The Influence of Developmental Age on the Early Transcriptomic Response of Children with Septic Shock

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Septic shock is a frequent and costly problem among patients in the pediatric intensive care unit (PICU) and is associated with high mortality and devastating survivor morbidity. Genome-wide expression patterns can provide molecular granularity of the host response and offer insight into why large variations in outcomes exist. We derived whole-blood genome-wide expression patterns within 24 h of PICU admission from children with septic shock. We compared the transcriptome between septic shock developmental-age groups defined as neonates (≤28 d, n = 17), infants (1 month to 1 year, n = 62), toddlers (2–5 years, n = 54) and school-age (≥6 years, n = 47) and age-matched controls. Direct intergroup comparisons demonstrated profound changes in neonates, relative to older children. Neonates with septic shock demonstrated reduced expression of genes representing key pathways of innate and adaptive immunity. In contrast to the largely upregulated transcriptome in all other groups, neonates exhibited a predominantly downregulated transcriptome when compared with controls. Neonates and school-age subjects had the most uniquely regulated genes relative to controls. Age-specific studies of the host response are necessary to identify developmentally relevant translational opportunities that may lead to improved sepsis outcomes.

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INTRODUCTION

Sepsis is a common and deadly condition that occurs in all patient age groups requiring intensive care. Survival and outcomes among children that develop septic shock are poor (1). Bacterial sepsis of the newborn is the seventh leading cause of infant death in the United States (2), and infection kills >1 million newborns worldwide annually (3).

Multiple developmental alterations in the host innate and adaptive immune responses highlight the age-related differences in the capacity to effectively respond to a sepsis challenge (4,5). Consequently, adjunctive sepsis therapies that prove useful in adults and older children may have little effect, or even completely lack biological plausibility, in less immunologically mature populations. Thus, clarification of the age-specific host response to sepsis is critically important to identify age-appropriate therapeutic strategies.

Unbiased genome-wide expression patterns are increasingly used to improve understanding of complex, heterogeneous diseases that have large variations in host response and outcomes. We and others have used this approach in children with septic shock to successfully identify mRNA expression patterns that enhance diagnostic accuracy, predict sepsis severity, stratify disease and identify novel signaling pathways (6–10).

We now show for the first time that significant differences in gene expression exist between developmental-age groups

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of children with septic shock, particularly within the neonatal group. Furthermore, the unique neonatal response we describe herein raises the question of whether adjunctive sepsis therapies that may be successful in older populations will retain utility or even pose increased risks in neonates.

MATERIALS AND METHODS

Patients and Data Collection

The study protocol was approved by the Institutional Review Boards of each participating institution (n = 11). Children ≤10 years of age admitted to the pediatric intensive care unit (PICU) and meeting pediatric-specific criteria for septic shock were eligible for enrollment (11). Age-matched controls were recruited from the ambulatory departments of participating institutions using published inclusion and exclusion criteria (10). All patients and controls were previously reported in microarray-based studies addressing hypotheses entirely different from those of the current report (6,8–10,12). All microarray data were deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (accession numbers GSE26440 and GSE26378). The patients in this study cohort were recruited between March 2003 and June 2010.

After informed consent from parents or legal guardians, blood samples were obtained within 24 h of initial presentation to the PICU with septic shock. Clinical and laboratory data were collected daily while in the PICU and stored using a Web-based database. Organ failure was defined using pediatric-specific criteria and tracked up to the first 7 d of PICU admission (11). Mortality was tracked for 28 d after enrollment. The developmental-age categories used in this analysis are as follows: neonate (≤28 d of age), infant (1 month through 1 year of age), toddler (2–5 years of age), and school-age (≥6 years of age) (11). All patients in the neonate group were products of full-term gestations.

RNA Extraction and Microarray Hybridization

Total RNA was isolated from whole blood using the PaxGene™ Blood RNA System (PreAnalytiX; Qiagen/Becton Dickson, Valencia, CA, USA). Microarray hybridization was performed as previously described using the Human Genome U133 Plus 2.0 GeneChip (Affymetrix, Santa Clara, CA, USA) (6,8–10,12).

