Adjunctive and novel therapies for sepsis

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Abstract. Primary goals of sepsis therapy include early, appropriate antimicrobial therapy and prompt recognition and reversal of shock. Despite these measures, however, sepsis remains an important source of pediatric morbidity and mortality. Here we review rationale and existing evidence in support of adjunctive sepsis therapies including extracorporeal support, immunomodulation, and mitochondria-targeted therapies. While each of these therapeutic modalities shows promise, additional studies are urgently needed to identify the right patients, the right timing, and the right context for these interventions.

Keywords: Sepsis, extra corporeal membrane oxygenation, renal dialysis, immunomodulation, mitochondria

1. Introduction

Despite advances in critical care, septic disease remains an important source of morbidity and mortality in the pediatric intensive care unit [1–3]. Thus, development of novel and adjunctive sepsis therapies represents a vitally important step in improving outcomes for critically ill children facing this potentially devastating disease process. The focus of this review will be extracorporeal therapies, immunomodulatory therapies, and mitochondria-targeted therapies in the setting of sepsis.

2. Extracorporeal therapies

As the collective understanding of the pathobiology of pediatric sepsis has progressed, so has the development of a host of devices designed for extracorporeal support of failing organ systems.

Extracorporeal membrane oxygenation (ECMO), continuous renal replacement therapy (CRRT), therapeutic plasma exchange (TPE) and cytokine adsorption have all been employed in the management of pediatric patients with severe sepsis and septic shock and/or their sequelae. These modalities are alike in that pumps and circuits move blood to and from the patient through devices, which act upon the blood to accomplish a physiologic change. Extracorporeal techniques are also similar in that each requires specially-trained personnel, thereby increasing the complexity and expense of care (and, in many cases, limit availability). They also invariably expose the child to side effects and complications. Although the use of certain extracorporeal adjuncts in the treatment of critically ill children suffering from severe sepsis/septic shock is supported by clinical practice parameters [4], large-scale clinical trials are lacking in many cases, and patient selection and timing of application are key areas for ongoing study.

When deployed in the venoarterial (VA) configuration, ECMO has the potential to benefit children with refractory septic shock by using the blood pump and oxygenator to supplement cardiac output and increase arterial oxygen content. Although ECMO support for the hemodynamically unstable patient is

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intuitively sensible, it is also highly resource-intensive and carries significant risks of complications, chiefly hemorrhage related to anti-coagulation. For these reasons, ECMO has typically been reserved for only the most severely ill patients with recalcitrant septic shock despite intense conventional therapy. This patient selection paradigm involves assessment of benefits and risks, which is difficult since patients selected for VA ECMO typically have high-predicted mortality. Beca and Butt [5] in 1994 reported a mortality of 45% for pediatric patients supported with VA ECMO for refractory septic shock. Most of the children in this nine patient cohort developed multiple organ failure (MOF) in addition to refractory hemodynamic instability. In 1997, Goldman et al. [6] reported a twelve patient cohort who underwent VA ECMO support for refractory meningococcal septic shock with 69% survival to discharge. These case series reversed the previously held notion that sepsis was a contraindication for ECMO. More recent case series have shown even better outcomes and have hinted that cannula configuration may play a role in the success of ECMO support. Maclaren et al. [7] reported VA ECMO survival rates for refractory septic shock as high as 73% in a single-institution retrospective study in 2007, despite a high prevalence of organ failure and cardiopulmonary resuscitation prior to cannulation. The same group subsequently reported 78% survival for 23 children with refractory septic shock supported with VA ECMO using open thoracic cannulation [8]. To date, prospective studies to determine ideal ECMO cannulation strategies for patients with septic shock have not been done.

In addition to the nonspecific circulatory support possible with VA ECMO, recent years have seen the development of other extracorporeal methods of organ support that are less invasive and target specific aspects of sepsis pathophysiology. CRRT, TPE, and cytokine adsorption modify the small molecule and/or protein composition of blood and have been used in the setting of severe sepsis to modulate inflammation. In the context of severe septic disease, these modalities differ from VA ECMO, which is primarily a supportive therapy, in that they have the potential to affect the course of MOF which can develop after the initial hemodynamic instability has been temporized.

