Early Immune Function and Duration of Organ Dysfunction in Critically Ill Children with Sepsis

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Abstract

Rationale: Late immune suppression is associated with nosocomial infection and mortality in adults and children with sepsis. Relationships between early immune suppression and outcomes in children with sepsis remain unclear.

Objectives: Prospective observational study to test the hypothesis that early innate and adaptive immune suppression are associated with longer duration of organ dysfunction in children with severe sepsis or septic shock.

Methods: Children younger than 18 years of age meeting consensus criteria for severe sepsis or septic shock were sampled within 48 hours of sepsis onset. Healthy control subjects were sampled once. Innate immune function was quantified by whole blood *ex vivo* LPS-induced TNF- α (tumor necrosis factor- α) production capacity. Adaptive immune function was quantified by *ex vivo* phytohemagglutinin-induced IFN- γ production capacity.

Measurements and Main Results: One hundred two children with sepsis and 35 healthy children were enrolled. Compared with healthy children, children with sepsis demonstrated

lower LPS-induced TNF- α production ($P < 0.0001$) and lower phytohemagglutinin-induced IFN- γ production ($P < 0.0001$). Among children with sepsis, early innate and adaptive immune suppression were associated with greater number of days with multiple organ dysfunction syndrome and greater number of days with any organ dysfunction. On multivariable analyses, early innate immune suppression remained independently associated with increased multiple organ dysfunction syndrome days (adjusted relative risk, 1.2; 95% confidence interval, 1.03–1.5) and organ dysfunction days (adjusted relative risk, 1.2; 95% confidence interval, 1.1–1.3).

Conclusions: Critically ill children with severe sepsis or septic shock demonstrate early innate and adaptive immune suppression. Early innate and adaptive immune suppression are associated with longer durations of organ dysfunction and may be useful markers to help guide future investigations of immunomodulatory therapies in children with sepsis.

Keywords: pediatrics; multiple organ failure; sepsis; immune system

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At a Glance Commentary

Scientific Knowledge on the

Subject: Although subacute or late immune suppression is associated with adverse outcomes in critically ill adults and children with sepsis, less is known about the relationships between early immune suppression and outcomes in pediatric sepsis. Similarly, relationships between immune function and duration of organ dysfunction are unknown in this population.

What This Study Adds to the

Field: We report here one of the largest studies of innate and adaptive immune function in critically ill children with severe sepsis/septic shock. In this cohort, suppression of both innate and adaptive immunity within 48 hours of sepsis onset were significantly associated with longer duration of organ dysfunction. Furthermore, the severity of early innate immune suppression remained independently associated with longer duration of organ dysfunction after accounting for baseline variables. Our findings suggest that early measures of innate and adaptive immune function may enhance risk stratification and provide therapeutic targets for future pediatric sepsis trials. Our findings also highlight duration of organ dysfunction as a potential outcome measure for future trials of immunomodulatory therapies in pediatric severe sepsis or septic shock.

Sepsis-induced organ dysfunction is an important source of morbidity and mortality in children worldwide, particularly when more than one organ is affected (1–3). Although the classic signs and symptoms of acute severe sepsis and septic shock are the result of an overwhelming inflammatory response, a growing body of literature suggests that a compensatory state of immune suppression frequently accompanies septic disease. Sepsis-related immune suppression can affect both the innate and adaptive arms of the immune system, likely represents the combination of endogenous (e.g., cytokines) and exogenous (e.g., medications) influences, and has been associated with

increased risk for nosocomial infection and death (4–7). Critical illness-associated innate immune suppression is typically occult but is detectable through provocative testing, characterized by a reduced ability of subjects' blood immune cells to secrete the proinflammatory cytokine TNF- α (tumor necrosis factor- α) in response to *ex vivo* stimulation with LPS. Adaptive immune function can similarly be assessed through the *ex vivo* stimulation of whole blood with phytohemagglutinin (PHA), with low IFN- γ production capacity representing adaptive immune suppression (8). Although transcriptomic studies suggest that early lymphocyte suppression can predict adverse outcomes in children with sepsis, few studies have investigated the functional lymphocyte response in this setting (8, 9).