Data Analysis

Analyses were performed using one patient sample per chip, and CEL files were preprocessed using Robust Multiple-Array Average (RMA) normalization and GeneSpring GX 7.3 software (Agilent Technologies, Palo Alto, CA, USA). All signal intensity–based data were used after RMA normalization, which specifically suppresses all but significant variation among lower-intensity probe sets (13). All chips representing patient samples were then normalized to the respective median values of controls.

Differences in mRNA abundance between the developmental age categories were measured by sequential expression and statistical filters using GeneSpring GX 7.3. For direct comparisons across the four developmental-age groups of patients with septic shock, we used a two-stage approach. In stage one, we applied an expression filter to determine the number of gene probes on the array (>80,000 gene probes) having ≥two-fold expression on the basis of all possible intergroup comparisons. In the second stage, we conducted a four-group analysis of variance (ANOVA) with a Benjamini-Hochberg false discovery rate of 1% to determine how many of the gene probes identified in stage one were differentially regulated among the four groups.

We also compared gene expression between patients with septic shock from each of the four respective developmental-age groups and normal age-matched controls. This analysis also occurred in two stages: more than twofold expression filter followed by ANOVA with a Benjamini-Hochberg false discovery rate of 1% for each of the four developmental-age groups and normal controls.

Gene lists of differentially expressed genes were analyzed using the ingenuity pathways analysis (IPA) application (Ingenuity Systems, Redwood City, CA, USA), which provides a tool for discovery of signaling pathways within the uploaded gene lists as previously described (12,14). Gene expression mosaics representing the expression patterns of differentially regulated genes were generated using the Gene Expression Dynamics Inspector (GEDI) (15–17). The signature graphic outputs of GEDI are expression mosaics that give microarray data a "face" that is intuitively recognizable via human pattern recognition. Additional technical details regarding GEDI can be found at http://www.childrenshospital.org/research/ingber/GEDI/gedihome.htm.

Ordinal and continuous clinical variables not normally distributed were analyzed via ANOVA on ranks. Dichotomous clinical variables were analyzed using a χ^2 test (SigmaStat Software; Systat Software, San Jose, CA, USA).

All supplementary materials are available online at www.molmed.org.

RESULTS

Demographics and Clinical Characteristics of the Developmental Age Groups

Table 1 provides the demographic and clinical characteristics of the four developmental-age groups. The neonate group had a higher mortality rate and Pediatric Risk of Mortality (PRISM) score than the other three groups. The neonate group also had a higher number of maximal organ failures than the toddler and school-age groups. In contrast, the neonate group had a lower proportion of subjects with comorbidities than the toddler and school-age groups. The neonate group had a higher proportion of infections with gram-positive bacteria than the school-age group and a lower pro-

Table 1. Demographic and clinical characteristics of the four developmental-age groups.

	Neonate	Infant	Toddler	School-age
Number of patients	17	62	54	47
Age (years)	0.1 (0.0–0.1)	1.0 (0.7–1.5)	3.0 (2.4–4.4)	8.6 (7.3–9.3)
Males/females (n)	13/4	37/25	29/25	30/17
Deaths (n (%))	8 (47)[a]	8 (13)	6 (11)	7 (15)
PRISM score	24 (14–35)[b]	16 (8–22)	15 (10–19)	11 (10–17)
Maximum number of organ failure[c]	3 (3–4)[d]	2 (2–3)	2 (2–3)	2 (2–3)
Days to death from presentation	2 (1.5–5)	7 (5–14.5)	2 (1–9)	2 (1–10)
White blood cell count × 10^3/mm^3	3.5 (2.3–9.9)[b]	16.1 (7.2–22.8)	13.4 (7.1–17.6)	13.2 (7.8–18.2)
Neutrophil count × 10^3/mm^3	0.9 (0.4–4.9)[b]	11.4 (4.7–18.5)	8.1 (3.9–14.2)	9.4 (3.4–13.8)
% Neutrophils	45 (30–55)[b]	70 (58–79)	73 (58–83)	77 (69–87)
Lymphocyte count × 10^3/mm^3	1.3 (0.8–3.0)	2.3 (1.1–4.5)[e]	2.3 (0.9–3.8)	1.3 (0.6–2.3)
% Lymphocytes	46 (34–59)[b]	19 (8–31)	18 (10–31)	11 (6–18)
Monocyte count × 10^3/mm^3	0.2 (0.0–0.3)[f]	0.8 (0.4–1.5)	0.6 (0.3–1.4)	0.3 (0.1–0.9)
% Monocytes	6 (2–9)	6 (3–8)	7 (3–10)	4 (2–8)
Number with gram-positive bacteria (%)	8 (47)[g]	18 (29)	15 (28)	6 (13)
Number with gram-negative bacteria (%)	0 (0)[h]	18 (29)	17 (31)	11 (23)
Number with other organism (%)[i]	2 (12)	5 (8)	4 (7)	5 (11)
Number with negative cultures (%)	7 (41)	21 (34)	18 (33)	25 (53)
Number with bacteremia (%)	8 (47)	23 (37)	20 (37)	9 (19)
Number with comorbidities (%)	2 (12)[d]	25 (40)	26 (48)	23 (49)