Numerous retrospective and prospective observational studies have examined CRRT in critically ill pediatric patients [9–14]. Although the majority of these studies have focused on MOF, it should be noted

that in the majority of patients in each study, sepsis was the inciting event. Goldstein et al. [9] reviewed 21 cases of MOF receiving CRRT and noted an association between fluid overload of 20% or more at the time of CRRT initiation and increased mortality. Foland et al. [14] noted a similar association between fluid overload and mortality in 113 pediatric MOF patients treated with CRRT. These early associations were confirmed by prospective observational work by the Prospective Pediatric CRRT Registry Group [10, 11]. From this registry, Goldstein et al. [10] in 2005 reported an additional 116 children with MOF and acute renal failure, the majority of whom were septic, and again noted an association between fluid overload of 20% at the time of CRRT and mortality. In 2010, the completed registry reported data on 297 critically ill children, with an overall incidence of sepsis of 32%. The authors of the study noted an association between fluid overload and mortality with a 3% increased risk of death for each 1% fluid overload present at the time of CRRT initiation [11]. In each of these studies, the association between fluid overload and mortality remained when controlling for covariables in multivariate analyses. Although these data provide convincing evidence for adverse outcomes associated with fluid overload in septic pediatric patients who develop MOF with renal failure, important unanswered questions are whether CRRT is able to ameliorate mortality and, if so, the optimal timing of CRRT initiation.

Since aggressive fluid resuscitation is a crucial aspect of septic shock management, it is counterintuitive to presume that fluid removal by CRRT will be beneficial until intravascular volume has been restored. However, CRRT also allows for filtration of blood without fluid removal when replacement fluids are prescribed to offset filtrate loss. Whether hemofiltration without fluid removal to reduce cytokine burden is useful has yet to be definitively determined. However, DiCarlo et al. [15] reported, in a 10 patient case series, an 80% survival rate in pediatric patients with ARDS after bone marrow transplantation using a high-clearance approach to hemodiafiltration (52 ± 17 mL/min/1.73m²). A phase II controlled trial in 24 septic adults using CRRT in the early stages of sepsis showed no benefit in cytokine removal or clinical endpoints [16]. In contrast, a retrospective adult study [17] showed that 40 patients treated with iso-volemic hemofiltration in the early stages of septic shock had improved survival, hemodynamics, vasopressor requirement, and gas exchange compared to

40 historical controls. To date, no sizeable studies of hemofiltration to modulate inflammation in infected children have been conducted.

It should be noted that the use of CRRT carries with it significant risks. These include the need for assiduous attention to the balance of fluid intake and output with, potentially, hourly adjustment of fluid removal or replacement rates. In centers that use a systemic heparinization strategy, CRRT poses the risk of life-threatening hemorrhage and requires careful titration of anticoagulation. In those that use a regional citrate-based approach to anticoagulation, attention must be paid to systemic calcium levels and citrate lock remains a concern in patients with liver failure. Lastly, little is known about the pharmacokinetics of most of the drugs used in the pediatric intensive care unit (ICU) in the context of extracorporeal therapies. It is important to consider the use of these therapies when dosing medications, particularly those for which drug levels can be obtained. Consultation with a therapeutic drug monitoring service can be invaluable in this setting.

Extracorporeal hemoperfusion with adsorption is a variation on the theme of inflammatory mediator removal whereby patient blood is made to perfuse columns with adsorptive properties in order to remove specific mediators. Resins, charcoal and artificial fiber cartridges have all been employed in animal models and small clinical studies to remove proinflammatory mediators from septic subjects [18, 19]. Fiber columns complexed with polymyxin B selectively remove bacterial lipopolysaccharide, thus reducing the inflammatory burden to infected patients [18, 20]. A randomized adult study of polymyxin B-fixed column hemoperfusion in the setting of abdominal sepsis was stopped early when interim analysis yielded a hazard ratio for death of 0.43 in the treatment group [21] indicating it would be unethical to randomize additional patients to standard therapy. Artificial columns have also been developed to remove cytokines from infected patients in the setting of septic disease. Multiple case series and pilot studies in adults have shown cytokine adsorptive columns, often used in conjunction with plasmafiltration, to be effective in reducing intotrope/vasopressor use [22, 23], improving organ failure [23, 24] and reducing inflammatory cytokine levels [25] in septic patients. As yet, the role of lipopolysaccharide (LPS) and cytokine adsorption in the treatment of pediatric septic shock has not been addressed in a large scale fashion.

