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Immunoparalysis in Pediatric Critical Care

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SYNOPSIS

While many forms of critical illness are initiated by a pro-inflammatory stimulus, it is now understood that a compensatory anti-inflammatory response can occur concurrently with systemic inflammation. When severe, this is termed immunoparalysis and it represents an important form of acquired immunodeficiency. Immunoparalysis can affect the innate and adaptive arms of the immune system and is characterized by reduced monocyte HLA-DR expression, reduced cytokine production capacities upon ex vivo stimulation of leukocytes or whole blood, the presence of lymphopenia, and by increase expression of inhibitory cell surface molecules such as PD-1. Immunoparalysis has been associated with increased risks for nosocomial infection and death in a wide variety of pediatric critical illnesses. Evidence suggests that immunoparalysis is reversible

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through treatment with immunostimulants such as GM-CSF or interferon- γ . Highly standardized, prospective immune monitoring regimens are needed in order to better understand the immunologic effects of ICU treatment regimens and to enrich clinical trials with subjects most likely to benefit from immunostimulatory therapies.

Keywords

Immunoparalysis; pediatric; critical care; immune; sepsis; trauma; cardiopulmonary bypass

Introduction

While the diagnoses that result in pediatric critical illness can vary (*e.g.* sepsis, trauma, cardiopulmonary bypass), the initial insult is often pro-inflammatory in nature.

It has long been understood that the degree of hyperinflammation associated with these diagnoses predicts adverse outcomes, with greater inflammation being linked to organ failure and mortality.^{1–3} Mounting evidence suggests, however, that a compensatory anti-inflammatory response syndrome (CARS) often occurs concurrently with the systemic inflammatory response syndrome (SIRS) (Figure 1). This anti-inflammatory response, when severe and persistent, has been termed immunoparalysis and it represents a form of acquired immune deficiency which is itself associated with adverse outcomes in the pediatric intensive care unit (PICU). Immunoparalysis can affect multiple arms of the immune system and is often occult, with no physical examination or routine clinical laboratory evidence of its presence. Specialized testing, however, can identify patients with critical illness-induced immune suppression and a growing body of evidence suggests that immunoparalysis is reversible with the potential for beneficial effects on clinical outcomes.

In order to place the concept of immunoparalysis in context, this review will begin with an overview of the immune system. Following this, the laboratory characteristics of immunoparalysis will be reviewed along with detailed discussions of its clinical implications across multiple forms of pediatric critical illness. The treatment of immunoparalysis through immunostimulatory strategies will also be reviewed. The overall goal of this review is to highlight the need for immunologic balance in children with critical illness and to identify immunoparalysis as an important contributor to clinical outcomes in the PICU.

The Immunology of Inflammation

The Innate Immune System

In general terms, the immune system can be divided into the innate and the adaptive arms (Table 1). The innate immune system is the body's first cellular line of defense. It includes cell types such as monocytes, macrophages, polymorphonuclear cells, natural killer (NK) cells and dendritic cells. Innate immune cells are key drivers of the initial acute immune response. Their diverse roles include recognition and phagocytosis of pathogens, presentation of antigens to adaptive immune cells, and secretion of mediators which modulate the overall immune response. A characteristic feature of innate immune cells is their ability to consistently respond to pathogens regardless of prior exposure history. This is

accomplished through the presence of constitutively expressed receptors on their plasma membranes. These receptors recognize broad classes of pathogen-associated molecular patterns. For example, bacterial lipopolysaccharide (LPS) is recognized by monocytes through its interaction with the toll-like receptor (TLR)-4 on the monocyte's cell surface. A monocyte which encounters LPS should respond vigorously regardless of whether or not it has been exposed to LPS in the past.