All data are median (interquartile range) unless otherwise noted.
[a]$P < 0.05$ versus infant, toddler and school-age (χ^2).
[b]$P < 0.05$ versus infant, toddler and school-age (ANOVA on ranks).
[c]Refers to maximal number of organ failures over the first 7 d of admission.
[d]$P < 0.05$ versus toddler and school-age.
[e]$P < 0.05$ versus school-age (ANOVA on ranks).
[f]$P < 0.05$ versus infant and toddler (ANOVA on ranks).
[g]$P < 0.05$ versus school-age (χ^2).
[h]$P < 0.05$ versus infant and toddler (χ^2).
[i]Refers to viral or fungal pathogens.

portion of infections with gram-negative organisms than the infant and toddler groups. There were a variety of significant differences between the developmental-age groups with respect to peripheral white blood cell counts.

Direct Comparison of Gene Expression Across the Four Developmental Age Groups

In this analysis, we directly compared gene expression across the four developmental-age groups of patients with septic shock as described in Materials and Methods. Table 2 provides the number of gene probes having ≥two-fold expression on the basis of all possible intergroup comparisons. The number of gene probes meeting the expression criteria increased in proportion to age differences. For example, the comparison between the neonate group and the school-age group yielded more than three times the number of gene probes relative to the comparison between the neonate group and the infant group. In contrast, the comparison between the infant group and the toddler group yielded no gene probes meeting the expression criteria.

The statistical test applied in stage II of this analysis yielded 1,638 gene probes differentially regulated between the four developmental-age groups. The top 100 (on the basis of *P* values) differentially expressed unique and well-annotated genes from these 1,638 gene probes are listed in Supplementary Table 1.

To derive a global view of the respective gene expression patterns, we uploaded the expression data for the 1,638 gene probes to the GEDI platform and generated gene expression mosaics for each developmental-age group. Figure 1A provides the average mosaic patterns for each group and demonstrates that the neonatal group had the most distinct global gene expression pattern.

To extract more specific biological information from the 1,638 gene probes, we uploaded the entire list of gene probes to the IPA platform and focused the data

Table 2. The number of gene probes having ≥two-fold expression difference between all possible developmental-age group comparisons among patients with septic shock.