Bridging the gap between CRRT and cytokine adsorption is TPE. Unlike cytokine adsorption, TPE does not specifically target individual mediators but rather uses apheresis techniques to replace patient plasma (presumably laden with deleterious mediators) with donor plasma (of theoretically more beneficial composition). The bulk removal of plasma contents mirrors the nonspecific hemofiltration characteristics of CRRT, but TPE does not alter patient fluid status appreciably. Multiple reports in adults and children provide evidence for the ability of TPE to ameliorate multiple organ dysfunction in the setting of sepsis. Stegmayr et al. [26] reported results from 76 patients, including six children, who underwent TPE as rescue therapy for sepsis-induced MOF with an overall cohort survival of 82% despite severity of illness-predicted survival of <20%. A small randomized pediatric trial of TPE for treatment of thrombocytopenia-associated multiple organ failure (TAMOF) reported improved organ failure in five treated patients, all of whom met criteria for sepsis, compared to control [27]. This study suggested that TPE can be effective in restoring plasma levels and activity of the von Willebrand factor cleaving protease ADAMTS13. This raises the possibility that TAMOF represents a form of thrombotic microangiopathy on the same spectrum as thrombotic thrombocytopenic purpura. A larger retrospective study recently reported 27% mortality in TPE-treated children with TAMOF versus 70% in a standard therapy control cohort [28]. Aside from supportive care for organ failure, TPE has also been employed in the early acute phase of septic shock in adults with conflicting results. Busund demonstrated improved survival in the TPE-treated adult patients with septic shock compared to standard therapy [29]. Hadem et al. [30] however, showed no statistically significant improvement in hemodynamics or vasopressor use in 23 patients. Whether TPE has a role in the acute management of pediatric patients with septic shock is unknown. As in other extracorporeal therapies, careful attention should be paid to drug dosing, as drug removal will occur along with other plasma constituents. Similarly, an “emergence phenomenon” can occur, particularly with centrifugal apheresis, in which acute drops in plasma levels of sedatives and/or cardiovascular agents may necessitate a temporary increase in drip rates while the therapy is ongoing.

Extracorporeal therapeutic adjuncts have evolved substantially over the past several decades from wholesale general organ support with VA ECMO

to molecularly-targeted cytokine adsorption technologies. Sound biological reasoning underlies the use of each, but nevertheless, limited availability, expense, and complications remain significant barriers to their use, as do unanswered questions of ideal patient selection and timing of therapy.

3. Immunomodulatory therapies

The immunologic response to sepsis is highly dynamic and complex. Certainly, the well-recognized signs and symptoms of sepsis (e.g. tachycardia, tachypnea, fever, leukocytosis) arise from an overly exaggerated immune response. However, it is increasingly recognized that even in the context of signs and symptoms of inflammation and high levels of circulating pro-inflammatory mediators, the body also produces high levels of anti-inflammatory mediators [31–35]. In many patients, the anti-inflammatory mediators begin to dominate and a state of acquired immune suppression develops. Across multiple studies of septic adults and children, an under-active or suppressed immune response is significantly associated with increased risks of nosocomial infection and mortality [36–38]. These data along with the failure of anti-inflammatory therapies to improve outcomes in sepsis have shifted the focus of immunomodulatory approaches to sepsis therapy toward restoration of immune function and a return to immunologic homeostasis [39–44].

4. Therapies to modulate innate immune function

The immune system is broadly categorized into innate and adaptive arms. Circulating monocytes are a key cell type in the innate immune response. Monocytes function as first responders by ingesting pathogens; digesting, processing and displaying antigens via MHC class II molecules (e.g. HLA-DR) on the cell surface; and when activated they secrete cytokines to further modulate the immune response. Monocytes from critically ill patients, particularly in the acute phase of illness, are often characterized by reduced cell surface expression of HLA-DR and significantly diminished ability to secrete pro-inflammatory cytokines (e.g. tumor necrosis factor (TNF)- α) in response to *ex vivo* stimulation with LPS [33, 35,

45, 46]. Severe and/or prolonged reductions in monocyte HLA-DR (mHLA-DR) expression and *ex vivo* TNF- α production capacity have both been associated with increased rates of nosocomial infection and/or mortality in septic adults and children [37, 38, 47].