Once stimulated, monocytes engulf and destroy microbes which are then processed into antigenic peptides. These are presented on the surfaces of innate immune cells on class II Major Histocompatibility Complex (MHC) molecules such as Human Leukocyte Antigen (HLA)-DR. Antigens presented in this way, along with co-stimulatory input from the innate immune cells, activate the adaptive arm of the immune response. Innate immune cells also secrete cytokines and chemokines which modulate the inflammatory response by recruiting and activating other immune effector cells and activating non-cellular aspects of the immune response such as the complement and coagulation cascades. Local production of proinflammatory cytokines such as tumor necrosis factor (TNF)- α results in activation of nearby immune cells and alters the vascular endothelium to promote cellular migration into the periphery. It is when these effects become systemic that the clinical signs and symptoms of hyperinflammation (fever, hemodynamic instability, and capillary leak) become evident.

The Adaptive Immune System

T and B lymphocytes are adaptive immune cells. In contrast to innate immunity, the adaptive immune system produces responses that are highly antigen-specific. Adaptive immune responses typically require antigen presentation by innate immune cells. The initial lymphocyte response to a pathogen takes more time and is of smaller magnitude than the initial innate response. Repeated exposure to an antigen, however, provokes a more rapid and powerful adaptive immune response due to the presence of antigen-specific T and B cell clones that can be maintained for decades.

Upon stimulation, B cells mature into plasma cells which produce antibodies that opsonize invaders, thus marking them for clearance by lymphoid organs and phagocytes. Activated T cells regulate the immune response through the production of cytokines (CD4+) and via cytotoxic killing (CD8+). Naïve CD4+ T cells can differentiate into one of a number of T cell subtypes depending upon the cytokine milieu in which they are activated. While a detailed review of T cell biology is beyond the scope of this review, several major classes of CD4+ T cells merit general discussion here. These include the T-helper (T_H)-1, T_H2, and regulatory T cells (T_{reg}). In the presence of proinflammatory cytokines, naïve T cells will differentiate into T_H1 cells. These cells in turn produce proinflammatory cytokines such as interferon (IFN)- γ and serve to perpetuate the inflammatory response. T_H2 cells, by contrast, arise when naïve T cells are activated in an anti-inflammatory environment. In addition to promoting host immunity against extracellular parasites (e.g. helminths) and stimulating IgE release by B cells, T_H2 cells produce anti-inflammatory cytokines including interleukin (IL)-10 with a resultant inhibitory effect on the inflammatory response. T_{reg} are even more potently anti-inflammatory, down-regulating pro-inflammatory responses through elaboration of IL-10 and transforming growth factor (TGF)- β along with direct inhibition of

leukocytes through cell-to-cell contact. These cells can be identified through their cell surface marker expression pattern (CD4⁺CD25⁺CD127^{lo}) and through their expression of transcription factor FOXP3.

Cytokines and Chemokines

Cytokines and chemokines are proteins that leukocytes and other cell types produce that modulate the local environment and/or recruit other immune cells to the area in order to combat infection (Table 2). Some cytokines (*e.g.* TNF α , IFN γ and IL-1 β) have proinflammatory effects while anti-inflammatory cytokines (*e.g.* IL-10 and TGF β) deactivate effector cells and inhibit the proinflammatory response. Chemokines, such as IL-8, are chemoattractants which result in cellular migration into an inflamed area. Some cytokines are pleiotropic, with both pro- and anti-inflammatory effects. For example, IL-6 is known to stimulate the hepatic acute phase response while it also induces an adrenal glucocorticoid response.^{4, 5} Restoring balance between pro- and anti-inflammatory forces, while maintaining the ability of immune cells to survey for and fight new infection and participate in tissue healing is the goal of immunomodulatory therapy in the PICU.

