchiche B, et al. Lack of recovery in monocyte human leukocyte antigen-DR expression is independently associated with the development of sepsis after major trauma. *Crit Care*. 2010;14:R208.
142. Landelle C, Lepape A, Voirin N, et al. Low monocyte human leukocyte antigen-DR is independently associated with nosocomial infections after septic shock. *Intensive Care Med*. 2010;36:1859–1866.
143. Venet F, Pachot A, Debard AL, et al. Human CD4⁺CD25⁺ regulatory T lymphocytes inhibit lipopolysaccharide-induced monocyte survival through a Fas/Fas ligand-dependent mechanism. *J Immunol*. 2006;177:6540–6547.
144. Monneret G, Venet F, Pachot A, Lepape A. Monitoring immune dysfunctions in the septic patient: A new skin for the old ceremony. *Mol Med*. 2008;14:64–78.
145. Carson WF, Cavassani KA, Dou Y, Kunkel SL. Epigenetic regulation of immune cell functions during post-septic immunosuppression. *Epigenetics*. 2011;6:273–283.
146. Ishii M, Wen H, Corsa CA, et al. Epigenetic regulation of the alternatively activated macrophage phenotype. *Blood*. 2009;114:3244–3254.
147. Saeed S, Quintin J, Kerstens HH, et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science*. 2014;345:1251086.
148. Poli A, Michel T, Theresine M, Andres E, Hentges F, Zimmer J. CD56bright natural killer (NK) cells: An important NK cell subset. *Immunology*. 2009;126:458–465.
149. Halstead ES, Carcillo JA, Schilling B, Greiner RJ, Whiteside TL. Reduced frequency of CD56 dim CD16 pos natural killer cells in pediatric systemic inflammatory response syndrome/sepsis patients. *Pediatr Res*. 2013;74:427–432.
150. Giamarellos-Bourboulis EJ, Tsaganos T, Spyridaki E, et al. Early changes of CD4-positive lymphocytes and NK cells in patients with severe Gram-negative sepsis. *Crit Care*. 2006;10:R166.
151. Souza-Fonseca-Guimaraes F, Parlato M, Philippart F, et al. Toll-like receptors expression and interferon-gamma production by NK cells in human sepsis. *Crit Care*. 2012;16:R206.
152. Chiche L, Forel JM, Thomas G, et al. Interferon-gamma production by natural killer cells and cytomegalovirus in critically ill patients. *Crit Care Med*. 2012;40:3162–3169.
153. Limaye AP, Kirby KA, Rubenfeld GD, et al. Cytomegalovirus reactivation in critically ill immunocompetent patients. *JAMA*. 2008;300:413–422.
154. Giamarellos-Bourboulis EJ. Natural killer cells in sepsis: Detrimental role for final outcome. *Crit Care Med*. 2014;42:1579–1580.
155. Broquet A, Roquilly A, Jacqueline C, Potel G, Caillon J, Asehnoune K. Depletion of natural killer cells increases mice susceptibility in a *Pseudomonas aeruginosa* pneumonia model. *Crit Care Med*. 2014;42:e441–450.
156. Grimaldi D, Louis S, Pene F, et al. Profound and persistent decrease of circulating dendritic cells is associated with ICU-acquired infection in patients with septic shock. *Intensive Care Med*. 2011;37:1438–1446.
157. Pastille E, Didovic S, Brauckmann D, et al. Modulation of dendritic cell differentiation in the bone marrow mediates sustained immunosuppression after polymicrobial sepsis. *J Immunol*. 2011;186:977–986.
158. Efron PA, Martins A, Minnich D, et al. Characterization of the systemic loss of dendritic cells in murine lymph nodes during polymicrobial sepsis. *J Immunol*. 2004;173:3035–3043.
159. Scumpia PO, McAuliffe PF, O'Malley KA, et al. CD11c⁺ dendritic cells are required for survival in murine polymicrobial sepsis. *J Immunol*. 2005;175:3282–3286.
160. Toliver-Kinsky TE, Lin CY, Herndon DN, Sherwood ER. Stimulation of hematopoiesis by the Fms-like tyrosine kinase 3 ligand restores bacterial induction of Th1 cytokines in thermally injured mice. *Infect Immun*. 2003;71:3058–3067.
