

Immune Modulation in Pediatric Sepsis

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Abstract

The initial host immune response to sepsis in children is characterized by a proinflammatory surge that can be associated with fever, capillary leak, and organ dysfunction. There is, however, a concurrent anti-inflammatory response that results in hyporesponsiveness of innate and adaptive immune cells. When severe, this response is termed immunoparalysis and is known to be associated with prolonged organ dysfunction, increased risk for nosocomial infection, and death in septic adults and children. Sepsis-induced immune suppression can be defined in the laboratory by reduced whole blood ex vivo-stimulated cytokine production capacities, reduced expression of human leukocyte antigen (HLA)-DR on circulating monocytes, and reduced absolute cell counts. While anti-inflammatory therapies have largely been unsuccessful at improving outcomes from adult and pediatric sepsis, the use of immunostimulatory therapies such as granulocyte macrophage colony-stimulating factor (GM-CSF) in patients with sepsis-induced immunoparalysis shows promise. A greater understanding of the risk factors for immunoparalysis along with the development and execution of immunophenotype-specific clinical trials of strategies to optimize innate and adaptive immune function are needed to further improve outcomes in septic children.

Keywords

- ▶ immune
- ▶ modulation
- ▶ pediatric
- ▶ sepsis
- ▶ HLA-DR
- ▶ TNF α

Introduction

The proinflammatory response to infection is typically responsible for the classical signs and symptoms of sepsis such as fever, tachycardia, tachypnea, capillary leak, and organ dysfunction. This element of the host response has been targeted in numerous clinical trials in septic adults, in which anti-inflammatory therapies were employed in an effort to ameliorate the proinflammatory surge. The failure of most of these trials to improve outcomes led to the investigation of the counter-regulatory immune response to sepsis. In the last two decades, multiple investigators have identified an overly robust compensatory anti-inflammatory response to sepsis in some patients. This state, termed "immunoparalysis," has been associated with adverse outcomes including increased risks for nosocomial infection,

prolonged organ dysfunction, and death. In this review, we will identify elements of the pro- and anti-inflammatory responses to pediatric sepsis, approaches to monitoring the immune response in septic children, and potential strategies for immunomodulation in pediatric sepsis with a goal of restoring immunologic balance.

Host Response to Sepsis

The production of proinflammatory cytokines and chemokines, when localized to infected tissues, makes the local environment more favorable in fighting infection. When these mediators spill over into the systemic circulation; however, they are responsible for the signs and symptoms of sepsis. The pathophysiologic features of acute sepsis are therefore largely attributable to the host's innate immune response to the offending pathogen. The innate immune system includes leukocytes such as

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neutrophils, dendritic cells, monocytes, and macrophages. These cells broadly recognize and are activated by pathogen-associated molecular patterns (PAMP), such as lipopolysaccharide (LPS) through the ligation of constitutively expressed cell surface receptors. The best described of these are the toll-like receptors (TLR¹). This family of PAMP receptors includes those that can recognize a wide variety of molecules that are expressed by pathogens but not human cells. Upon activation, innate immune cells can ingest pathogens and kill them through enzymatic action or exposure to reactive oxygen species. Innate immune cells then digest the pathogen's proteins into antigenic peptides that are then loaded onto class II major histocompatibility complex (MHC) molecules, such as human leukocyte antigen (HLA)-DR. These antigens can then be presented to lymphocytes, members of the adaptive immune system. Finally, activated innate immune cells make proinflammatory cytokines which modulate the local environment to promote clearance of infection, along with chemokines which serve to attract other leukocytes to the area along concentration gradients. The innate immune response does not require prior exposure to a pathogen and should be of the same magnitude with each exposure.

The adaptive immune system consists of T and B lymphocytes. These cells typically, though not always, require activation through interaction with an antigen-bearing innate immune cell. The response is highly antigen-specific and is of greater magnitude and rapidity with subsequent exposures to an antigen. Activated B cells develop into plasma cells which produce antibody. T cells can be divided, based on cell surface marker expression, into CD4⁺ helper T cell and CD8⁺ cytotoxic T cell populations. Cytotoxic T cells promote cellular killing through elaboration of lytic enzymes. Helper T cells produce cytokines that modulate and perpetuate the immune response. Naïve helper T cells can differentiate into one of many subtypes depending on the signals they receive from antigen-presenting cells and the cytokine milieu in which they are activated. T helper (T_H)-1 cells are proinflammatory and produce cytokines including interferon (IFN)- γ and interleukin (IL)-2. T_H2 cells promote anti-inflammatory and autoimmune/allergic responses through elation