To date, the majority of studies evaluating immunostimulatory drugs in critical illness have focused on therapies targeting restoration of innate immune responses (Table 1). Initial studies evaluated the ability of interferon gamma (IFN γ) to restore innate immune function and improve infection-related outcomes in critically injured adults [48, 49]. IFN γ is a pro-inflammatory cytokine, which functions to activate monocytes and macrophages and promote MHC class II expression. Two randomized, controlled trials demonstrated improvements in monocyte HLA-DR expression in adult trauma patients after treatment with IFN γ , though effects on clinical outcomes were mixed [48, 49]. In an early randomized, controlled trial of critically injured adults at high risk for infection due to bacterial contamination, Polk et al. [49] demonstrated improvement in mHLA-DR expression in patients treated with 10 days of IFN γ versus placebo but failed to demonstrate differences in rates of major or minor infection. Notably, mHLA-DR expressions in the treated group fell to the levels of the placebo group after day 10, suggesting that longer therapy may have been beneficial. Accordingly, in a subsequent randomized controlled trial of 416 critically injured adults, Dries et al. [48] administered IFN γ versus placebo for 21 days or until hospital discharge and demonstrated lower incidence of infection-related mortality in the treated group. These data suggest that IFN γ may show promise in reducing post-trauma infectious complications, but further studies are needed.

Regarding IFN γ treatment in sepsis, data are limited. In two small un-randomized studies, septic adults were treated with IFN γ with improvements noted in both monocyte HLA-DR expression and TNF- α production capacity after treatment [50, 51]. In both cases however, small numbers and non-randomized study designs limit the ability to draw conclusions about clinical efficacy. Currently there are no data from large randomized-controlled trials of IFN γ therapy in sepsis, though a single-center randomized controlled trial evaluating the use of IFN γ in adults with septic shock (NCT 01649921) is currently ongoing and may provide additional insights.

Granulocyte colony stimulating factor (G-CSF) has also been evaluated as an immunostimulatory agent to

Table 1
Selected clinical trials evaluating immunostimulatory drugs in critically ill patients

Study	Design	Population	Number	Findings
Drugs targeting innate immune suppression				
IFN γ				
Polk et al. [49], 1992	RCT	Severely injured adults	193	Enhanced mHLA-DR in treated group. No difference in rates of minor or major infection
Dries et al. [48], 1994	RCT	Severely injured adults	416	Lower incidence of infection-related mortality in treated group
Döcke et al. [51], 1997	Non-randomized, interventional	Septic adults with low mHLA-DR	9	Restoration of mHLA-DR expression and TNF α production capacity after treatment
Wasserman et al. [103], 1998	RCT	Adults with severe burn injury	216	No differences in infection-related mortality
G-CSF				
Nelson et al. [52], 2000	RCT	Hospitalized adults with pneumonia	480	No difference in therapeutic failure rates. Trend toward lower mortality in treated group among subjects with positive <i>S. pneumoniae</i> blood culture
Root et al. [53], 2003	RCT	Adults with pneumonia and severe sepsis	701	No differences in mortality or new organ dysfunction between groups
GM-CSF				
Bilgin et al. [54], 2001*	RCT	Septic neonates with neutropenia	60	Lower mortality in treated group
Drossou-Agakidou et al. [55], 2002*	RCT	Septic neonates	56	Earlier restoration of mHLA-DR in GM-CSF treated group compared to G-CSF or placebo
Presneill et al. [59], 2002	RCT	Septic adults with pulmonary dysfunction	18	Improved neutrophil function and improved oxygenation in treated group
Nierhaus et al. [57], 2003	Non-randomized, interventional	Septic adults with low mHLA-DR	9	Restoration of mHLA-DR expression and TNF α production capacity after treatment
Rosenbloom et al. [60], 2005	RCT	Septic adults	33	No difference in mortality. Higher rate of resolution/improvement of infection in treated group
Orozco et al. [58], 2006	RCT	Adults with abdominal sepsis	58	Shorter hospital stay and fewer infectious complications in treated group
Meisel et al. [56], 2009	RCT	Septic adults with low mHLA-DR	38	Increased mHLA-DR and shorter durations of mechanical ventilation, intensive care unit and hospital stay in treated group