161. Ochoa JB, Bernard AC, O'Brien WE, et al. Arginase I expression and activity in human mononuclear cells after injury. *Ann Surg*. 2001;233:393–399.
162. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol*. 2012;12:253–268.
163. Cuenca AG, Delano MJ, Kelly-Scumpia KM, et al. A paradoxical role for myeloid-derived suppressor cells in sepsis and trauma. *Mol Med*. 2011;17:281–292.
164. Makarenkova VP, Bansal V, Matta BM, Perez LA, Ochoa JB. CD11b⁺/Gr-1⁺ myeloid suppressor cells cause T cell dysfunction after traumatic stress. *J Immunol*. 2006;176:2085–2094.
165. Kelly-Scumpia KM, Scumpia PO, Delano MJ, et al. Type I interferon signaling in hematopoietic cells is required for survival in mouse polymicrobial sepsis by regulating CXCL10. *J Exp Med*. 2010;207:319–326.

166. Scumpia PO, Kelly-Scumpia KM, Delano MJ, et al. Cutting edge: Bacterial infection induces hematopoietic stem and progenitor cell expansion in the absence of TLR signaling. *J Immunol.* 2010;184:2247–2251.
167. Cartwright GE, Athens JW, Wintrobe MM. The kinetics of granulopoiesis in normal man. *Blood.* 1964;24:780–803.
168. McCabe A, Zhang Y, Thai V, Jones M, Jordan MB, MacNamara KC. Macrophage-lineage cells negatively regulate the hematopoietic stem cell pool in response to interferon gamma at steady state and during infection. *Stem Cells.* 2015;33:2294–2305.
169. Mathias B, Delmas AL, Ozrazgat-Baslanti T, et al. Human myeloid-derived suppressor cells are associated with chronic immune suppression after severe sepsis/septic shock. *Ann Surg.* 2016 May 9. Epub ahead of print.
170. Terashima A, Okamoto K, Nakashima T, Akira S, Ikuta K, Takayanagi H. Sepsis-induced osteoblast ablation causes immunodeficiency. *Immunity.* 2016;44:1434–1443.
171. Hotchkiss RS, Coopersmith CM, Karl IE. Prevention of lymphocyte apoptosis—a potential treatment of sepsis? *Clin Infect Dis.* 2005;41(Suppl 7):S465–469.
172. Lang JD, Matute-Bello G. Lymphocytes, apoptosis and sepsis: Making the jump from mice to humans. *Crit Care.* 2009;13:109.
173. Hotchkiss RS, Swanson PE, Cobb JP, Jacobson A, Buchman TG, Karl IE. Apoptosis in lymphoid and parenchymal cells during sepsis: Findings in normal and T- and B-cell-deficient mice. *Crit Care Med.* 1997;25:1298–1307.
174. Hotchkiss RS, Osmon SB, Chang KC, Wagner TH, Coopersmith CM, Karl IE. Accelerated lymphocyte death in sepsis occurs by both the death receptor and mitochondrial pathways. *J Immunol.* 2005;174:5110–5118.
175. Liu ZG, Ni SY, Chen GM, et al. Histones-mediated lymphocyte apoptosis during sepsis is dependent on p38 phosphorylation and mitochondrial permeability transition. *PLoS ONE.* 2013;8:e77131.
176. Grailer JJ, Fattahi F, Dick RS, Zetoune FS, Ward PA. Cutting edge: Critical role for C5aRs in the development of septic lymphopenia in mice. *J Immunol.* 2015;194:868–872.
177. Unsinger J, McGlynn M, Kasten KR, et al. IL-7 promotes T cell viability, trafficking, and functionality and improves survival in sepsis. *J Immunol.* 2010;184:3768–3779.
178. Brahmamdam P, Inoue S, Unsinger J, Chang KC, McDunn JE, Hotchkiss RS. Delayed administration of anti-PD-1 antibody reverses immune dysfunction and improves survival during sepsis. *J Leukoc Biol.* 2010;88:233–240.
179. Holtmeier W, Kabelitz D. gammadelta T cells link innate and adaptive immune responses. *Chem Immunol Allergy.* 2005;86:151–183.