Laboratory Characteristics of Immunoparalysis

The innate and adaptive arms of the immune system can both be affected in the setting of immunoparalysis. The two measures of reduced innate immune function that have been most consistently associated with adverse PICU outcomes are monocyte HLA-DR expression and whole blood *ex vivo* LPS-induced cytokine production capacity. As noted above, monocytes activate the adaptive immune response through the presentation of antigens on cell-surface class II MHC molecules, of which there are several subtypes. Human leukocyte antigen (HLA)-DR is the antigen-presenting molecule which has been best characterized in critical illness, and its expression on circulating monocytes can be quantified by flow cytometry. It has been clearly shown that monocytes, which normally express HLA-DR very strongly, internalize their HLA-DR molecules in the setting of immunoparalysis.⁶ Early work in critically ill adults suggested that if < 30% of subjects' circulating monocytes stained strongly for HLA-DR then their risk of nosocomial infection and/or death was significantly higher than those with higher HLA-DR expression.^{7–10} This has also been demonstrated in children with multiple organ dysfunction syndrome (MODS).¹¹ More recently, assays have been developed that can determine the number of HLA-DR molecules per monocyte, also using flow cytometry.¹² This method, through the use of standard beads, has the potential to avoid variability between antibody lots and cytometers, leading to more generalizable results. A threshold of < 8,000 HLA-DR molecules/cell has been used to define immunoparalysis, with < 5,000 HLA-DR molecules per cell signifying severe immunoparalysis.¹³ While few studies have used this method to evaluate pediatric innate immune function, one small study showed that failure to increase monocyte HLA-DR expression by at least 1,000 molecules/cell over the first week of pediatric septic shock was associated with mortality.¹⁴

The other commonly used method for quantification of innate immune function in pediatric critical illness is through measurement of the capacity of whole blood to produce the

proinflammatory cytokine TNF α when stimulated *ex vivo*. This type of measurement does not reflect circulating TNF α levels in the plasma, but rather reflects the *ability* of a patient's blood to make TNF α when it is needed. While a normal subject's whole blood will produce large amounts of TNF α when incubated with LPS, a sample from an immunoparalyzed subject's will not. In this way TNF α production capacity, when measured using highly standardized stimulation reagents and protocols, can serve as a readout of innate immune responsiveness, with a low value representing immune suppression. The largest body of evidence in critically ill children associating low innate immune function with adverse outcomes, spanning multiple diagnostic groups, uses this method of immune function testing.^{11, 15–18}

The adaptive immune system can fail in the context of immunoparalysis as well.

Apoptosis of lymphocytes within lymphoid organs such as the spleen is common in the setting of immunoparalysis.^{19–21} Similarly, persistent systemic lymphopenia with an absolute lymphocyte count < 1,000 cells/mm³ has been associated with nosocomial infection and death in the setting of pediatric MODS.²⁰ In addition to low cell numbers, lymphocyte responsiveness can also be reduced in the ICU. Reduction in the ability of lymphocytes to produce interferon (IFN)- γ when incubated *ex vivo* with the lymphocyte stimulants phytohemagglutinin (PHA) or anti-CD3/anti-CD28 antibody has been associated with infections complications and death in the setting of pediatric and adult sepsis.^{21, 22} These findings are in agreement with mRNA studies which suggest that early suppression of lymphocyte signaling pathways are associated with pediatric sepsis mortality.²³ Lastly, expression of co-inhibitory molecules such as programmed death (PD)-1 on lymphocytes, and its ligands PD-L1 and PD-L2 on antigen-presenting cells, is known to be upregulated in critically ill adults, with higher expression levels predicting adverse outcomes.^{21, 24}

Clinical Implications of Immunoparalysis

Trauma

Trauma surgeons were among the first clinicians to study the innate immune response in the setting of critical illness. In 1986, Polk *et al* undertook a systematic evaluation of adults with severe trauma to assess immunologic factors associated with the development of secondary infection and late mortality.²⁵ The results of their analyses indicated that persistently impaired monocyte antigen presenting capacity was associated with the development of nosocomial sepsis. The ability of HLA-DR expression to predict outcome in traumatized adults was strengthened by the work of Cheadle *et al* in 1989 in a study of serial monocyte HLA-DR measurements in 60 trauma victims beginning the first 24 hours after injury.²⁶ In their cohort, the depth and persistence of HLA-DR expression was predictive of the development of secondary infection. *Ex vivo* TNF α production has also been correlated with outcome in clinical trauma studies. Majetschak *et al* in 1999 performed *ex vivo* LPS-induced stimulation of whole blood samples from 46 adult blunt trauma victims.²⁷ They documented a profound reduction in subjects' TNF α production capacity compared to healthy controls. The degree of reduction in the TNF α response was associated with the severity of injury and this reduction was detectable in samples obtained within 90 minutes after trauma. Patients requiring surgery to treat their injuries experienced a further impairment in their TNF α .

response. In 2014, Muszynski *et al* reported similar findings in a 76-subject cohort of critically injured children, with early reduction in *ex vivo* LPS-induced TNF α production capacity (< 48 hours from injury) being associated with nosocomial infection risk.¹⁸ Children who went on to develop nosocomial infection also had lower TNF responses over the first week following injury compared to children who recovered without infection.