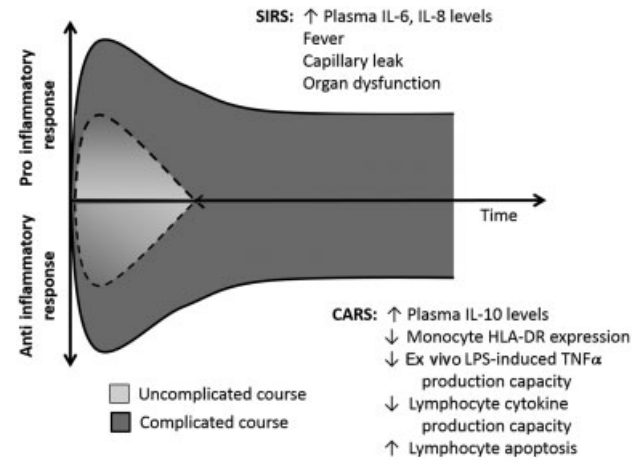


Fig. 1 Schematic of the dynamic inflammatory response to sepsis. CARS, compensatory anti-inflammatory response syndrome; SIRS, systemic inflammatory response syndrome.

ation of IL-10, IL-4, and IL-13. IL-17 that produces T_H17 cells are potently proinflammatory while regulatory T cells (T_{reg}) are anti-inflammatory through production of IL-10, transforming growth factor (TGF)- β , and through direct, cell-contact-mediated inhibition. Innate and adaptive immune cells, their actions, and mediators are listed in **Table 1**.

Immunologic Balance

The systemic inflammatory response syndrome (SIRS) represents the clinical manifestation of the systemic proinflammatory response. The clinical phenotype of fever, tachycardia, tachypnea, and capillary leak is common to most inflammatory conditions including sepsis. However, every major biologic pathway has a counter-regulatory system. The compensatory anti-inflammatory response syndrome (CARS) is the “off switch” for the proinflammatory response (**Fig. 1**). The CARS response has no clinical phenotype but is characterized by several important physiologic elements. First, within minutes to hours of the proinflammatory response becoming activated, innate and adaptive leukocytes can down-regulate intracellular signaling and become hyporesponsive to

Table 1 Immune effector cells, actions, and mediators

	Cell type	Primary actions	Primary mediators
Innate	Neutrophil	Phagocytosis, intracellular killing	Reactive oxygen species, lytic enzymes, TNF α
	Monocyte	Phagocytosis, intracellular killing, antigen presentation	TNF α , IL-1 β , IL-6, IL-8, IL-10
	Macrophage	Phagocytosis, intracellular killing, antigen presentation	TNF α , IL-1 β , IL-6, IL-8, IL-10
	Dendritic cell	Phagocytosis, intracellular killing, antigen presentation	IFN α , IL-12
	Natural killer cell	Extracellular killing	Perforin, granzyme
Adaptive	CD4 ⁺ T cell	Cytokine production	T _H 1, T _H 17: IFN γ , IL-2, IL-17 T _H 2, T _{reg} : IL-10, TGF β , IL-4, IL-13
	CD8 ⁺ T cell	Extracellular killing	Perforin, granzyme
	B cell/plasma cell	Antibody production	Antibody

Abbreviations: IFN, interferon; IL, interleukin; TGF, transforming growth factor; T_H, T helper; TNF, tumor necrosis factor; T_{reg}, regulatory T cell.

stimulation. This period of quiescence serves to rein in the proinflammatory response and potentially avoid systemic spillover of cytokines. Second, leukocyte cell death can occur either as a result of their initial activation (e.g., neutrophils) or as the result of apoptosis (e.g., lymphocytes). Third, surviving innate and adaptive leukocytes can skew their cytokine production toward the production of anti-inflammatory mediators, such as IL-10. While it is likely beneficial to the host if brief and modest in magnitude, the CARS response can become pathological if severe and prolonged. In this setting, it is termed immunoparalysis and it represents an important form of acquired immune deficient which is very prevalent in the intensive care unit (ICU).