180. Andreu-Ballester JC, Tormo-Calandin C, Garcia-Ballesteros C, et al. Association of gammadelta T cells with disease severity and mortality in septic patients. *Clin Vaccine Immunol.* 2013;20:738–746.
181. Grimaldi D, Le Bourhis L, Sauneuf B, et al. Specific MAIT cell behaviour among innate-like T lymphocytes in critically ill patients with severe infections. *Intensive Care Med.* 2014;40:192–201.
182. Tomasello E, Bedoui S. Intestinal innate immune cells in gut homeostasis and immunosurveillance. *Immunol Cell Biol.* 2013;91:201–203.
183. Szabo PA, Anantha RV, Shaler CR, McCormick JK, Haeryfar SM. CD1d- and MR1-restricted T cells in sepsis. *Front Immunol.* 2015;6:401.
184. Gutcher I, Becher B. APC-derived cytokines and T cell polarization in autoimmunity inflammation. *J Clin Invest.* 2007;117:1119–1127.
185. Hotchkiss RS, Tinsley KW, Swanson PE, et al. Sepsis-induced apoptosis causes progressive profound depletion of B and CD4⁺ T lymphocytes in humans. *J Immunol.* 2001;166:6952–6963.
186. O'Sullivan ST, Lederer JA, Horgan AF, Chin DH, Mannick JA, Rodrick ML. Major injury leads to predominance of the T helper-2 lymphocyte phenotype and diminished interleukin-12 production associated with decreased resistance to infection. *Ann Surg.* 1995;222:482–490; discussion 490–482.
187. Pachot A, Monneret G, Voirin N, et al. Longitudinal study of cytokine and immune transcription factor mRNA expression in septic shock. *Clin Immunol.* 2005;114:61–69.
188. Romani L. Immunity to fungal infections. *Nat Rev Immunol.* 2011;11:275–288.
189. Wu HP, Chung K, Lin CY, Jiang BY, Chuang DY, Liu YC. Associations of T helper 1, 2, 17 and regulatory T lymphocytes with mortality in severe sepsis. *Inflamm Res.* 2013;62:751–763.
190. Smeekens SP, Ng A, Kumar V, et al. Functional genomics identifies type I interferon pathway as central for host defense against *Candida albicans*. *Nat Commun.* 2013;4:1342.
191. Spec A, Shindo Y, Burnham CA, et al. T cells from patients with *Candida* sepsis display a suppressive immunophenotype. *Crit Care.* 2016;20:15.
192. Zanin-Zhorov A, Cahalon L, Tal G, Margalit R, Lider O, Cohen IR. Heat shock protein 60 enhances CD4⁺CD25⁺ regulatory T cell function via innate TLR2 signaling. *J Clin Invest.* 2006;116:2022–2032.
193. Scumpia PO, Delano MJ, Kelly KM, et al. Increased natural CD4⁺CD25⁺ regulatory T cells and their suppressor activity do not contribute to mortality in murine polymicrobial sepsis. *J Immunol.* 2006;177:7943–7949.
194. Scumpia PO, Delano MJ, Kelly-Scumpia KM, et al. Treatment with G1TR agonistic antibody corrects adaptive immune dysfunction in sepsis. *Blood.* 2007;110:3673–3681.
195. Cavassani KA, Carson W, Moreira AP, et al. The post sepsis-induced expansion and enhanced function of regulatory T cells create an environment to potentiate tumor growth. *Blood.* 2010;115:4403–4411.
196. Mauri C, Bosma A. Immune regulatory function of B cells. *Annu Rev Immunol.* 2012;30:221–241.
197. Monserrat J, de Pablo R, Diaz-Martin D, et al. Early alterations of B cells in patients with septic shock. *Crit Care.* 2013;17:R105.
198. Kelly-Scumpia KM, Scumpia PO, Weinstein JS, et al. B cells enhance early innate immune responses during bacterial sepsis. *J Exp Med.* 2011;208:1673–1682.
199. Rauch PJ, Chudnovskiy A, Robbins CS, et al. Innate response activator B cells protect against microbial sepsis. *Science.* 2012;335:597–601.