Central nervous system damage represents a particularly immunosuppressive form of injury. Injury to the brain and/or spinal cord can promote immune cell deactivation through direct neural effects mediated by the vagus nerve, through the hypothalamo-adrenal axis, and through elaboration of immunomodulatory mediators that cross the damaged blood-brain barrier into the systemic circulation.²⁸ It is not clear if these mechanisms are similar between pediatric and adult brain-injured patients, but in the Muszynski cohort, severe traumatic brain injury was associated with severe impairment of innate immune function and dramatically increased risk for nosocomial infection.¹⁸

Sepsis

Critical illness-induced immune suppression has also been well described in the setting of adult and pediatric sepsis. In the mid-1990s Volk *et al* studied monocyte HLA-DR expression in 247 septic adults.⁷ In their cohort, an HLA-DR expression level < 30% for five days or more was associated with a 12% survival rate. This was in contrast to a survival rate of 88% in patients whose reduction in monocyte HLA-DR expression was transient or less severe. Monneret *et al* in 2006 reported monocyte HLA-DR expression data from 93 adult patients with septic shock and showed that those who survived had recovery of HLA-DR expression by Day 3 or 4, while those who eventually died failed to recover HLA-DR expression.⁹ Multivariate logistic regression analysis showed that a monocyte HLA-DR expression < 30% on day 3 or 4 was associated with mortality after adjusting for confounders including initial severity of illness and presence of co-morbidities. We found similar results in children with MODS, in that those who demonstrated monocyte HLA-DR expression < 30% for more than three days had significantly increased risks for the development of nosocomial infection and death.¹¹ It is notable that immunoparalysis was highly prevalent in this population, representing approximately a third of subjects.

As in the setting of trauma, impairment of the *ex vivo* LPS-induced TNF α response has also been shown to occur following the onset of septic disease and is also associated with adverse outcomes. Ploder *et al* described a series of adult patients with sepsis in whom the degree of depression of the *ex vivo* TNF α response on the day of sepsis onset was more predictive of death than was the level of monocyte HLA-DR expression.²⁹ In a cohort of 28 children with MODS (25 of whom had septic shock as the inciting event) we showed that nonsurvivors demonstrated lower *ex vivo* LPS-induced TNF α production over the first two weeks of MODS compared to survivors.³⁰

Innate immune function has been evaluated in single- and multi-center cohorts of children with critical illness due to viral infection. In 2013 we reported results from a 15-center, 55-subject study of critical influenza that spanned the recent H1N1 pandemic.³¹ Nonsurvivors had high levels of both pro- and anti-inflammatory mediators in their serum but had marked reduction in their TNF α response in samples obtained within 72 hours of ICU admission

compared to survivors and healthy controls. Severe innate immune suppression remained associated with mortality risk after adjusting for initial severity of illness, secondary infection, and co-morbidities. Interestingly, the risk of severe reduction in TNF α response was greatest in children presenting with influenza plus *Staphylococcus aureus* secondary infection. Mella *et al* similarly showed, in a cohort of infants hospitalized with respiratory syncytial virus infection, that reduction in the *ex vivo* LPS-induced TNF α response was associated with higher severity of illness and longer lengths of PICU stay.¹⁷