Table 1
(continued)

Study	Design	Population	Number	Findings
Hall et al. [37], 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 et al. [104], 2012	RCT	Adults with ALI/ARDS	132	No difference in ventilator-free days
Drugs targeting adaptive immune suppression IL-7				
Venet et al. [68], 2012	<i>Ex-vivo</i>	Adults with septic shock	10	Restoration of stimulated lymphocyte proliferation and IFN γ production after <i>ex-vivo</i> treatment with recombinant human IL-7
Anti PD-1				
Chang et al. [77], 2014	<i>Ex-vivo</i>	Septic adults	19	Increased IFN γ production and decreased lymphocyte apoptosis after <i>ex-vivo</i> treatment with antibodies to block PD-1 pathway

*Indicates pediatric data. IFN γ = Interferon gamma; RCT = Randomized controlled trial; mHLA-DR = Monocyte human leukocyte antigen-DR expression; G-CSF = Granulocyte colony stimulating factor; GM-CSF = Granulocyte macrophage colony stimulating factor; MODS = Multiple organ dysfunction syndrome; TNF α = Tumor necrosis factor alpha. ALI = Acute lung injury; ARDS = Acute respiratory distress syndrome; IL-7 = interleukin-7; PD-1 = Programmed death-1.

augment the treatment of sepsis and severe infections with mixed results. G-CSF is an endogenous molecule, which increases neutrophil production in the bone marrow, and has been used for decades in recombinant form for restoration of neutrophil counts following myeloablative chemotherapy. Two large randomized, controlled trials have been conducted to evaluate the addition of G-CSF therapy to antimicrobials to treat hospitalized adults with pneumonia [52, 53]. The first study enrolled 480 patients requiring IV antibiotics for multi-lobar pneumonia [52]. No significant differences were found between treated and placebo groups with respect to the primary outcome measure of therapeutic failure rate. Similarly, overall mortality and intensive care unit lengths of stay were not different between groups, though a mortality benefit was seen for the subset of patients with *Streptococcus pneumoniae* bacteremia. However, a subsequent study of 701 hospitalized adults with pneumonia and severe sepsis failed to show a mortality benefit with G-CSF therapy [53].

The majority of studies of immunostimulatory agents in sepsis or critical illness, including the only studies in pediatric patients, have evaluated the use

of granulocyte macrophage-colony stimulating factor (GM-CSF) [37, 54–59]. GM-CSF is a cytokine, which functions to promote increased numbers and function of innate immune cells including those of both the neutrophil and monocyte lineages. Clinical studies in septic adults have consistently demonstrated improvement in markers of innate immune function with recombinant human GM-CSF therapy [56, 57, 59]. Importantly GM-CSF therapy has been well tolerated in the setting of critical illness, and markers of systemic inflammation (such as plasma IL-6 levels) have not been shown to increase in treated groups. Regarding clinical outcomes, Rosenbloom et al. [60] reported improved innate immune function and faster rates of infection resolution in septic adults treated with GM-CSF compared to placebo, though there were no differences in mortality between groups. Meanwhile, Orozco et al. [58] demonstrated shorter hospital stay and fewer infectious complications in adults with abdominal sepsis treated with GM-CSF versus placebo. Neither of these studies incorporated baseline measures of immune function in patient selection or randomization. More recently, Meisel et al. [56] randomized 38 adults with sepsis and severely

reduced mHLA-DR expression to 8-day treatment with GM-CSF versus placebo. While the study was underpowered to detect a mortality benefit, treated patients had improved mHLA-DR expression and *ex vivo* TNF α production capacity along with shorter durations of ICU stay and mechanical ventilation.