Immunoparalysis in Sepsis

The CARS response is quantifiable in the research laboratory and several approaches to the diagnosis of immunoparalysis have been undertaken in septic patients. The antigen presenting molecule HLA-DR should be expressed robustly on the surface of circulating monocytes. HLA-DR molecules are internalized as part of the CARS response and this can be quantified using flow cytometry. In a state of health, > 90% of circulating monocytes should stain positive for HLA-DR, while a monocyte HLA-DR (mHLA-DR) expression level of < 30% is associated with increased risks for nosocomial infection and death.²⁻⁴ An alternate method allows for the quantitation of molecules of HLA-DR per cell, also using flow cytometry. A threshold of < 8,000 molecules per cell has been used in adult studies to define immunoparalysis with < 5,000 molecules per cell representing the most severe suppression.^{5,6} Thresholds of mHLA-DR expression that are associated with adverse outcomes from pediatric sepsis are less clear, with one recent study suggesting that the change in mHLA-DR expression over time may be more important than absolute thresholds. Manzoli et al found that mortality, in a 30-subject cohort of septic children, was associated with failure to increase mHLA-DR by at least 1,000 molecules per cell over the first week after sepsis onset.⁷

An alternative method to the diagnosis of immunoparalysis is the use of ex vivo stimulation of whole blood to identify subjects whose leukocytes are hyporesponsive. The whole blood ex vivo LPS-induced tumor necrosis factor (TNF)- α production capacity assay measures the ability of circulating innate immune cells to respond to a new stimulus. In this assay, whole blood is added to a tube containing standardized LPS stimulation reagent and incubated for a period of time after which TNF α levels are measured in the supernatant. Healthy children's blood should produce large amounts of TNF α upon incubation with LPS. Marked reduction in the TNF α response is a feature of CARS with severe reductions being characteristic of immunoparalysis. It is important to note that the use of the TNF α response to evaluate innate immune function does not assign particular significance to TNF α itself or to the TLR4/CD14 pathway through which LPS signals. Rather, the TNF α response is used as a surrogate for the broader responsiveness of the innate immune cell by virtue of TNF α 's favorable kinetics and relative ease of measurement. In the most widely reported

approach to this type of testing, a TNF α response less than approximately 200 pg/mL has been associated with increased risks for the development of nosocomial infection, prolonged organ dysfunction, and death in single- and multicenter cohorts of septic children.^{2,8,9} Thresholds of TNF α response that are associated with adverse outcomes may vary depending on the type of LPS used, blood volume used, incubation conditions, and the outcome being considered.

The adaptive immune system can also be affected as part of the CARS response. Lymphocyte apoptosis is common in the aftermath of sepsis, with peripheral lymphopenia and splenic lymphocyte depletion, having been associated with multiple organ dysfunction syndrome (MODS) and death in septic adults and children.¹⁰⁻¹² Downregulation of lymphocyte responsiveness has similarly been associated with adverse outcomes from sepsis. Wong et al performed mRNA profiling on peripheral blood leukocytes early in the course of pediatric septic shock and evaluated expression of 100 genes corresponding to adaptive immunity and glucocorticoid receptor signaling.¹³ They identified two distinct patterns of gene expression or endotypes that were prevalent in the cohort, one with predominately suppressed gene expression and another with a substantially greater proportion of activated genes. Risks for mortality and prolonged MODS were significantly higher in children with the more suppressed endotype. Lymphocyte suppression at the protein level has also been shown to predict outcomes from sepsis. Adaptive immune testing can also utilize ex vivo stimulation as a tool for evaluating cellular responsiveness. Instead of using LPS as a stimulant, one must use a lymphocyte stimulant such as phytohemagglutinin (PHA) or anti-CD3/anti-CD28 antibodies. Multiple cytokines have been evaluated as readouts from lymphocyte stimulation in this setting but reduced capacity to produce IFN γ in response to ex vivo stimulation has been associated with the development of infectious complications, prolonged organ failure, and death in septic children and adults.^{9,11,14,15}

The timing of innate and adaptive immune suppression is somewhat controversial in the field. Early work with innate immune function in septic adults suggested that very early suppression (e.g., days 1-2 of illness) was less predictive of adverse outcomes than were samples obtained later in the course (e.g., days 3-4).^{3,4} The first report of the TNF α response in children with MODS found increased risks for nosocomial infection and death in subjects whose immunoparalysis persisted beyond day 3 of MODS, though very early samples were rare in that cohort.² Subsequent single- and multicenter studies have shown increased risks for prolonged organ dysfunction and death associated with severe suppression of the TNF α response and/or lymphocyte responsiveness as early as 1 to 2 days after sepsis onset.^{8,9} The significance of early versus late immunoparalysis as it relates to reversibility and outcomes remains unclear.