Because an intact immune response is important to help clear initial infection, prevent secondary infection, and prevent latent virus reactivation, immune function is likely highly relevant in sepsis. However, there are several important gaps in our understanding of sepsis-induced immune suppression in children. First, the timing of sepsis-induced immune suppression remains controversial, with some adult data suggesting that early measurements of immune function (within the first 48 h of illness) may not predict outcomes as well as measurements taken later in the first week of illness (10, 11). Other data from critically ill children without sepsis suggest that early innate immune suppression is associated with increased nosocomial infection and mortality risk (5–7, 12). Second, the relationship between innate and adaptive immune function at these early time points is also poorly understood. Last, the relationships between early immune function and organ failure outcomes are unknown in pediatric sepsis. Understanding these relationships is necessary to consider the use of organ failure duration as an outcome variable in future clinical trials of immunomodulation in this population.

We therefore performed a prospective observational study to test the hypothesis that in critically ill children with severe sepsis or septic shock, both innate and adaptive immune suppression measured within 48 hours of sepsis onset will be associated with longer durations of organ dysfunction. We anticipate that these data will support the concepts of early immune function monitoring in children with sepsis and the use of organ failure recovery as an outcome variable in future trials of immunomodulatory therapies.

Methods

Setting

The pediatric ICU at Nationwide Children's Hospital is a 40-bed quaternary-care medical-surgical ICU with nearly 3,000 annual admissions. Admissions to a separate cardiothoracic ICU were not included in this study.

Subjects

The protocol was approved by the Institutional Review Board at Nationwide Children's Hospital. Informed consent was obtained from subjects' legal guardians and, when appropriate, informed assent was obtained from subjects. Children younger than 18 years of age who were admitted to the pediatric ICU, met consensus criteria for severe sepsis or septic shock (13), and were within 48 hours of sepsis onset were eligible for enrollment. Children were excluded if there was a limitation-of-care order in place.

Healthy children were recruited from the outpatient phlebotomy area and Clinical Research Services at Nationwide Children's Hospital. Healthy children were excluded if they had subjective or measured fever within the past 24 hours, history of systemic corticosteroid use within the past month, aspirin or nonsteroidal antiinflammatory drug use in the past 48 hours, or a history of a chronic inflammatory disease, malignancy, or transplantation. Because our previous studies indicate that immune suppression in critical illness occurs across multiple diagnoses and is not limited to sepsis, we chose to compare children with sepsis to healthy children rather than ICU control subjects (5–7).

Immune Function Measures

Details of blood sampling and plasma cytokine and immune function measurements are provided in the online supplement. Innate immune function was assessed by whole blood *ex vivo* LPS-induced TNF- α production capacity, and adaptive immune function was assessed by *ex vivo* PHA-induced IFN- γ production capacity (5, 7, 14). Plasma IL-6, a marker of systemic inflammation and predictor of adverse outcomes in multiple studies of adult and pediatric sepsis, was quantified in unstimulated samples (15–17).

Clinical Data and Outcome Measures

Clinical definitions and details of statistical analysis are provided in the online supplement. Primary outcome measures include number of days with organ

dysfunction in 14 days and number of days with multiple organ dysfunction syndrome (MODS) in 14 days. Organ dysfunction was defined using previously published criteria (18), with the following modifications: use of venoarterial extracorporeal membrane oxygenation was considered as fulfilling criteria for cardiovascular and respiratory dysfunction, use of venovenous extracorporeal membrane oxygenation was considered as fulfilling criteria for respiratory dysfunction, and use of renal replacement therapy was considered as fulfilling criteria for renal dysfunction. Any patient who died was assigned the maximum number of MODS days (14). Prolonged MODS was defined as death or 7 or more days with MODS, as previously published (19).