200. Weber GF, Chousterman BG, He S, et al. Interleukin-3 amplifies acute inflammation and is a potential therapeutic target in sepsis. *Science.* 2015;347:1260–1265.
201. Hotchkiss RS, Sherwood ER. Immunology. Getting sepsis therapy right. *Science.* 2015;347:1201–1202.
202. Lieschke GJ, Grail D, Hodgson G, et al. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. *Blood.* 1994;84:1737–1746.
203. Petit I, Szyper-Kravitz M, Nagler A, et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol.* 2002;3:687–694.
204. Nelson S, Belknap SM, Carlson RW, et al. A randomized controlled trial of filgrastim as an adjunct to antibiotics for treatment of hospitalized patients with community-acquired pneumonia. CAP Study Group. *J Infect Dis.* 1998;178:1075–1080.
205. Root RK, Lodato RF, Patrick W, et al. Multicenter, double-blind, placebo-controlled study of the use of filgrastim in patients hospitalized with pneumonia and severe sepsis. *Crit Care Med.* 2003;31:367–373.
206. Francisco-Cruz A, Aguilar-Santelises M, Ramos-Espinosa O, et al. Granulocyte-macrophage colony-stimulating factor: Not just another haematopoietic growth factor. *Med Oncol.* 2014;31:774.
207. Paine R 3rd, Standiford TJ, Dechert RE, et al. A randomized trial of recombinant human granulocyte-macrophage colony stimulating factor for patients with acute lung injury. *Crit Care Med.* 2012;40:90–97.

208. Meisel C, Schefold JC, Pchowski R, et al. Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: A double-blind, randomized, placebo-controlled multicenter trial. *Am J Respir Crit Care Med.* 2009;180:640–648.
209. Hall MW, Knatz NL, Vetterly C, et al. Immunoparalysis and nosocomial infection in children with multiple organ dysfunction syndrome. *Intensive Care Med.* 2011;37:525–532.
210. Bo L, Wang F, Zhu J, Li J, Deng X. Granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) for sepsis: A meta-analysis. *Crit Care.* 2011;15:R58.
211. Mathias B, Szpila BE, Moore FA, Efron PA, Moldawer LL. A review of GM-CSF therapy in sepsis. *Medicine (Baltimore).* 2015;94:e2044.
212. Nalos M, Santner-Nanan B, Parnell G, Tang B, McLean AS, Nanan R. Immune effects of interferon gamma in persistent staphylococcal sepsis. *Am J Respir Crit Care Med.* 2012;185:110–112.
213. Dries DJ, Jurkovich GJ, Maier RV, et al. Effect of interferon gamma on infection-related death in patients with severe injuries. A randomized, double-blind, placebo-controlled trial. *Arch Surg.* 1994;129:1031–1041; discussion 1042.
214. Cheng SC, Scicluna BP, Arts RJ, et al. Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. *Nat Immunol.* 2016;17:406–413.
215. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol.* 2013;13:227–242.
216. Day CL, Kaufmann DE, Kiepiela P, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature.* 2006;443:350–354.
217. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med.* 2012;366:2443–2454.
218. Zhang Y, Li J, Lou J, et al. Upregulation of programmed death-1 on T cells and programmed death ligand-1 on monocytes in septic shock patients. *Crit Care.* 2011;15:R70.
219. Wang JF, Li JB, Zhao YJ, et al. Up-regulation of programmed cell death 1 ligand 1 on neutrophils may be involved in sepsis-induced immunosuppression: An animal study and a prospective case-control study. *Anesthesiology.* 2015;122:852–863.
220. Chang KC, Burnham CA, Compton SM, et al. Blockade of the negative co-stimulatory molecules PD-1 and CTLA-4 improves survival in primary and secondary fungal sepsis. *Crit Care.* 2013;17:R85.
221. Mackall CL, Fry TJ, Gress RE. Harnessing the biology of IL-7 for therapeutic application. *Nat Rev Immunol.* 2011;11:330–342.
222. Perales MA, Goldberg JD, Yuan J, et al. Recombinant human interleukin-7 (CYT107) promotes T-cell recovery after allogeneic stem cell transplantation. *Blood.* 2012;120:4882–4891.