	Infant	Toddler	School-age
Neonate	410	1,057	1,498
Infant		0	40
Toddler			9

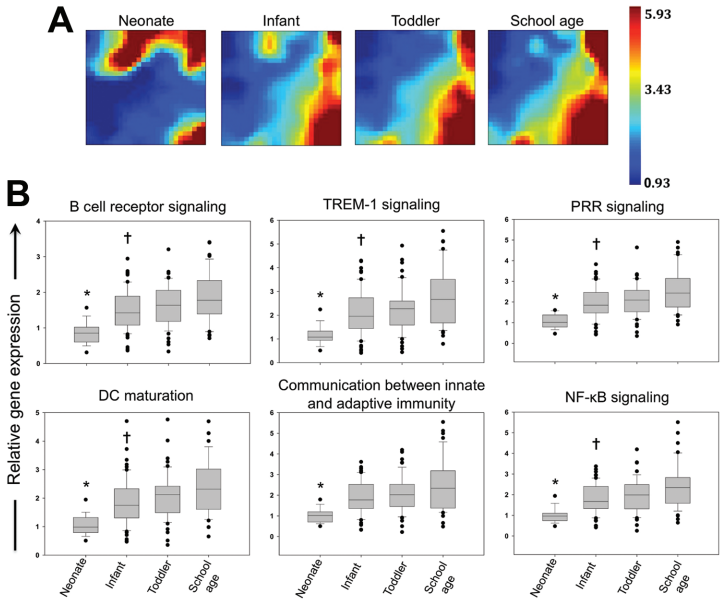

Figure 1. (A) GEDI-generated gene expression mosaics for each of the developmental-age groups. Each mosaic represents the average expression pattern of the same 1,638 gene probes from patients with septic shock in each of the four developmental-age groups. The degree of red intensity correlates with increased gene expression and the degree of blue intensity correlates with decreased gene expression. (B) Relative expression of genes corresponding to the indicated signaling pathways among patients with septic shock from each developmental-age group. Data are expressed as medians with interquartile ranges and were analyzed using ANOVA on ranks. *$P < 0.05$ versus infant, toddler and school-age groups. †$P < 0.05$ versus school-age group. (C) Relative expression of genes corresponding to the NF-κB pathway in neonates and school-age children with septic shock. An illustrative example of expression data is shown in B. Greater intensity of the gene node coloring represents greater change in expression (green: downregulated; red: upregulated).

output on enrichment for genes corresponding to signaling pathways directly related to inflammation and immune function. Table 3 provides the results of this analysis and demonstrates enrichment for genes corresponding to signaling pathways highly relevant to sepsis biology. We next extracted the normalized expression values for each of the genes corresponding to the signaling pathways in Table 3 and calculated the respective median relative gene expression values for each of the four developmental-age groups (Figure 1B). The neonatal group had significantly lower expression of the genes corresponding to all six signaling pathways compared with the other three developmental-age groups.

To provide more detail regarding the signaling pathway data provided in Figure 1B, we generated pathway-specific diagrams that depict gene expression by red (upregulation) and green (downregulation) node coloring. We limited these comparisons to the neonate and school-age groups because they had the largest degree of variation. Figure 1C shows gene expression corresponding to the nuclear factor (NF)-κB pathway and demonstrates generalized downregulation of NF-κB pathway–related genes in the neonate group. Similar diagrams for the pathogen recognition receptor and triggering receptor expressed on myeloid cells-1 (*TREM-1*) pathways are shown in Supplementary Figures 1 and 2, respectively.

Collectively, this analysis based on direct comparison of gene expression across the four developmental-age groups demonstrates that developmental age strongly influences the early whole blood transcriptomic response during pediatric septic shock. The degree of differential gene expression increases in proportion to the difference between developmental ages. In addition, neonatal responses are characterized by decreased expression of genes corresponding to key inflammation- and immunity-related signaling pathways, relative to the responses of older children.

Gene Expression Patterns Relative to Controls

A direct comparison across the four developmental-age groups with septic shock has the potential to overlook important gene expression profiles that more directly reflect perturbations from a normal state. Accordingly, we also conducted an analysis in which we compared gene expression between patients with septic shock from each of the four respective developmental-age groups and normal age-matched controls, as described in Materials and Methods.

Relative to controls, neonates had a significantly greater proportion of downregulated gene probes than the three other groups, and the school-age group had the largest total number of differentially expressed genes compared with controls (Table 4). To broadly compare gene expression patterns for each developmental-age group, relative to controls, we constructed Venn diagrams of all possible group comparisons (Figure 2). The Venn diagrams demonstrate that between 805 and 1,408 gene probes were common across any one of the four possible group comparisons. In addition, the Venn diagrams demonstrate that in all of the comparisons, either the neonate group or the school-age group had the largest number of uniquely regulated genes.

We next uploaded the individual lists of upregulated and downregulated genes in Table 4 to the IPA application and again focused the analytical output on enrichment for genes corresponding to inflammation- and immunity-related sig-