Secondary outcomes included development of new or progressive multiple organ dysfunction (NP-MODS), hospital mortality, and development of new nosocomial infection. NP-MODS was defined as any day beyond the first 48 hours with any new or worsening organ dysfunction. New nosocomial infection was defined by CDC criteria and included bloodstream infection, urinary tract infection, central nervous system infection, ventilator-associated pneumonia, and/or ventilator-associated tracheobronchitis (20). Nosocomial infections were identified by a single investigator before knowledge of immune function laboratory data.

Results

Subjects

One hundred two children with sepsis were enrolled. Baseline characteristics are listed in Table 1. About half of subjects had a history of a complex chronic condition. The most common complex chronic conditions were neurologic, including developmental delay and/or seizure disorder ($n = 14$). Sixteen children had a history of baseline immune compromise, including two subjects with rheumatologic disease, six subjects with an oncologic diagnosis, three subjects with a congenital immunodeficiency, and five subjects with a history of bone marrow or solid organ transplant. Septic shock was present in 78% of subjects, and nearly 60% presented with two or more organ dysfunctions. The most common bacterial organisms isolated included *Staphylococcus aureus* ($n = 16$; 9 with methicillin-susceptible

Table 1. Subject Characteristics

Characteristic	N = 102
Male	61 (60)
Age, mo	74.5 (6–160)
Complex chronic condition	48 (47)
Baseline immune compromise	16 (16)
Shock	79 (78)
Sepsis source*	
Bloodstream	21 (21)
Respiratory	50 (49)
Urine	9 (9)
Abdomen	4 (4)
CNS	4 (4)
Community acquired	96 (94)
Organism(s)	
Bacterial	37 (36)
Fungal	2 (2)
Viral	22 (22)
Viral plus bacterial	11 (11)
Culture negative	30 (29)
Initial severity of illness [†]	
PRISM III	9.0 (5–14)
PELOD 2	7.0 (5–9)
Invasive mechanical ventilation	76 (75)
Vasoactive inotrope score [‡]	11.5 (5–20)
Hydrocortisone use [‡]	31 (30)
RBC transfusion [‡]	48 (47)
Initial number of organ dysfunctions [‡]	
0	7 (7)
1	36 (35)
2	46 (45)
3	12 (12)
4	2 (2)
Renal replacement therapy	7 (7)
Renal replacement therapy in first 48 h	2 (2)
ECMO	4 (4)
ECMO in first 48 h	3 (3)

Definition of abbreviations: CNS = central nervous system; ECMO = extracorporeal membrane oxygenation; PELOD = pediatric logistic organ dysfunction; PRISM = pediatric risk of mortality; RBC = red blood cell.

Data are median (interquartile range) or n (%).

*Some subjects had more than one site of infection.

[†]Worst values within 24 hours of sepsis onset.

[‡]Within 48 hours of sepsis onset.

S. aureus and 7 with methicillin-resistant *S. aureus*), Streptococcal species ($n = 13$), and enteric gram-negative bacilli ($n = 11$). The most common viral species included respiratory syncytial virus ($n = 11$) and rhino/enterovirus species ($n = 8$).

Clinical outcomes for subjects with sepsis are presented in Table 2. Overall, rates of hospital mortality, new nosocomial infection, and new or progressive MODS were 6%, 10%, and 11%, respectively.

Early Innate and Adaptive Immune Function in Children with Sepsis versus Healthy Controls

Lowest values of *ex vivo* cytokine production capacity in the first 48 hours were used in analyses for all subjects with sepsis. For

subjects with both Day 1 and Day 2 samples available ($n = 28$), the lowest LPS-induced TNF- α response was obtained on sepsis Day 1 in 54% and on Day 2 in 46%. For logistical reasons, about 16% of subjects with sepsis did not undergo testing for their *ex vivo* PHA-induced IFN- γ response. There were no significant differences in baseline characteristics or outcomes comparing subjects with or without PHA-induced IFN- γ response data available (see Table E1 in the online supplement).