223. Demaret J, Dupont G, Venet F, et al. STAT5 phosphorylation in T cell subsets from septic patients in response to recombinant human interleukin-7: A pilot study. *J Leukoc Biol.* 2015;97:791–796.
224. Kinter AL, Godbout EJ, McNally JP, et al. The common gamma-chain cytokines IL-2, IL-7, IL-15, and IL-21 induce the expression of programmed death-1 and its ligands. *J Immunol.* 2008;181:6738–6746.
225. Levy Y, Sereti I, Tambussi G, et al. Effects of recombinant human interleukin 7 on T-cell recovery and thymic output in HIV-infected patients receiving antiretroviral therapy: Results of a phase I/IIa randomized, placebo-controlled, multicenter study. *Clin Infect Dis.* 2012;55:291–300.
226. Pelletier M, Ratthe C, Girard D. Mechanisms involved in interleukin-15-induced suppression of human neutrophil apoptosis: Role of the anti-apoptotic Mcl-1 protein and several kinases including Janus kinase-2, p38 mitogen-activated protein kinase and extracellular signal-regulated kinases-1/2. *FEBS Lett.* 2002;532:164–170.
227. Inoue S, Unsinger J, Davis CG, et al. IL-15 prevents apoptosis, reverses innate and adaptive immune dysfunction, and improves survival in sepsis. *J Immunol.* 2010;184:1401–1409.
228. Tracey KJ. The inflammatory reflex. *Nature.* 2002;420:853–859.
229. Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. *J Clin Invest.* 2007;117:289–296.
230. Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature.* 2000;405:458–462.
231. Wang H, Liao H, Ochani M, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat Med.* 2004;10:1216–1221.
232. Ulloa L. The vagus nerve and the nicotinic anti-inflammatory pathway. *Nat Rev Drug Discov.* 2005;4:673–684.
233. Tracey KJ. Reflex control of immunity. *Nat Rev Immunol.* 2009;9:418–428.
234. Song XM, Li JG, Wang YL, et al. The protective effect of the cholinergic anti-inflammatory pathway against septic shock in rats. *Shock.* 2008;30:468–472.
235. van Westerloo DJ, Giebelen IA, Florquin S, et al. The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis. *J Infect Dis.* 2005;191:2138–2148.
236. Rosas-Ballina M, Tracey KJ. Cholinergic control of inflammation. *J Intern Med.* 2009;265:663–679.
237. Kox M, Pickkers P. Modulation of the innate immune response through the vagus nerve. *Nephron.* 2015;131:79–84.
238. Koopman FA, Chavan SS, Miljko S, et al. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. *Proc Natl Acad Sci USA.* 2016;113:8284–8289.
239. Gabanyi I, Muller PA, Feighery L, Oliveira TY, Costa-Pinto FA, Mucida D. Neuro-immune interactions drive tissue programming in intestinal macrophages. *Cell.* 2016;164:378–391.
240. Tracey KJ. Reflexes in immunity. *Cell.* 2016;164:343–344.
241. Kempner W. The nature of leukemic blood cells as determined by their metabolism. *J Clin Invest.* 1939;18:291–300.
242. Newsholme P, Curi R, Gordon S, Newsholme EA. Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. *Biochem J.* 1986;239:121–125.
243. Newsholme P, Newsholme EA. Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture. *Biochem J.* 1989;261:211–218.
244. Oren R, Farnham AE, Saito K, Milofsky E, Karnovsky ML. Metabolic patterns in three types of phagocytizing cells. *J Cell Biol.* 1963;17:487–501.
245. Levene PA, Meyer GM. On the action of leucocytes on glucose. *J Biol Chem.* 1912;12:265–273.
246. Ardawi MS, Newsholme EA. Maximum activities of some enzymes of glycolysis, the tricarboxylic acid cycle and ketone-body and glutamine utilization pathways in lymphocytes of the rat. *Biochem J.* 1982;208:743–748.
247. Newsholme EA, Crabtree B, Ardawi MS. Glutamine metabolism in lymphocytes: Its biochemical, physiological and clinical importance. *Q J Exp Physiol.* 1985;70:473–489.