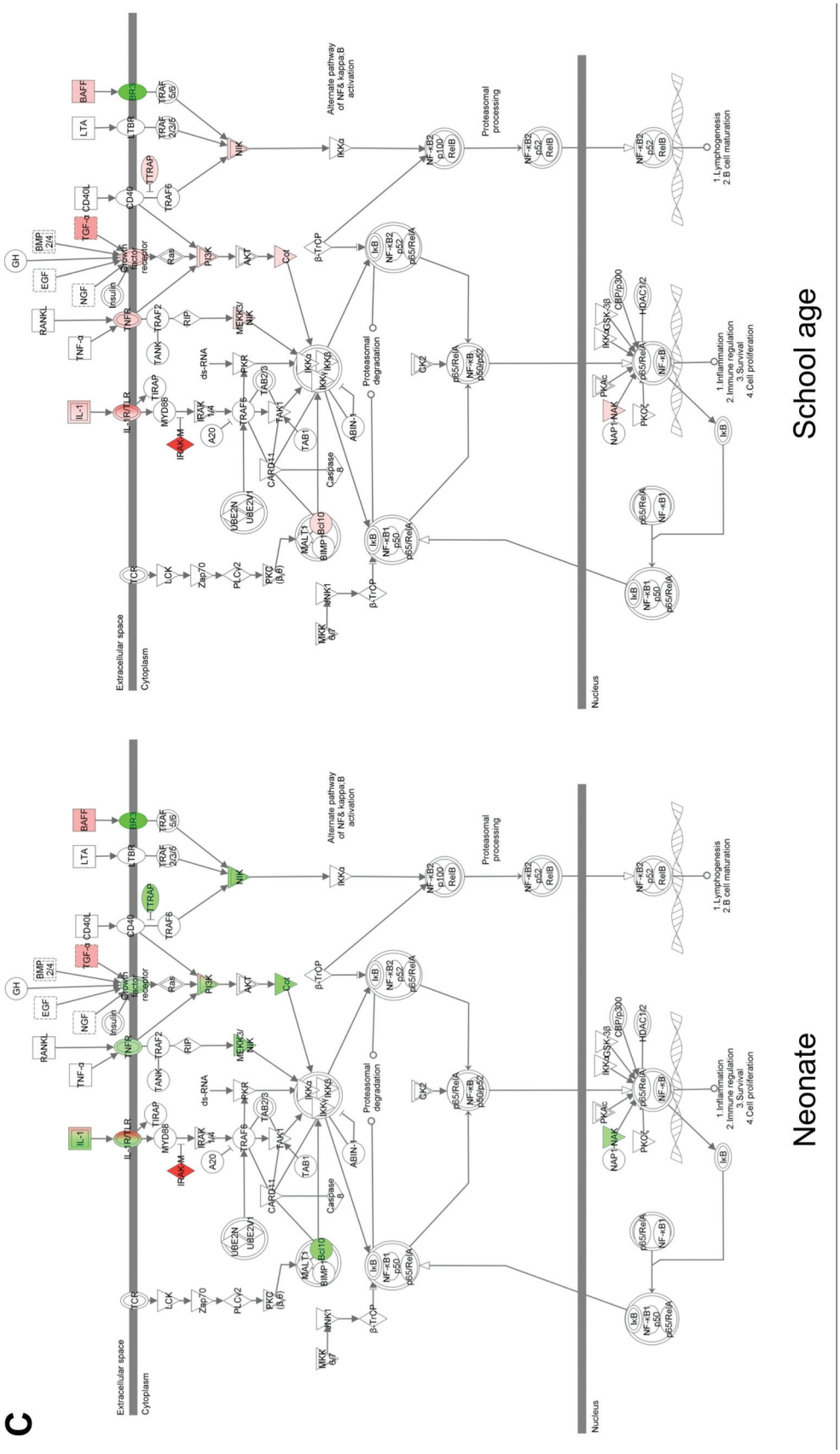

Figure 1. *Continued.*

Table 3. Top inflammation- and immunity-related signaling pathways represented in the 1,638 gene probes differentially regulated between patients with septic shock in the four developmental-age groups.

Signaling pathway	*P*	Number of genes
B-cell receptor signaling	$2.1x10^{-8}$	27
TREM1 signaling	$1.5x10^{-7}$	15
Pattern recognition receptor signaling	$3.6x10^{-7}$	17
NF-κB signaling	$1.8x10^{-6}$	25
Dendritic cell maturation	$1.6x10^{-5}$	22
Communication between innate and adaptive immunity	$1.7x10^{-4}$	13

Table 4. Differential gene expression between controls and patients with septic shock from each of the respective developmental-age groups.