As noted above, lymphocyte suppression has also been associated with adverse sepsis outcomes. Hotchkiss *et al* have repeatedly shown that lymphocyte apoptosis and impairment of lymphocyte cytokine production capacity are characteristic of sepsis deaths in adults.^{19, 21} Muszynski *et al* demonstrated, in children with septic shock, that infectious complications were associated with lower lymphocyte counts and reduced ability of lymphocytes to respond to *ex vivo* stimulation with PHA.²² This reduction was not limited to pro-inflammatory cytokines such as IFN γ but extended to anti-inflammatory cytokines as well, implying global down-regulation of cytokine responses. These data were consistent with transcriptome profiling done by Wong *et al* which suggested that early down-regulation of lymphocyte signaling pathways predicts mortality and organ failure duration in pediatric sepsis.²³ In addition, data from septic adults suggest that regulatory T cells may be resistant to the apoptotic surge seen in sepsis, resulting in a T_{reg}-predominant state that may perpetuate immunoparalysis.^{32–34} This T_{reg}-predominance has not been seen in pediatric sepsis to date.²²

Cardiopulmonary Bypass

The inflammatory stimuli of leukocyte exposure to cardiopulmonary bypass circuitry and ischemia-reperfusion injury frequently result in systemic inflammation following pediatric open heart surgery. The compensatory anti-inflammatory response, however, can also be prevalent in this population. Allen *et al* studied 82 infants and children undergoing cardiopulmonary bypass (CPB) for repair of congenital heart defects and documented reduced monocyte HLA-DR expression in all patients post-CPB compared with pre-CPB levels.³⁵ Prolonged reduction of HLA-DR expression was associated with the development of SIRS and sepsis after surgery. These results were supported by the same group in a 2006 follow-up study in which 36 children undergoing CPB were evaluated.¹⁶ Immune function was monitored serially by *ex vivo* LPS stimulation assays and IL-10 measurements. Higher levels of plasma IL-10 were associated with the greatest risk of developing more severe innate immune dysfunction. Patients with more profoundly reduced *ex vivo* TNF α responses as early as Day 2 following CPB had a greater likelihood of experiencing post-operative complications including nosocomial sepsis. More recently, Cornell *et al* demonstrated an association between reduced TNF α response on post-operative day 1 and subsequent development of sepsis in a 92 subject cohort of children undergoing cardiopulmonary bypass.¹⁵ Intriguingly, an epigenetic modification in the IL-10 promotor region that is predicted to enhance IL-10 production appeared to be triggered by cardiopulmonary bypass in some children.

Reversibility of Immunoparalysis

Evidence suggests that host immune function can be restored in the setting of immunoparalysis through several therapeutic approaches. Hershman *et al* reported in 1989 that monocytes collected from immunoparalyzed adult trauma victims upregulated their HLA-DR expression upon *ex vivo* culture with IFN γ .³⁶ In a similar cohort, Flohe *et al* showed restoration of the TNF α response in monocytes that were cultured *ex vivo* with the cytokine granulocyte macrophage colony-stimulating factor (GM-CSF).³⁷ More recently, Lendemanns *et al* showed that *ex vivo* treatment with IFN γ or GM-CSF (but not granulocyte colony stimulating factor [GCSF]) resulted in improvement in both monocyte HLA-DR expression and whole blood LPS-induced cytokine production in blood samples from injured patients.³⁸

Reversal of immunoparalysis *in vivo* appears to be possible as well. In 1997 Kox *et al* demonstrated restoration of monocyte HLA-DR expression and *ex vivo* TNF α response in nine of 10 adult septic patients with immunoparalysis upon treatment with subcutaneous IFN γ .³⁹ In 2002 Nakos *et al* conducted a small randomized placebo-controlled trial of inhaled IFN γ in 21 trauma patients with reduced HLA-DR expression on alveolar macrophages obtained by bronchoalveolar lavage (BAL).⁴⁰ Although there was no difference in mortality between groups, the IFN γ -treated subjects showed increased HLA-DR expression on alveolar macrophages, decreased IL-10 levels in subsequent BAL fluid, and a reduced rate of ventilator-associated pneumonia compared to the placebo group.