Thirty-five healthy children were enrolled. Healthy children had a median age of 126 (interquartile range [IQR], 44–165) months, and 57% were male ($P = 0.13$ and $P = 0.84$ vs. septic cohort, respectively). As expected, children with sepsis demonstrated

Table 2. Subject Outcomes

Outcome	N = 102
Organ dysfunction	
MODS days in 14 d	2 (1–3)
Organ dysfunction days in 14 d	4 (2–9)
New or progressive MODS	11 (11)
Prolonged MODS	14 (14)
Hospital mortality	6 (6)
New nosocomial infection	10 (10)

Definition of abbreviation: MODS = multiple organ dysfunction syndrome.
Data are median (interquartile range) or *n* (%).

systemic inflammation, with a median plasma IL-6 concentration of 78.9 pg/ml (IQR, 22–261 pg/ml) versus 4 pg/ml (4–4) pg/ml for healthy control subjects ($P < 0.0001$). Children with sepsis also demonstrated significantly suppressed innate and adaptive immune responses and lower absolute monocyte and lymphocyte counts relative to healthy control subjects (Figure 1).

Early Innate and Adaptive Immune Function and Organ Dysfunction

Children with sepsis who went on to develop NP-MODS had lower LPS-induced TNF- α responses than those who did not (median [IQR], 246.5 [51.7–408] vs. 442.5 [163.5–892] pg/ml; $P = 0.019$); and nonsurvivors tended to have lower LPS-induced TNF- α responses than survivors (median [IQR], 134 [48–401] vs. 442 [155–879] pg/ml; $P = 0.05$). Early innate immune function was not associated with subsequent development of nosocomial infection ($P = 0.49$). Early adaptive immune suppression was significantly associated with both development of NP-MODS (PHA-induced IFN- γ , median [IQR], 8.2 [1.6–19] vs. 15 [7–55] pg/ml; $P = 0.044$) and with mortality (PHA-induced IFN- γ , median [IQR], 7.4 [2.4–10.3] vs. 15 [6.7–44] pg/ml; $P = 0.046$). Similar to the innate immune response, early adaptive immune suppression was not associated with subsequent nosocomial infection ($P = 0.27$).

Variables associated with number of days with multiple organ dysfunction in 14 days on univariate analyses are listed in Table 3. On multivariable analysis, higher initial pediatric logistic organ dysfunction (PELOD 2) score, presence of a complex chronic condition, and lower innate immune response were independently associated with longer duration of multiple

organ dysfunction (Figure 2A). Variables associated with number of days with any organ dysfunction in 14 days on univariate analyses are listed in Table 4. On multivariable analysis, older age, higher initial PELOD 2 score, the presence of a complex chronic condition, and lower innate immune response remained independently associated with longer duration of any organ dysfunction (Figure 2C). Relationships between adaptive immune response and organ dysfunction were evaluated in separate multivariable models. Although lower adaptive immune response was also associated with both duration of multiple organ dysfunction and duration of any organ dysfunction on univariate analyses, these relationships failed to meet statistical significance on multivariable analyses (Figures 2B and 2D).

To evaluate whether the subset of subjects with baseline immune compromise

($n = 16$) was driving these associations, we performed sensitivity analyses in which these subjects were excluded from the multivariable models. In the analyses for MODS days, the coefficients in the models excluding subjects with baseline immune compromise were within 20% of the coefficients from the original models (coefficient change: -6.8% , adjusted relative risk [aRR] change from 1.23 to 1.21 for *ex vivo* TNF- α response; coefficient change: -14.69% , aRR change from 1.17 to 1.14 for *ex vivo* IFN- γ response), suggesting that inclusion of children with baseline immune compromise did not significantly influence the results of the original models. In the analyses for organ dysfunction days, the percentage change values for the coefficients were larger, although the adjusted relative risks remained greater than 1 (coefficient percentage change: 37% , aRR change from 1.18 to 1.11 for *ex vivo*

Figure 1. (A and C) Among all subjects with sepsis, (A) innate immune function and (C) absolute monocyte counts measured within the first 48 hours of sepsis onset were significantly lower for subjects with sepsis than for healthy children. (B and D) Among subjects with sepsis with adaptive immune function data available ($n = 86$), (B) adaptive immune function and (D) absolute lymphocyte counts measured within the first 48 hours of sepsis onset were also lower than in healthy children. Data presented are median, interquartile range, and range. PHA = phytohemagglutinin; TNF = tumor necrosis factor.