	Upregulated gene probes (relative to controls)	Downregulated gene probes (relative to controls)	Total gene probes
Neonate	599	1,224	1,823
Infant	930	636	1,566
Toddler	1,184	745	1,929
School-age	1,632	1,286	2,918

naling pathways. Figure 3 provides the top signaling pathways represented by the upregulated and downregulated genes for each of the four developmental-age groups. For the upregulated genes, the level of significance generally increased for each signaling pathway in proportion to increasing developmental-age group. In addition, for all of the upregulated signaling pathways, except *interleukin (IL)-8*, the level of significance was lowest for the neonate group. To further illustrate these differences in gene expression, we generated a pathway-specific diagram that depicts the degree and number of upregulated genes corresponding to the *IL-10* pathway for the neonate and school-age groups, respectively (Figure 4). Figure 4 further illustrates that the neonate group had a substantially lower number of upregulated genes corresponding to the *IL-10* pathway, compared with that of the school-age group.

The downregulated genes were highly enriched for signaling pathways corresponding to adaptive immunity (Figure 3). The level of significance for each signaling pathway was generally higher for the neonate group, indicating that the neonate group had a proportionally larger number of downregulated genes corresponding to adaptive immunity signaling. To further illustrate these differences in gene expression, we generated a pathway-specific diagram that depicts the degree and number of downregulated genes corresponding to the antigen presentation pathway for the neonate and school-age groups, respectively (Figure 5). Figure 5 further illustrates that the neonate group had a substantially higher number of downregulated genes corresponding to the antigen presentation pathway, compared with that of the school-age group.

Collectively, this analysis comparing each individual developmental-age group to controls further demonstrates that developmental age strongly influences the early whole-blood transcriptomic response during pediatric septic shock. While the groups shared some common patterns of gene expression, the two extremes of developmental-age groups in this cohort (that is, neonate and school-age) had a relatively large number of uniquely regulated gene sets. Among the upregulated genes that correspond to inflammation- and immunity-related signaling pathways, the proportion of genes that were upregulated for a given pathway increased in proportion to developmental age. Notably, the downregulated genes corresponded to adaptive immunity-related signaling pathways, and the neonate group tended to have the highest proportion of downregulated genes corresponding to adaptive immunity.

DISCUSSION

This study represents the first developmental-age group comparison of the transcriptomic response of children with septic shock. We show that developmental age strongly influences the early whole blood transcriptomic response. This assertion is supported by direct comparisons of patients with septic shock across four developmental-age groups and by comparisons between the respective developmental-age groups and age-matched controls. The direct comparisons demonstrated minimal differences between the infant, toddler and school-age groups with septic shock. In contrast, age-specific alterations in host

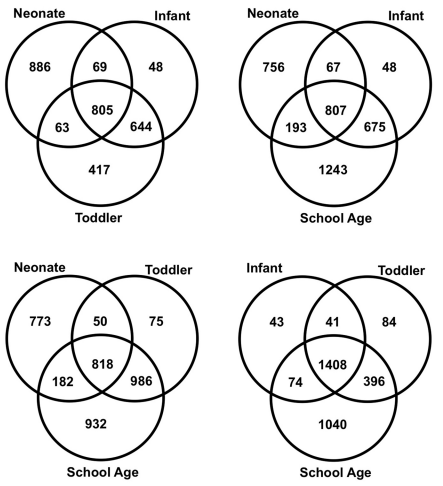

Figure 2. Total differentially regulated genes between patients with septic shock in each developmental-age category and age-matched controls from across all possible group comparisons. Venn diagrams represent differential gene expression between age-matched controls and patients with septic shock from each of the respective developmental-age groups.