Recombinant human GM-CSF has also been used to treat ICU patients with immunoparalysis. This drug is particularly favorable for this purpose as it has been approved by the Food and Drug Administration (FDA) since 1991 for reconstitution of bone marrow after chemotherapy in adults and children. Its side effect profile is low and it is known to increase granulocyte and monocyte numbers as well as improve the function of existing cells of those lineages. In 2003 Nierhaus *et al* reported a case series in which nine septic adults with immunoparalysis received GM-CSF by subcutaneous injection daily for three days.⁴¹ All patients experienced increases in both monocyte HLA-DR expression and *ex vivo* LPS-induced TNF α production within 24 hours of initiation of GM-CSF treatment. A small randomized, controlled trial (RCT) in septic adults showed improvement in monocyte HLA-DR expression and faster resolution of infection with GM-CSF compared to placebo.⁴² In 2001 Bilgin *et al* reported the results of an RCT of GM-CSF therapy versus placebo for neutropenic neonates with sepsis.⁴³ Neonates receiving GM-CSF demonstrated significant improvement in mortality rate (10%) compared to those in the placebo group (30%). These RCTs, however, did not employ *a priori* measurement of the innate immune response. It is likely, therefore, that some subjects assigned to receive GM-CSF did not have severe reductions in innate immune function, thereby diluting the treatment effect.

Two small clinical trials, one in adults and one in children, have been completed using innate immune function monitoring to screen subjects for immunoparalysis prior to drug treatment. Meisel *et al* reported data from 38 patients who had immunoparalysis as evidenced by monocyte HLA-DR expression < 8,000 molecules/cell for two days.¹³ Subjects were randomized to receive GM-CSF or placebo for eight days. Subjects in GM-CSF group

showed higher HLA-DR expression and TNF α production capacity over time compared to the placebo group, with lower illness severity over time and lower resource utilization. A smaller, 14-subject RCT has been performed in children with MODS, comparing GM-CSF treatment to standard care in subjects found to have severe reduction in TNF α response.¹¹ In this study, GM-CSF therapy was associated with normalization of the TNF α response and dramatic reduction in risk of nosocomial infection. Importantly, in none of these studies was GM-CSF therapy associated with worsening of systemic inflammation. In fact, plasma levels of pro-inflammatory markers such as IL-6 and IL-8 were the same or lower after treatment with GM-CSF.

There are several ongoing studies of GM-CSF for the restoration of innate immune function in critical illness. These include a French RCT in septic adults with immunoparalysis, targeting reduction in nosocomial infection risk (NCT02361528) and a smaller British RCT in septic adults, targeting improvement in neutrophil function (NCT01653665). The only such trial in pediatrics is the GM-CSF for Immunomodulation Following Trauma (GIFT) study which is currently being performed by the Collaborative Pediatric Critical Care Research Network (NCT01495637). In this multi-center study, critically injured children undergo immune function testing in the first few days following injury using a highly standardized and centralized approach. Immune testing kits are produced and quality controlled at the Immune Surveillance Laboratory (ISL) at The Research Institute at Nationwide Children's Hospital and are then shipped to study sites. LPS stimulation and HLA-DR staining are done at each site, with the resulting supernatants and flow cytometry tubes being sent back to the ISL for TNF α and monocyte HLA-DR quantitation. Only children who are shown to have significant reduction in their TNF α production capacity go on to get GM-CSF. This open-label, dose-finding study is designed to determine the lowest immunostimulatory, tolerable dose of GM-CSF that will restore the TNF α response and monocyte HLA-DR expression above thresholds thought to be protective against nosocomial infection.

Less work has been done to investigate stimulation of the adaptive arm of the immune system in critical illness. Experimental evidence suggests, however, that blockade of the PD-1 pathway may confer a survival advantage in animal models of sepsis.^{44, 45} Recombinant human IL-7 has been shown to have similar effects in animal models.⁴⁶ As blocking antibodies against co-inhibitory molecules (checkpoint inhibitors) such as PD-1 have been tested in the adult oncology population, investigators are beginning to evaluate them as therapies for critical illness-induced immune suppression. A multi-center clinical trial of anti-PD-L1 is currently ongoing in adult septic patients with lymphopenia (NCT02576457).