History of Immunomodulation in Sepsis

The 1980s and 1990s saw multiple phase II and III clinical trials of therapeutics designed to reduce the proinflammatory response to sepsis in adults. These included drugs to

block or bind LPS, IL-1 β , TNF α , and bradykinin as well as potent glucocorticoids, such as methylprednisolone or dexamethasone.^{16–23} These treatments consistently failed to improve outcomes and were occasionally associated with increased mortality. There were a few notable exceptions including the MONARCS trial in which a mortality benefit was seen in septic adults treated with anti-TNF α therapy but only if their initial plasma IL-6 levels were > 1,000 pg/mL.²⁴ Similarly, a secondary analysis of a large trial of IL-1 receptor antagonist suggested benefit in the subset of subjects who had hepatobiliary dysfunction and disseminated intravascular coagulation (chosen as a surrogate for macrophage activation syndrome [MAS]).²⁵ Lastly, anecdotal evidence has suggested improved outcomes from the use of anti-IL-6 antibodies in children with severe cytokine release syndrome following chimeric antigen receptor (CAR) T cell therapy.²⁶ Together, these data suggest that broad use of anti-inflammatory therapies likely has little place in acute sepsis management. Rather, there may be a role for the targeted use of selected anti-inflammatory drugs in septic patients with documented, severe hyperinflammation or in specific pathophysiologic states such as MAS.

Immunostimulatory Therapies in Sepsis

One possible reason for the failure of anti-inflammatory therapies in sepsis is that they may exacerbate or perpetuate sepsis-induced immune suppression. Immunoparalysis itself has become a target for immunomodulation in recent years, prompting a paradigm shift toward the use of immunostimulatory therapies in adults and children with severe immune suppression. The approaches were developed in response to the finding that immunoparalysis appears to be reversible. Fumeaux and Pugin were able to demonstrate restoration of HLA-DR expression in immunoparalyzed monocytes after ex vivo incubation with anti-IL-10 neutralizing antibodies.²⁷ Since then several approaches to immunostimulation in septic patients have been evaluated (► **Table 2**).^{2,6,28–39}

The use of recombinant IFN γ has been shown, in several small European case series, to be associated with improved mHLA-DR expression and TNF α response in septic adults with immunoparalysis.^{28,29} Interferon- γ is currently being evaluated for the reversal of immunoparalysis (mHLA-DR < 30%) in a multicenter, European randomized controlled trial (RCT) in septic adults (NCT03332225).

Granulocyte macrophage colony-stimulating factor (GM-CSF) is a cytokine that is made by T_H1 lymphocytes but is also available in recombinant form. GM-CSF was approved by the Food and Drug Administration (FDA) in 1991 for the restoration of bone marrow function following bone marrow transplantation and chemotherapy. In addition to its favorable side effect profile, GM-CSF represents an attractive candidate for the reversal of immunoparalysis in that it is capable of improving immune function in existing cells in addition to promoting the development of new cells of the monocyte and neutrophil lineages. Rosenbloom et al showed, in a small single-center RCT, that treatment with GM-CSF can result in improved mHLA-DR expression in septic adults, though enrollment was not limited to those with immunoparaly-

sis.³⁴ Meisel et al subsequently showed, in a 38-subject RCT of septic adults with proven, severe reduction in mHLA-DR expression that GM-CSF treatment was associated with prompt recovery of mHLA-DR expression and TNF α response.⁶ A similar single-center RCT was performed in septic children with MODS who demonstrated severe reduction in TNF α response (< 200 pg/mL).² In this study, 14 subjects were randomized to a week of GM-CSF or standard therapy. Those receiving GM-CSF showed recovery of the TNF α response to normal levels by the end of the week of treatment, while the standard therapy arm had a much more modest response (► **Fig. 2**). Interestingly, all of the subjects in the standard therapy arm developed a nosocomial infection while none were noted in the GM-CSF-treated group.