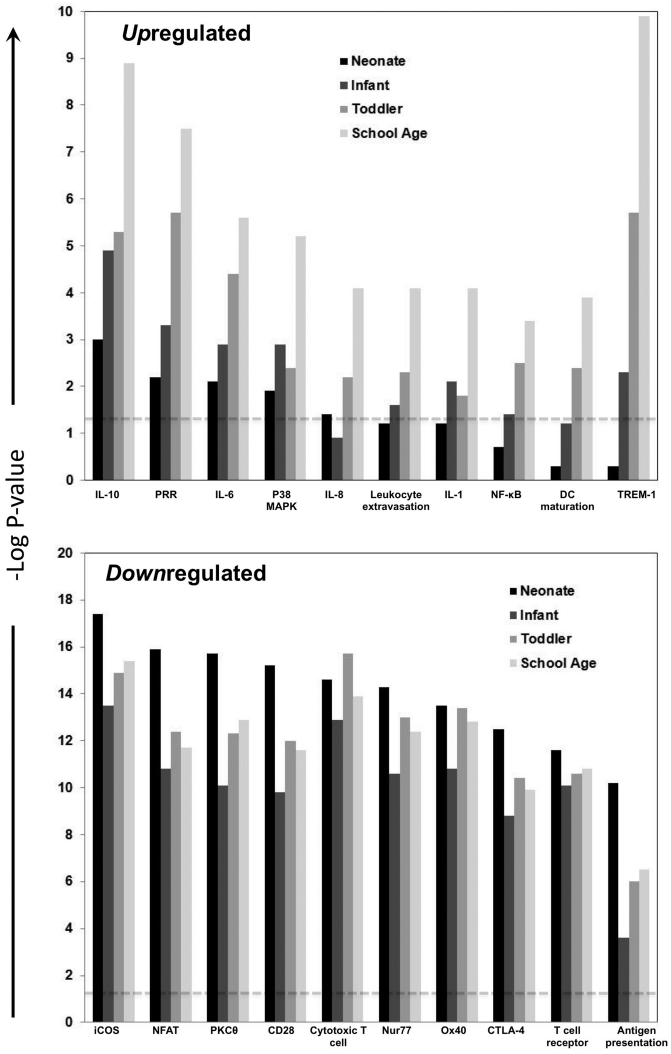

Figure 3. Top signaling pathways represented by the differentially regulated (upregulated and downregulated) genes between patients with septic shock in each developmental-age category versus age-matched controls. The *y*-axis is depicted as the –log(*P* value) and provides an indication of how likely a gene list is enriched for a given pathway by chance alone. The –log for a *P* value of 0.05 is ~1.3 and is indicated by the horizontal dashed line. In contrast, the –log for a *P* value of 1.0×10^{-8} is ~10. The level of significance for a given pathway is directly proportional to the number of genes in a given gene list that correspond to the pathway and indirectly proportional to the total number of genes in the list. PRR, pattern recognition receptor; MAPK, mitogen-activated protein kinase; iCOS, inducible costimulator; NFAT, nuclear factor of activated T cells; PKCθ, protein kinase C theta; Nur77, NR4A nuclear receptor family member Nur77; CTLA-4, cytotoxic T lymphocyte antigen-4.

response were most profound in the neonate group, as demonstrated by reduced expression of genes representative of several key pathways of the innate and adaptive immune systems.

In the comparisons to age-matched controls, the neonate and school-age groups had the largest number of uniquely regulated genes. The upregulated genes corresponded to several key inflammatory/immunity pathways. Importantly, the number of upregulated genes corresponding to these pathways increased in proportion to developmental age. In contrast, the downregulated genes derived from these comparisons corresponded to adaptive immunity-related pathways, and the number of downregulated genes in each pathway was greatest in the neonate group.

The innate immune system plays a critical role in a successful host response to sepsis, particularly in the neonate (18). Multiple developmental alterations of innate host response capabilities are present in neonates compared with older age groups, including pathogen recognition receptors, inflammatory signaling pathways and overall innate immune cellular function (19–23). Consistent with these observations, our current data demonstrate reduced expression of genes corresponding to the pathogen recognition receptor and *TREM-1* pathways, as well as their relevant downstream signaling molecules (for example, *Janus kinase 2* [*JAK2*], *signal transducer and activator of transcription 5* [*STAT5*] and *extracellular signal regulated kinase 1/2* [*ERK1/2*]; Supplementary Figure 2) in the neonate group.

TREM-1 signaling is critical for amplification of the inflammatory responses to microbial products in adults. Inhibition of *TREM-1* signaling through antibody-mediated blockade reduced mortality in septic adult animals and has been proposed as a potential therapeutic target for septic shock (24). Our current data indicate that *TREM-1* pathway-related genes are not substantially expressed in neonates with septic shock. Thus, blockade of *TREM* signaling may not be biologically warranted in neonates. The notion of a *TREM-1*–limited reduced neonatal capacity to produce an intense innate response to a septic challenge is also supported by the attenuated inflammatory response seen in septic murine neonates compared with septic young adult mice (25). Taken together, these data suggest that the neonate has a relatively reduced capacity to generate as robust an innate immune response to septic shock as seen in older age groups, which may be in part related to alterations in *TREM-1* signaling.