Iatrogenic Immunosuppression

Many patients in the PICU receive systemic immunosuppressive therapies for the management of malignancy, autoimmune disease, or transplantation. At present, most transplant specialists follow drug levels and monitor end-organ function in order to titrate immunosuppressive therapy (*e.g.* tacrolimus, cyclosporine). While these agents are classically thought of as T cell inhibitors, they also exert a polarizing effect, skewing both

innate and adaptive immune systems toward a T_H2-like phenotype. Similarly, glucocorticoids promote lymphocyte apoptosis and impair pro-inflammatory signaling. The tapering or withholding of these medications in the context of life-threatening infection is thought to be essential. Reinke *et al* described a cohort of 45 adult kidney transplant recipients who developed nosocomial sepsis in the setting of immunoparalysis.⁴⁷ Patients who underwent rapid tapering of their calcineurin inhibition experienced a 90% survival rate (30/33) with 98% graft survival. In contrast, patients who did not undergo tapering had an 8% survival rate (1/12) with the sole survivor experiencing graft loss. Pediatric data on this subject are limited, but Hoffman *et al* described a series of 13 pediatric lung transplant recipients who underwent monocyte HLA-DR monitoring weekly following transplantation.⁴⁸ Those who developed pneumonia had lower monocyte HLA-DR expression over the four-week study period than those who remained infection-free. Monocyte HLA-DR expression measurement and quantification of the *ex vivo* stimulated cytokine responses both appear to have the potential to provide insight into the degree of functional immunosuppression following transplantation and are deserving of future study.

Perhaps less obvious is the fact that most of the medications and therapies that are employed in the PICU are, in some way, immunomodulatory (Table 3). Catecholamines, sedatives, analgesics, insulin, and diuretics can all directly or indirectly affect leukocyte function with the bulk of their effects being immunosuppressive. The net effect of these drugs' immunologic influences in a given patient is poorly understood. Another example of unintended immunomodulation can be found in the transfusion literature. Transfusion-related immunomodulation has historically been associated with the proinflammatory effects of mediators in stored blood products, but we now know that stored blood products can be immunosuppressive (reviewed in⁴⁹). These examples highlight the need for prospective immune function screening in our critically ill patients in order to develop immunophenotype-specific management strategies designed to promote immunologic balance.

Conclusion and Future Directions

While anti-inflammatory strategies were once thought to be promising in the setting of critical illness, the repeated failure of studies designed to reduce the inflammatory response in the ICU called this into question. The immunologic response to critical illness is now known to be highly dynamic and often includes a phase in which systemic inflammation and functional immunosuppression coexist. Immune monitoring strategies now exist which can identify critical illness-induced immune suppression, or immunoparalysis. These include quantification of monocyte HLA-DR expression, whole blood *ex vivo* stimulation testing, and measurement of lymphocyte counts and co-inhibitor molecule expression (*e.g.* PD-1). While these tests are not yet FDA-approved for the clinical laboratory, they can be used in the research setting for the design and testing of immune monitoring and modulation trials. By prospectively identifying patients with immunoparalysis, studies can be enriched with subjects most like to benefit from immunostimulatory strategies. Among the highest research priorities in the field is the development of a better understanding of the disease-, treatment-, and host-specific factors that promote immunoparalysis in the ICU. It similarly remains unknown if immunostimulatory strategies should target the innate or adaptive

immune system or both. The approach of real-time, multi-center, highly standardized immunophenotype-driven clinical trials has great potential to answer these questions and improve the outcomes and lives of critically ill children.

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KEY POINTS

- The pro-inflammatory surge that accompanies the onset of pediatric critical illness often occurs concurrently with a compensatory anti-inflammatory response.
- When severe and persistent, this anti-inflammatory response has been termed immunoparalysis and is associated with increased risks for nosocomial infection and mortality across a wide variety of PICU diagnoses.
- Immunoparalysis is quantifiable by tests that measure monocyte HLA-DR expression, *ex vivo* stimulated cytokine production capacities, and cell counts.
- Promising therapies exist which have the potential to reverse immunoparalysis and improve outcomes in the PICU.
- Standardized immune monitoring regimens are needed to guide clinical management and inform enrollment into clinical trials aimed at restoring immune function in the PICU.