248. Vachharajani V, Liu T, McCall CE. Epigenetic coordination of acute systemic inflammation: Potential therapeutic targets. *Expert Rev Clin Immunol.* 2014;10:1141–1150.
249. Palsson-McDermott EM, O'Neill LA. The Warburg effect then and now: From cancer to inflammatory diseases. *BioEssays.* 2013;35:965–973.
250. Warburg O. On the origin of cancer cells. *Science.* 1956;123:309–314.
251. Lu H, Forbes RA, Verma A. Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. *J Biol Chem.* 2002;277:23111–23115.
252. Sun Q, Chen X, Ma J, et al. Mammalian target of rapamycin up-regulation of pyruvate kinase isoenzyme type M2 is critical for aerobic glycolysis and tumor growth. *Proc Natl Acad Sci USA.* 2011;108:4129–4134.
253. Cheng SC, Quintin J, Cramer RA, et al. mTOR- and HIF-1 α -mediated aerobic glycolysis as metabolic basis for trained immunity. *Science.* 2014;345:1250684.

254. Rodriguez-Prados JC, Traves PG, Cuenca J, et al. Substrate fate in activated macrophages: A comparison between innate, classic, and alternative activation. *J Immunol.* 2010;185:605–614.
255. Tannahill GM, Curtis AM, Adamik J, et al. Succinate is an inflammatory signal that induces IL-1 β through HIF-1 α . *Nature.* 2013;496:238–242.
256. Freemerman AJ, Johnson AR, Sacks GN, et al. Metabolic reprogramming of macrophages: Glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. *J Biol Chem.* 2014;289:7884–7896.
257. Davenport EE, Burnham KL, Radhakrishnan J, et al. Genomic landscape of the individual host response and outcomes in sepsis: A prospective cohort study. *Lancet Respir Med.* 2016;4:259–271.
258. Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve—an integrative interface between two supersystems: The brain and the immune system. *Pharmacol Rev.* 2000;52:595–638.
259. Muthu K, Deng J, Romano F, et al. Thermal injury and sepsis modulates beta-adrenergic receptors and cAMP responses in monocyte-committed bone marrow cells. *J Neuroimmunol.* 2005;165:129–138.
260. Cohen MJ, Shankar R, Stevenson J, Fernandez R, Gamelli RL, Jones SB. Bone marrow norepinephrine mediates development of functionally different macrophages after thermal injury and sepsis. *Ann Surg.* 2004;240:132–141.
261. Rudiger A, Singer M. Mechanisms of sepsis-induced cardiac dysfunction. *Crit Care Med.* 2007;35:1599–1608.
262. Rudiger A. Beta-block the septic heart. *Crit Care Med.* 2010;38:5608–612.
263. Evans DG, Miles AA, Niven JS. The enhancement of bacterial infections by adrenaline. *Br J Exp Pathol.* 1948;29:20–39.
264. Lyte M, Freestone PP, Neal CP, et al. Stimulation of *Staphylococcus epidermidis* growth and biofilm formation by catecholamine inotropes. *Lancet.* 2003;361:130–135.
265. Freestone PP, Haigh RD, Lyte M. Specificity of catecholamine-induced growth in *Escherichia coli* O157:H7, *Salmonella enterica* and *Yersinia enterocolitica*. *FEMS Microbiol Lett.* 2007;269:221–228.
266. Freestone PP, Lyte M, Neal CP, Maggs AF, Haigh RD, Williams PH. The mammalian neuroendocrine hormone norepinephrine supplies iron for bacterial growth in the presence of transferrin or lactoferrin. *J Bacteriol.* 2000;182:6091–6098.
267. Ohtsuka T, Hamada M, Hiasa G, et al. Effect of beta-blockers on circulating levels of inflammatory and anti-inflammatory cytokines in patients with dilated cardiomyopathy. *J Am Coll Cardiol.* 2001;37:412–417.
268. Friese RS, Barber R, McBride D, Bender J, Gentilello LM. Could Beta blockade improve outcome after injury by modulating inflammatory profiles? *J Trauma.* 2008;64:1061–1068.