Table 3. Univariate Associations with Multiple Organ Dysfunction Syndrome Days in 14 Days

Variable	Relative Risk (95% CI)	P Value
Male*	0.96 (0.56–1.64)	0.87
Age*	1 (0.995–1.002)	0.49
Complex chronic condition*	1.99 (1.2–3.31)	0.0078
Baseline immune compromise	1.49 (0.74–3)	0.27
Initial PRISM III*	1.09 (1.05–1.1)	<0.0001
Initial PELOD 2*	1.15 (1.08–1.2)	<0.0001
Vasoactive inotrope score*	1.02 (1.01–1.03)	0.0012
Hydrocortisone use	3.5 (2.2–5.6)	<0.0001
RBC transfusion in first 48 h	3.3 (2–5.4)	<0.0001
Lower absolute monocyte count [†]	1.04 (0.98–1.1)	0.2
Lower absolute lymphocyte count [†]	1.09 (0.8–1.31)	0.5
Higher plasma IL-6 [†]	1.2 (1.05–1.3)	0.005
Lower <i>ex vivo</i> TNF- α response* [†]	1.34 (1.09–1.63)	0.0051
Lower <i>ex vivo</i> IFN- γ response* [†]	1.28 (1.06–1.5)	0.012

Definition of abbreviations: CI = confidence interval; PELOD = pediatric logistic organ dysfunction; PRISM = pediatric risk of mortality; RBC = red blood cell; TNF = tumor necrosis factor.

*Variables considered for inclusion in multivariable models.

[†]Worst values in the first 48 hours of sepsis onset.

TNF- α response; coefficient change: 38%, aRR change from 1.09 to 1.05 for *ex vivo* IFN- γ response).

Among patients with sepsis, there were statistically significant correlations between early LPS-induced TNF- α response and both absolute monocyte counts and absolute lymphocyte counts, although the strength of correlation was moderate to weak (Figure E1). Similarly, early PHA-induced IFN- γ response was statistically significantly, although weakly, correlated with absolute lymphocyte count. Compared with absolute cell counts, both LPS-induced TNF- α response and PHA-induced IFN- γ response better predicted subjects who went on to develop prolonged MODS as defined by number of MODS days greater than 7 (19). Areas under the receiver operating characteristic (ROC) curves to predict prolonged MODS were 0.71 for LPS-induced TNF- α response and 0.75 for PHA-induced IFN- γ response, compared with 0.66 and 0.57 for absolute monocyte count and absolute lymphocyte count, respectively (Figure 3). Regarding innate immune function, the optimum threshold for the LPS-induced TNF- α response to predict the development of prolonged MODS by ROC curve analysis was less than 186 pg/ml. In terms of adaptive immune function, ROC curve analysis suggested an optimal threshold for PHA-induced IFN- γ response of less than 8.6 pg/ml to predict prolonged MODS.

Discussion

We and others have described associations between innate and adaptive immune suppression and adverse outcomes from pediatric critical illness, but this is the first report of early suppression of immune responsiveness being associated with prolonged organ dysfunction in pediatric sepsis. In this cohort of critically ill children with sepsis, early innate and adaptive immune responses were both frequently suppressed. The severity of early innate immune suppression was independently associated with longer durations of both single and multiple organ dysfunction.