There are pediatric data evaluating GM-CSF therapy in the setting of sepsis. Two randomized controlled trials of GM-CSF therapy have been conducted in neonatal sepsis (54,55). Drossou-Agakidou et al. [55] randomized 56 neonates with suspected sepsis to receive GM-CSF, G-CSF, or placebo. Infants who received GM-CSF showed more rapid recovery of monocyte HLA-DR expression compared to infants in the G-CSF or placebo groups. Clinical outcome measures were not evaluated. Bilgin et al. [54] evaluated 7-day treatment with GM-CSF versus placebo in neonates with culture positive sepsis and neutropenia. The treated group demonstrated more rapid improvements in absolute neutrophil and monocyte counts as well as significantly lower mortality (10% versus 30% for placebo group). In the pediatric intensive care unit, Hall et al. [37] randomized 14 children with multiple organ dysfunction syndrome and severely low TNF α production capacities to 7-day treatment with GM-CSF or placebo. Treated patients demonstrated significant improvements in TNF α production capacities over time compared to the placebo group. While every patient in the placebo group subsequently developed nosocomial infection, there were no nosocomial infections in the treated group.

Taken together, the literature to date suggests that GM-CSF may be a promising adjunct for restoration of innate immune function, particularly for septic patients with documented immune suppression. However, larger studies capable of drawing definitive conclusions have yet to be conducted.

5. Therapies to modulate adaptive immune function

Emerging evidence suggests that adaptive immune suppression may also play an important role in sepsis-related morbidity and mortality [36, 61–63]. The major cell types of the adaptive immune response are B and T lymphocytes. Unlike innate immune cells, lymphocytes typically respond very specifically to pathogens, with lymphocyte stimulation occurring in response to interaction with innate immune cells, which process

and present pathogenic antigens. Upon stimulation, B cells mature to plasma cells, which produce antibodies. T cells are grouped into cytotoxic (CD 8+) T cells, which serve to promote killing of intracellular organisms and helper (CD 4+) T cells, which serve to modulate the overall immune response via the production and release of cytokines.

Sepsis-related lymphopenia and apoptotic cell death of both B and T lymphocytes have been well recognized in both adults and children [61, 64]. In addition to lymphopenia, recent studies of children with septic shock reveal evidence of suppressed lymphocyte function (e.g. cytokine production capacity) as well as down-regulation in genes coding for adaptive inflammatory pathways [62, 63]. Markers of adaptive immune suppression have been demonstrated early in the course of disease (within 24–48 hr of sepsis onset) with early reductions in lymphocyte cytokine production capacity being associated with infection-related complications [62]. These data suggest that reversal of early sepsis-related adaptive immune suppression may represent a therapeutic target.

To date, studies of drugs specifically targeting adaptive immune suppression have been limited to pre-clinical models and *ex vivo* experiments. One approach has been to administer cytokines aimed at restoring lymphocyte number and function. For example, treatment with recombinant human IL-7 or IL-15 has shown promise in augmenting adaptive immune function and improving survival in murine sepsis models [65–67]. Human data regarding cytokine therapy to improve adaptive immune function in sepsis are limited. In an *ex vivo* study, Venet et al. [68] administered IL-7 to peripheral blood mononuclear cells isolated from adults with septic shock. Treated lymphocytes demonstrated improvements in both proliferative capacity and stimulated INF γ production capacity, suggesting that sepsis-associated lymphocyte suppression may be pharmacologically reversible.

In addition to immunostimulatory cytokine therapy, an alternate approach to restore adaptive immune function involves blocking negative co-stimulatory molecules such as programmed death (PD)-1 or cytotoxic T-lymphocyte antigen-4 (CTLA-4). Both PD-1 and CTLA-4, which function to suppress adaptive immune responses, are upregulated in the setting of sepsis, as a counter-regulatory function [36, 69–71]. Antibodies to block either the PD-1 pathway or CTLA-4 provide a survival benefit along with improved lymphocyte survival and cytokine production capacity

in animal models of sepsis or fungal infection [72–75]. Human studies evaluating PD-1 or CTLA-4 blockade are few. Singh et al. [76] used blocking antibodies to block the PD-1 pathway in peripheral blood mononuclear cells isolated from adults with pulmonary tuberculosis. In these *ex vivo* studies, PD-1 pathway blockade reduced effector T cell apoptosis and improved lymphocyte cytokine production. Likewise, Chang et al. [77] used a similar strategy to block the PD-1 pathway in peripheral blood mononuclear cells isolated from critically ill septic adults and similarly reported decreases in lymphocyte apoptosis with improvements in cytokine production capacities after *ex vivo* treatment. Both recombinant human IL-7 and anti-PD-1 antibody therapy have been studied *in vivo* in humans as adjuvant therapies for human malignancy and may have a role in future clinical trials in sepsis.