269. Tian X, Zhang L, Hou Y, et al. Effects of cAMP and beta-adrenergic receptor antagonists on the function of peripheral T helper lymphocytes in patients with heart failure. *NeuroImmunoModulation.* 2011;18:73–78.
270. Bergmann M, Gornikiewicz A, Sautner T, et al. Attenuation of catecholamine-induced immunosuppression in whole blood from patients with sepsis. *Shock.* 1999;12:421–427.
271. Christensen S, Johansen MB, Tonnesen E, et al. Preadmission beta-blocker use and 30-day mortality among patients in intensive care: A cohort study. *Crit Care.* 2011;15:R87.
272. Herndon DN, Rodriguez NA, Diaz EC, et al. Long-term propranolol use in severely burned pediatric patients: A randomized controlled study. *Ann Surg.* 2012;256:402–411.
273. Arbabi S, Campion EM, Hemmila MR, et al. Beta-blocker use is associated with improved outcomes in adult trauma patients. *J Trauma.* 2007;62:56–61; discussion 61–52.
274. Hart DW, Wolf SE, Ramzy PI, et al. Anabolic effects of oxandrolone after severe burn. *Ann Surg.* 2001;233:556–564.
275. Demling RH, Orgill DP. The anticatabolic and wound healing effects of the testosterone analog oxandrolone after severe burn injury. *J Crit Care.* 2000;15:12–17.
276. Wolf SE, Edelman LS, Kemalyan N, et al. Effects of oxandrolone on outcome measures in the severely burned: A multicenter prospective randomized double-blind trial. *J Burn Care Res.* 2006;27:131–139; discussion 140–131.
277. Delano MJ, Moldawer LL. The origins of cachexia in acute and chronic inflammatory diseases. *Nutr Clin Pract.* 2006;21:68–81.
278. Haney M, Gunderson EW, Rabkin J, et al. Dronabinol and marijuana in HIV-positive marijuana smokers. Caloric intake, mood, and sleep. *J Acquir Immune Defic Syndr.* 2007;45:545–554.
279. Li YY, Li YN, Ni JB, et al. Involvement of cannabinoid-1 and cannabinoid-2 receptors in septic ileus. *Neurogastroenterol Motil.* 2010;22:350–e388.
280. Gallily R, Yamin A, Waksman Y, et al. Protection against septic shock and suppression of tumor necrosis factor alpha and nitric oxide production by dexanabinol (HU-211), a nonpsychotropic cannabinoid. *J Pharmacol Exp Ther.* 1997;283:918–924.
281. Pierrakos C, Vincent JL. Sepsis biomarkers: A review. *Crit Care.* 2010;14:R15.
282. Monneret G, Venet F. Monocyte HLA-DR in sepsis: Shall we stop following the flow? *Crit Care.* 2014;18:102.
283. Monneret G, Venet F. Sepsis-induced immune alterations monitoring by flow cytometry as a promising tool for individualized therapy. *Cytometry B Clin Cytom.* 2015;90:376–386.
284. Shao R, Fang Y, Yu H, Zhao L, Jiang Z, Li CS. Monocyte programmed death ligand-1 expression after 3–4 days of sepsis is associated with risk stratification and mortality in septic patients: A prospective cohort study. *Crit Care.* 2016;20:124.
285. Sironi M, Cagliani R, Forni D, Clerici M. Evolutionary insights into host-pathogen interactions from mammalian sequence data. *Nat Rev Genet.* 2015;16:224–236.
286. Opal SM, Dellinger RP, Vincent JL, Masur H, Angus DC. The next generation of sepsis clinical trial designs: What is next after the demise of recombinant human activated protein C?*. *Crit Care Med.* 2014;42:1714–1721.
287. Coffey CS, Kairalla JA. Adaptive clinical trials: Progress and challenges. *Drugs in R&D.* 2008;9:229–242.
288. Press WH. Bandit solutions provide unified ethical models for randomized clinical trials and comparative effectiveness research. *Proc the Natl Acad Sci USA.* 2009;106:22387–22392.
289. Petroni GR, Wages NA, Paux G, Dubois F. Implementation of adaptive methods in early-phase clinical trials. *Stat Med.* 2016; Feb 29. Epub ahead of print.