The immune system is essential for host defense against new infection, for clearance of existing infection, and for remodeling of injured tissues (21). It is therefore not surprising that an intact immune response would be desired in the setting of severe sepsis. The compensatory antiinflammatory response that occurs in the setting of sepsis has been increasingly well described in adults and children and includes features such as lymphocyte apoptosis, downregulation of monocyte antigen presenting capacity (e.g., reduced human leukocyte antigen [HLA]-DR expression), and reduction in the ability of circulating leukocytes to produce cytokines in response to *ex vivo* incubation with stimulants such as LPS (innate) and PHA (adaptive). Severe abnormalities in these immune parameters have been repeatedly associated with

nosocomial infection and death from pediatric critical illnesses (4–7, 12). Despite this, the optimal timing of these measurements has been unclear. Prior investigations into critical illness-induced immune suppression suggested that values obtained before Day 3 of illness may not predict outcomes as well those obtained after Day 3, although these studies focused largely on monocyte HLA-DR expression (10, 11, 22). Impaired TNF- α production capacity within 72 hours of the onset of critical illness has been associated with mortality and nosocomial infection risk in children after trauma (7), critical influenza (5), and cardiopulmonary bypass (6, 12). The current study is the first to show a relationship between early reduction in innate immune function and adverse outcomes from pediatric sepsis. To place these values in context with other forms of pediatric critical illness we have studied to date, the TNF- α production capacity values in the current cohort (433 [143–827] pg/ml) were similar to those seen on postoperative Day 1 after pediatric cardiopulmonary bypass (466 [306–796] pg/ml) (6) but lower than those seen within 48 hours of critical pediatric trauma (908 [506–1,193]) (7). Additional investigations are ongoing into the relationships between longitudinal immune function and outcomes in children with sepsis. This information is crucial for the design of future clinical trials of immunomodulation in children with sepsis.

The optimum threshold LPS-induced TNF- α production of less than 186 pg/ml as a predictor of prolonged MODS in this study is similar to previously published thresholds of approximately 200 pg/ml that were predictive of mortality in critically ill children using similar methods (4, 5, 23). It is important to note that this threshold is dependent on the specific methodology used, including the LPS source and preparation. Our study takes advantage of a highly standardized approach to the quantification of *ex vivo* stimulated cytokine production capacity that has been successfully used in single-center and multicenter studies of immune function in critically ill children (5–8, 14). LPS and PHA stimulation tubes are manufactured on site and rigorously quality controlled, and cytokine production is quantified using a good laboratory practices instrument.

Impairment of the adaptive immune response has also been associated with

Figure 2. (A and B) Among all subjects with sepsis ($n = 102$), lower LPS-induced $\text{TNF-}\alpha$ (tumor necrosis factor- α) response was independently associated with (A) a greater number of days with multiple organ dysfunction in 14 days and with (B) a greater number of days with any organ dysfunction in 14 days. (C and D) For subjects with adaptive immune function data available ($n = 86$), associations between phytohemagglutinin-induced $\text{IFN-}\gamma$ response and (C) number of days with multiple organ dysfunction or (D) with any organ dysfunction failed to meet statistical significance after accounting for baseline characteristics. Organ dysfunction was defined using a modification of previously published criteria (21). Data are adjusted relative risks and 95% confidence intervals. MODS = multiple organ dysfunction syndrome; PELOD = pediatric logistic organ dysfunction; PHA = phytohemagglutinin.

adverse sepsis outcomes in adults (24, 25). Pediatric data are more limited and include associations between sepsis mortality and prolonged lymphopenia (26) and early

suppression of leukocyte mRNA expression (27). Our group published data from a 22-subject cohort of children with sepsis that suggested that early reduction in

lymphocyte cytokine production capacity was associated with adverse infectious outcomes (8). The current study expands on that observation and newly identifies impaired lymphocyte responsiveness in the first 48 hours of sepsis as a risk factor for prolonged organ failure. Our data are in strong agreement with those published by Wong and colleagues, in which downregulation of mRNA expression in adaptive immune signaling pathways was associated with increased mortality and longer duration of organ failure in children with sepsis (28). Subsequent studies are needed to validate this threshold and to better understand the relative importance of innate versus adaptive immune suppression in sepsis-induced organ dysfunction.