Taken together, these data suggest that immunomodulating therapies aimed at restoring adaptive immune function may be beneficial adjuncts to sepsis therapy, though clinical trials are needed to determine efficacy at the bedside.

As an alternate approach rather than re-activating adaptive immune cell function, investigators have trialed the use of polyclonal intravenous immunoglobulin (IVIG) to provide exogenous antibodies in the setting of sepsis. While IVIG may be beneficial in the setting of streptococcal toxic shock syndrome, its utility in the setting of sepsis in general is less clear [78]. A recent meta-analysis evaluated 25 placebo controlled trials of IVIG therapy for sepsis, severe sepsis or septic shock, including five trials in neonates [79]. While there was a modest mortality benefit with IVIG therapy when all studies of septic adults were included, this benefit was not apparent when only studies with low risk of bias were included. When neonatal studies were evaluated, a mortality benefit was not observed with IVIG treatment. This updated review contained a recent large randomized-controlled trial of IVIG as adjunct therapy for neonatal sepsis, which failed to show benefit in the primary outcome measure of death or major disability at 2 yr of age or in any of the secondary outcomes measured [80]. Given the evidence to date, the routine use of IVIG for pediatric sepsis cannot be supported outside of the setting of toxic shock syndrome. However, it should be noted that previous studies have enrolled and randomized septic patients without regard for underlying nutritional status, immune function, or immunoglobulin levels. It is possible that IVIG therapy may be beneficial for select populations of septic

children, and this should be the subject of future investigation.

While not specific to sepsis, the CRISIS prevention trial is perhaps the largest pediatric study evaluating an immunomodulatory approach to prevent immune suppression in critical illness [81]. This multicenter randomized trial enrolled 293 critically ill, mechanically ventilated children between the ages of 1 and 17 yr. Subjects were randomized to receive either placebo or a combination prophylactic therapy with zinc, selenium, glutamine and the dopamine-2 antagonist metoclopramide designed to promote lymphocyte survival and prevent critical illness-associated lymphopenia. The trial was stopped early due to futility and there were no overall differences between treatment groups with respect to the primary outcome variable of time from pediatric ICU admission to the development of nosocomial infection. However, on subgroup analysis of the 25 patients with identified baseline immunodeficiency, zinc/selenium/glutamine/metoclopramide therapy was associated with a reduction in nosocomial infections, suggesting that a more targeted approach to patient selection may be beneficial.

6. Summary of immunomodulatory therapies

The data supporting the use of immunomodulatory therapy for sepsis are either mixed or at best limited to small studies. It is likely that the failure of immunomodulatory therapies to consistently improve outcomes in sepsis may be related to patient selection. Given the complexity of the immunologic response to severe sepsis and septic shock, it is likely that different patients will respond most favorably to different immunomodulatory approaches, or that the same patient may require different approaches at different points in time. Without comprehensive, standardized assessments of immune function, the clinician is left blinded to the therapies which may prove most beneficial. Future interventional trials, therefore, should be driven by prospective immune function monitoring, with enrollment targeting high-risk subjects with clearly documented immune dysfunction. Armed with more comprehensive analyses of immune function over time, we can further our ability to provide patient-tailored adjunct immunomodulatory therapies to improve sepsis outcomes in the future.