The adaptive immune system may also represent a target for immunostimulation in septic patients with immunoparalysis. While lymphocyte-specific colony stimulating factors are not available, several other approaches have shown promise in preclinical models. IL-7 inhibits lymphocyte apoptosis and enhances lymphocyte responsiveness in vitro. In murine studies, administration of recombinant IL-7 was associated with improved lymphocyte function and survival in a two-hit model of peritonitis and candida pneumonia.⁴⁰ The programmed death (PD)-1 pathway represents another potential target for immunomodulatory therapy in sepsis. PD-1 is a coinhibitory molecule expressed by lymphocytes and binding by its ligands PD-L1 or PD-L2 on antigen presenting cells results in lymphocyte deactivation and/or apoptosis. Anti-PD-1 antibodies are FDA-approved as immunotherapy to promote lymphocyte responses against some forms of cancer. These have also been evaluated in murine models of sepsis, with improvements in innate and adaptive immune function and improved survival reported.^{41,42} At least one blocking antibody in the PD-1 family has been evaluated in human sepsis (NCT02576457) though results have not yet been reported. Interestingly, preclinical data suggest that combinatorial therapy with anti-PD-1 antibodies and recombinant IL-7 may more beneficial than either alone.³⁹

Unintended Immunomodulation

While the prior section focused on intentional immunomodulation, it must be acknowledged that a great many therapies that are routinely employed in the care of children with sepsis have unintended immunologic effects, most of which are immunosuppressive. Red blood cell (RBC) transfusion, for example, may reduce leukocyte function as has been demonstrated in vitro and in septic children.^{43,44} The use of therapeutic hypothermia is known to reduce neutrophil function.⁴⁵ Many of the drugs in the ICU (intensive care unit) pharmacopeia are, in fact, immunomodulators (► **Table 3**). The clinical relevance of the individual and net effects of these therapies on immune function remains unknown and represents a crucial gap in our understanding of ICU pathophysiology. In addition to identifying treating patients with immunoparalysis, there is a great need for the development and testing of immune-friendly treatment regimens that may prevent the development of immunoparalysis in some cases.

Table 2 Summary of clinical trials of immunostimulation for treatment of sepsis-induced immune suppression

Study	Design	Population	n	Findings
IFN-γ				
Döcke, 1997	Case series	Septic adults with low mHLA-DR expression	9	↑ mHLA-DR expression and TNF α production capacity after treatment
Delsing, 2014	Case series	Adults with severe invasive fungal sepsis	8	Increased mHLA-DR expression and enhanced antifungal proinflammatory cytokine production
GM-CSF				
Bilgin, 2001	RCT	Septic neonates with neutropenia	60	↓ Mortality in treatment group
Drossou-Agakidou, 2002	RCT	Septic neonates	56	↑mHLA-DR in GM-CSF treated group compared with G-CSF or placebo
Presneill, 2002	RCT	Septic adults with pulmonary dysfunction	18	↑Neutrophil function and improved oxygenation in treated group
Nierhaus, 2003	Case series	Septic adults with low mHLA-DR expression	9	↑ mHLA-DR expression and TNF- α production capacity after treatment
Rosenbloom, 2005	RCT	Septic adults	33	↑ mHLA-DR expression and faster resolution of infection in treated group
Orozco, 2006	RCT	Adults with abdominal sepsis	58	↓ Hospital stay and ↓ infectious complications in treated group
Meisel, 2009	RCT	Septic adults with low mHLA-DR	38	↑ mHLA-DR expression and ↓ durations of mechanical ventilation and ICU stay in treated group
Hall, 2011	RCT	Children with MODS and low TNF α production capacity	14	Enhanced TNF α production capacity and lower incidence of nosocomial infection in treated group.
Paine, 2012	RCT	Adults with ALI/ARDS	132	No difference in ventilator-free days
IL-7				
Venet, 2012	Ex vivo (cell culture)	Adults with septic shock	10	↑Lymphocyte proliferation and INF- γ production
Thampy, 2018	Ex vivo (cell culture)	Septic adults	26	↑ IFN- γ production
Anti-PD-1 antibodies				
Chang, 2014	Ex vivo (cell culture)	Septic adults	19	↑ IFN- γ production and decreased lymphocyte apoptosis
Thampy, 2018	Ex vivo (cell culture)	Septic adults	26	↑IFN- γ production

Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; ICU, intensive care unit; IFN- γ , interferon gamma; IL, interleukin; mHLA-DR, monocyte human leukocyte antigen-DR expression; MODS, multiple organ dysfunction syndrome; PD, programmed death; RCT, randomized controlled trial; TNF, tumor necrosis factor.