Generally speaking, innate immune cells (e.g., monocytes) play a central role in early host defense, whereas adaptive immune cells (e.g., lymphocytes) are responsible for longer-term immunologic modulation. It is therefore notable that our

Table 4. Univariate Associations with Organ Dysfunction Days in 14 Days

Variable	Relative Risk (95% CI)	P Value
Male*	1.02 (0.73–1.4)	0.89
Age*	0.998 (0.996–1)	0.07
Complex chronic condition*	1.4 (1.01–1.9)	0.04
Baseline immune suppression	1.31 (0.85–2)	0.22
Initial PRISM III*	1.05 (1.03–1.07)	<0.0001
Initial PELOD 2*	1.12 (1.1–1.2)	<0.0001
Vasoactive inotrope score*	1.01 (1.004–1.02)	0.0023
Hydrocortisone use	1.7 (1.25–2.4)	0.0008
RBC transfusion in first 48 h	2.02 (1.5–2.7)	<0.0001
Lower absolute monocyte count [†]	1.03 (0.99–1.07)	0.1
Lower absolute lymphocyte count [†]	1.06 (0.89–1.2)	0.47
Higher plasma $\text{IL-6}^{\dagger}$	1.13 (1.05–1.2)	0.002
Lower ex vivo $\text{TNF-}\alpha$ response* [†]	1.25 (1.1–1.4)	0.0005
Lower ex vivo $\text{IFN-}\gamma$ response* [†]	1.15 (1.03–1.29)	0.013

Definition of abbreviations: CI = confidence interval; PELOD = pediatric logistic organ dysfunction; PRISM = pediatric risk of mortality; RBC = red blood cell; TNF = tumor necrosis factor.

*Variables considered for inclusion in multivariable models.

[†]Worst values in the first 48 hours of sepsis onset.

Figure 3. Receiver operating characteristic curve analysis suggests that (A) early innate immune function is predictive of prolonged multiple organ dysfunction syndrome (MODS), with fair discrimination and an optimum threshold of 186 pg/ml (sensitivity, 64%; 95% confidence interval [CI], 35–87%; specificity, 75%; 95% CI, 65–84%). (C) Early adaptive immune function demonstrated fair discrimination to predict prolonged MODS with an optimum threshold of 8.6 pg/ml (sensitivity, 77%; 95% CI, 46–95%; specificity, 74%; 95% CI, 62–84%). (B) Early absolute monocyte counts and (D) early absolute lymphocyte counts were both poorly predictive of prolonged MODS. Prolonged MODS was defined as persistence of two or more organ dysfunctions for 7 or more days, as previously published (26). AMC = absolute monocyte counts; ALC = absolute lymphocyte counts; AUC = area under the curve; ROC = receiver operating characteristic; TNF = tumor necrosis factor.

data suggest that early dysfunction of both lineages may be clinically relevant in pediatric sepsis. Although early adaptive immune suppression was associated with mortality, it was not independently associated with longer durations of organ dysfunction after adjusting for covariates. This was likely influenced by the slightly smaller number of children who underwent adaptive immune function testing, potentially resulting in those analyses being underpowered. It is also possible that longitudinal measures of immune function over time may reveal stronger relationships between later adaptive immune suppression and organ dysfunction. Both innate and adaptive immune function, as well as cross-talk between the two arms of the immune system, deserve future study in larger, multicenter cohorts of children with sepsis.

Our findings replicate our prior work, which showed that early immune suppression occurs simultaneously with elevated levels of circulating inflammatory plasma cytokines (5, 7, 8). Surveillance of plasma biomarkers alone may therefore not fully capture the immunologic profile of a given patient. Similarly, the associations between cytokine production capacities and absolute cell counts were weak, although statistically significant. Evaluation of absolute cell counts alone is unlikely to identify all children with sepsis-induced immune suppression. Furthermore, provocative functional immune testing was able to identify children in our cohort who went on to develop prolonged MODS better than absolute monocyte or lymphocyte counts. Thus, the use of *ex vivo* stimulation studies may enhance

subject selection or stratification in immunomodulatory therapy trials.