7. Therapies targeting mitochondrial function

While immune function remains an important target for emerging sepsis therapies, recent literature suggests that sepsis-induced organ dysfunction and morbidity may be associated with mitochondrial dysfunction. An emerging theory to explain the development of sepsis-related multiple organ dysfunction (MODS) suggests that in addition to global hypoperfusion in the setting of septic shock - or even in the absence of shock - cellular oxygen consumption may be impaired [82–84]. This would result in a state of cellular dysoxia and resulting cellular dysfunction, which persists despite adequate restoration of oxygen delivery. A state of impaired cellular function as an etiology for MODS (as opposed to structural cellular damage) is supported by autopsy evidence revealing ultrastructurally normal parenchymal cells in the context of severe sepsis-related organ dysfunction [85]. As mitochondria make up by far the largest component of cellular oxygen utilization, it stands to reason that altered mitochondrial function likely plays a role in this process.

A primary function of mitochondria is to produce energy (ATP) via transfer of electrons in oxidative phosphorylation. Mitochondria also serve vital roles in regulating the production and turnover of reactive oxygen species, in apoptosis signaling, and in innate inflammatory signaling [86–91]. Evidence from observational studies and animal models reveal a number of alterations in mitochondrial function in the setting of sepsis [92–96] (Table 2). Among septic adults, mitochondrial dysfunction as evidenced by decreased electron transport chain activity and decreased ATP production has been associated with increased shock severity and mortality [97]. Likewise, genomic analysis of healthy adults after endotoxin challenge revealed down-regulation of a number of genes within ATP synthesis pathways suggesting a probable link between inflammation and mitochondrial function [98]. Whether these changes represent potential causes of sepsis-related organ dysfunction or responses to inflammation and organ dysfunction remains to be determined [99]. It is notable however, that in pre-clinical animal models of sepsis a number of therapeutic approaches directed at restoring mitochondrial function are associated with improved markers of organ function and survival (reviewed in [100]). These include providing exogenous substrates and essential co-factors to support oxidative phosphorylation,

Table 2
Alterations in mitochondrial function associated with sepsis and potential therapeutic targets

Alteration in mitochondrial function	Potential therapy
↓ Oxidative phosphorylation	Anti-oxidant
↑ Reactive oxygen species/oxidative stress	Anti-oxidant
↓ Mitophagy	Anti-oxidant
↓ Mitochondrial biogenesis	Mitochondrial transcription factor A

antioxidants, reactive oxygen species scavengers, and mitochondrial membrane stabilizers.

Clinical trials of mitochondria-targeted therapies in sepsis are fewer. Galley et al. [101] performed a phase I dose escalation study of melatonin in healthy adults coupled with *in vitro* assessments of melatonin's anti-oxidant properties. *In vivo*, melatonin was generally well tolerated without significant adverse effects. *In vitro*, blood cells treated with melatonin in addition to inflammatory stimulus (LPS and peptidoglycan) had improved mitochondrial function and measures of oxidative stress compared to cells treated with LPS and peptidoglycan alone, suggesting that inflammation-related mitochondrial dysfunction may be preventable. In a separate study, Torracco et al. [102] evaluated the anti-oxidant potential of levosimendan in a randomized, controlled trial of 26 adults with septic shock. In this cohort, levosimendan therapy was associated with improvements in mitochondrial electron transport chain function and degree of oxidative stress, suggesting that sepsis-related mitochondrial dysfunction may be reversible *in vivo*. Though currently there are insufficient clinical data to support the routine use of anti-oxidants or other mitochondria-targeted therapies as adjuncts to sepsis care, mitochondrial dysfunction remains a promising therapeutic target and the subject of much ongoing research.

8. Conclusions

Rapid administration of appropriate antibiotic coverage and prompt recognition and reversal of shock remain cornerstones of pediatric sepsis care. Despite these interventions, however septic children remain at risk of adverse outcomes. Successful employment of adjunctive therapies for septic shock requires proper patient selection. Extracorporeal therapies,

immunomodulatory therapies, and mitochondrial-targeted therapies all represent potential additions to the armamentarium of the pediatric intensivist in the management of septic children. There is a spectrum of evidence supporting the use of these therapies *in vivo*, but in general, more studies are urgently needed to identify the right patients, the right timing, and right context for these interventions. Indeed, we must advance our understanding of cellular and molecular mechanisms of sepsis and more thoroughly phenotype septic children in order to devise and evaluate personalized adjunctive therapies aimed at improving pediatric sepsis outcomes.

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