Note: Up arrows indicate increase, down arrows indicate decrease.

Summary and Future Directions

The immune response to sepsis is highly dynamic and has both pro- and anti-inflammatory components. The presence of high levels of systemic inflammation, with accompanying symptoms of SIRS, does not rule out the presence of a compensatory anti-inflammatory response which may in itself be pathologic. Early attempts at immunomodulation in sepsis were likely flawed due to their singular focus on reduction of the proinflammatory response without measuring resultant effects on immune cell function. While some patients with severe inflammation may benefit from anti-inflammatory therapies, it is likely that these therapies should be carefully targeted, of

short duration, and accompanied by immune function monitoring. Conversely, many patients with sepsis may benefit from immune stimulation to reverse immunoparalysis and restore the ability to clear infection, survey for and combat new infection, and remodel injured tissues. There are several therapies that show promise for the reversal of innate immune suppression in sepsis of which GM-CSF has the largest body of evidence to date. Strategies that target restoration of lymphocyte function in sepsis are under development as well. It is likely that a combinatorial approach, driven by prospective immune function testing of the innate and adaptive arms of the immune system, will be needed in some patients to achieve

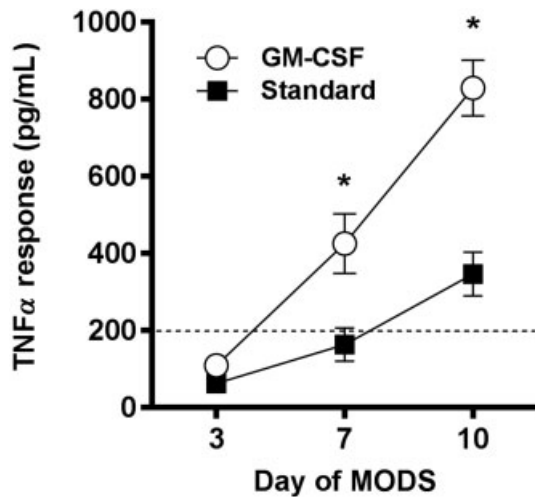


Fig. 2 Reversal of pediatric sepsis-induced immunoparalysis using GM-CSF. Treatment with GM-CSF (open circles) was associated with more rapid and more robust restoration of the TNF α response compared with standard therapy (dark squares) in a small RCT in children with sepsis-induced MODS and immunoparalysis ($n=14$; adapted from Hall, 2011²). $p < 0.05$. Note: TNF α response values from healthy children are approximately 1,000 pg/mL. GM-CSF, granulocyte macrophage colony stimulating factor; MODS, multiple organ dysfunction syndrome.

Table 3 Unintended immunomodulatory effects of common ICU therapies

	Immune effect	Putative mechanisms
RBC transfusion	↑	Allogeneic transfusion reactions, passenger leukocytes ⁴⁶
	↓	Inhibition of cytokine production ⁴⁶
Antibacterial agents	↑	Release of PAMPs upon pathogen death ⁴⁷
	↓	Bone marrow suppression, inhibition of cytokine production ⁴⁸
Benzodiazepines	↓	Cortisol induction, direct leukocyte inhibition ⁴⁹
Epinephrine/norepinephrine	↑	Stimulation of α receptors ⁵⁰
	↓	Stimulation of β receptors ⁵⁰
Dopamine	↓	Inhibition of prolactin secretion ⁵¹
Furosemide	↓	Inhibition of cytokine production ⁵²
Insulin	↓	Inhibition of proinflammatory signaling pathways ⁵³
Opiates	↓	Inhibition of cytokine production, induction of apoptosis ⁵⁴

Abbreviations: ICU, intensive care unit; PAMPs, pathogen-associated molecular patterns; RBC, red blood cell.

Note: Up arrows indicate increased inflammatory response, down arrows indicate decreased inflammatory response.

prompt and lasting restoration of immune function. Lastly, though the largest body of data on the TNF α response in critical illness comes from the pediatric population, the vast majority of immunomodulation data can be found in the adult literature. The development of multicenter, prospective immune function monitoring, and modulation studies in septic children will be essential to safely bring these therapies into the pediatric ICU and improve outcomes in this vulnerable population.

Conflict of Interest
None declared.

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