The identification of meaningful and practical outcome variables remains a challenge in pediatric sepsis. Although some studies suggest that pediatric severe sepsis mortality can be greater than 20% (29), most U.S. estimates suggest a mortality rate less than 10% (30). Nosocomial infection rates are also dropping in pediatric ICUs, likely as a result of quality-improvement initiatives related to central line management (31) and ventilator care (32, 33). Although these adverse outcomes still represent important clinical problems, the sample size estimates for clinical trials targeting pediatric sepsis mortality or nosocomial infection reduction may be prohibitively large. Improvement in organ dysfunction and/or prevention of the

development of new organ dysfunction represents an attractive alternative to mortality as an outcome variable, as all children with septic shock are, by definition, experiencing organ dysfunction. Clinical trials targeting the prevention of new or progressive pediatric MODS are already underway in the United States and Canada (e.g., NCT 01977547) although these do not specifically target restoration of immune function. Although Maddux and colleagues recently demonstrated a relationship between reduced cytokine production capacity and organ failure in adults with sepsis (34), the current study is the first to establish the plausibility of using organ dysfunction duration as a primary outcome variable for clinical trials targeting normalization of immune function in pediatric severe sepsis/septic shock. Such trials could use a precision medicine approach in which children are screened for early immune suppression, and only those with severe impairment of the innate and/or adaptive immune response receive therapy. Granulocyte macrophage colony-stimulating factor is currently being evaluated for the reversal of critical trauma-induced immune suppression in children (NCT 01495637) using this approach, and similar studies could be designed for children with sepsis.

Mechanisms underpinning the relationships between immune suppression and sepsis-induced organ dysfunction may include failure to resolve primary infection (8), viral reactivation (35), or altered tissue repair mechanisms (21). Although the current study is unable to shed light on these potential mechanisms, our findings support the hypothesis that early sepsis-related immune suppression may hinder

recovery of organ function by mechanisms other than nosocomial infection risk alone. The etiology of sepsis-induced immune suppression remains similarly poorly understood in many children. Therapies that are known to be immunomodulatory, including corticosteroids and red blood cell transfusions (36), were associated with longer durations of organ dysfunction in our analyses. The extent to which these treatments, the majority of which were administered before immune function testing in our cohort, may contribute to both sepsis-induced immune suppression and to clinical outcomes remains an important area of ongoing study by our group and others.

Our study has several important limitations. Although it is one of the largest observational studies of immune function in pediatric sepsis, our single-center design and relatively small sample size limited our ability to determine generalizable relationships between immune function and outcomes. Likewise, this study is underpowered to determine the relative contributions of innate immune suppression, adaptive immune suppression, or both to clinical outcomes. Our sensitivity analyses were likely similarly underpowered to address the effects of subjects with baseline immune compromise on our findings, although we were encouraged to note that inclusion of those subjects was unlikely to have affected the multivariable regression models for MODS days. In addition, because our study focused on early time points, we are unable to comment on relationships between immune response over time and clinical outcomes. It is possible that early immune function measures combined with the trajectory of immune function over time may better

inform clinical outcome predictions. This is an important area of active investigation for our group. Similarly, we primarily tested one aspect of innate and adaptive immune function, cytokine production capacity, as a measure of immune responsiveness. It is possible that other measures of immune system function, such as cell surface marker expression, phagocytosis, intracellular killing, and proliferation, may have different relationships with organ failure in children with sepsis. However, many of these measures are poorly suited to multicenter study, given high processing demands and/or the need to use fresh cells for testing. Our approach is highly standardized, requires a low degree of on-site processing, and is well suited to multicenter clinical trials. Last, our current work was not designed to provide insight into mechanisms of sepsis-induced immune suppression. Rather, it was designed to provide novel evidence for the associations between early suppression of the functional immune response and outcomes from pediatric sepsis to better inform the potential uses of these assays in future clinical trials.

In conclusion, early reductions in innate and adaptive immune function as measured by *ex vivo* stimulated cytokine production assays were associated with prolonged organ dysfunction in this cohort of critically ill children with sepsis. Further work is needed to confirm these findings in larger multicenter studies, to validate thresholds of severe innate and adaptive immune suppression, and to further understand mechanisms of sepsis-induced immune suppression. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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