Figure 1. The dynamic nature of the immune response to critical illness

Severe and persistent inflammation is associated with the classic symptoms of SIRS with fever, capillary leak, and organ dysfunction. A compensatory anti-inflammatory response syndrome (CARS) can occur concurrently with systemic inflammation. This can also be pathogenic when severe and prolonged, with associated increases in risk of nosocomial infection and death. More modest and transient deviations from immunologic homeostasis are typically associated with uncomplicated recovery.

Both SIRS and CARS can be quantified through specific laboratory testing.

Table 1

Elements of the innate and adaptive immune systems.

Innate	Adaptive
Cellular Elements	Cellular Elements
<i>Phagocytosis</i>	<i>Antibody production</i>
Monocytes / Macrophages	B cells / Plasma cells
Polymorphonuclear leukocytes	<i>Cytotoxic killing</i>
Dendritic cells	CD8+ T cells
<i>Antigen presentation</i>	<i>Cytokine / chemokine production</i>
Monocytes / Macrophages	CD4+ T cells
Dendritic cells	T _H 1 cells (proinflammatory)
<i>Cytotoxic killing</i>	T _H 2 cells (anti-inflammatory)
Natural killer cells	T _{reg} cells (anti-inflammatory)
Polymorphonuclear leukocytes	
<i>Cytokine / chemokine production</i>	
All of the above	

Table 2

Selected cytokines and their effects

	Cytokine	Primary Producer(s)	Action(s)
Proinflammatory	IL-1 β	Monocytes / macrophages	Fever, vasodilation, activation of T cells, monocytes / macrophages
	TNF α	Monocytes / macrophages, T cells (T _H 1), NK cells	Fever, vasodilation, apoptosis, activation of T cells, monocytes / macrophages
	IL-18	Macrophages	Activation of T cells and monocytes
	IL-12	Macrophages, DCs	Activation of NK cells
	IL-17	T cells (T _H 17)	Induction of proinflammatory cytokines
	IFN γ	T cells (T _H 1), NK cells	Activation of monocytes / macrophages
	GM-CSF	T cells, macrophages	Increases production and promotes growth and activation of monocytes, macrophages, PMNs, DCs
	IL6	Monocytes, macrophages, Vascular endothelium	Promotes acute phase response (<i>pro</i>), activates adrenal axis (<i>anti</i>)
Anti-inflammatory	IL-10	Monocytes / macrophages, T cells (T _H 2, Treg)	Inhibits monocyte / macrophage activation
	TGF β	Monocytes, T cell (T _H 2, Treg)	Inhibits monocyte / macrophage proliferation and activation
	IL-13	T cells (T _H 2)	Inhibits monocyte / macrophage cytokine production
	IL-1ra	Hepatocytes, monocytes/ macrophages, PMNs	Inhibits IL-1 action by blocking the IL-1 receptor

IL: interleukin, TNF: tumor necrosis factor, IFN: interferon, GM-CSF: granulocyte macrophage colony-stimulating factor, TGF: transforming growth factor, ra: receptor antagonist, NK: natural killer, DC: dendritic cell; Treg: regulatory T cell, PMN: polymorphonucleocyte

Table 3

Potential sources of unintended immunomodulation in the PICU

Drug/Therapy	Immune effect	Potential mechanisms
Antibiotics	↑	Release of bacterial components upon cell death
		Direct enhancement of intracellular killing
	↓	Bone marrow suppression (β lactams)
		Decreased pro-inflammatory cytokine production (macrolides)
Benzodiazepines	↓	Upregulation of cortisol axis
Catecholamines	↑	Stimulation of α adrenergic receptors
	↓	Stimulation of β adrenergic receptors
Dexmedetomidine	↓	CNS- and peripherally-mediated reduction in cytokine production
Furosemide	↓	Decreased pro-inflammatory cytokine production
Insulin	↓	Indirect reduction in hyperglycemia-induced pro-inflammatory cytokine production
Opiates	↓	Induction of leukocyte apoptosis, upregulation of TGF β and down-regulation of IFN γ
RBC transfusion	↑	Immune activation through cytokines and other mediators in stored blood products
	↓	Inhibition of host pro-inflammatory cytokine production capacity

CNS: central nervous system, RBC: red blood cell