In stark contrast to the largely upregulated transcriptomic responses from all

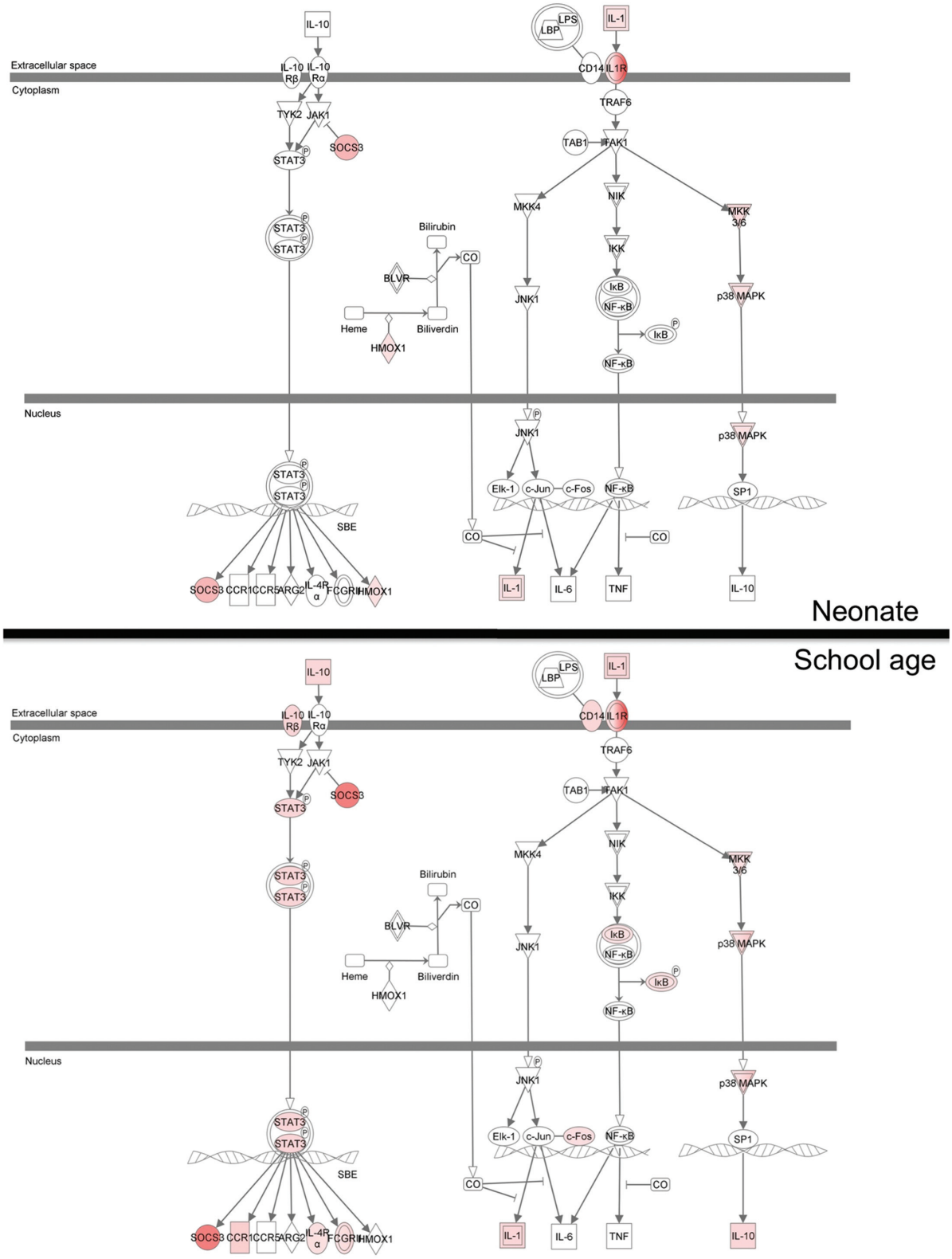

Figure 4. Differential regulation of genes in the IL-10 pathway in neonates and school-age children with septic shock versus age-matched controls. An illustrative example of the upregulated pathway shown in Figure 3 is demonstrated. The greater intensity of red color represents a greater degree of upregulation in gene expression.

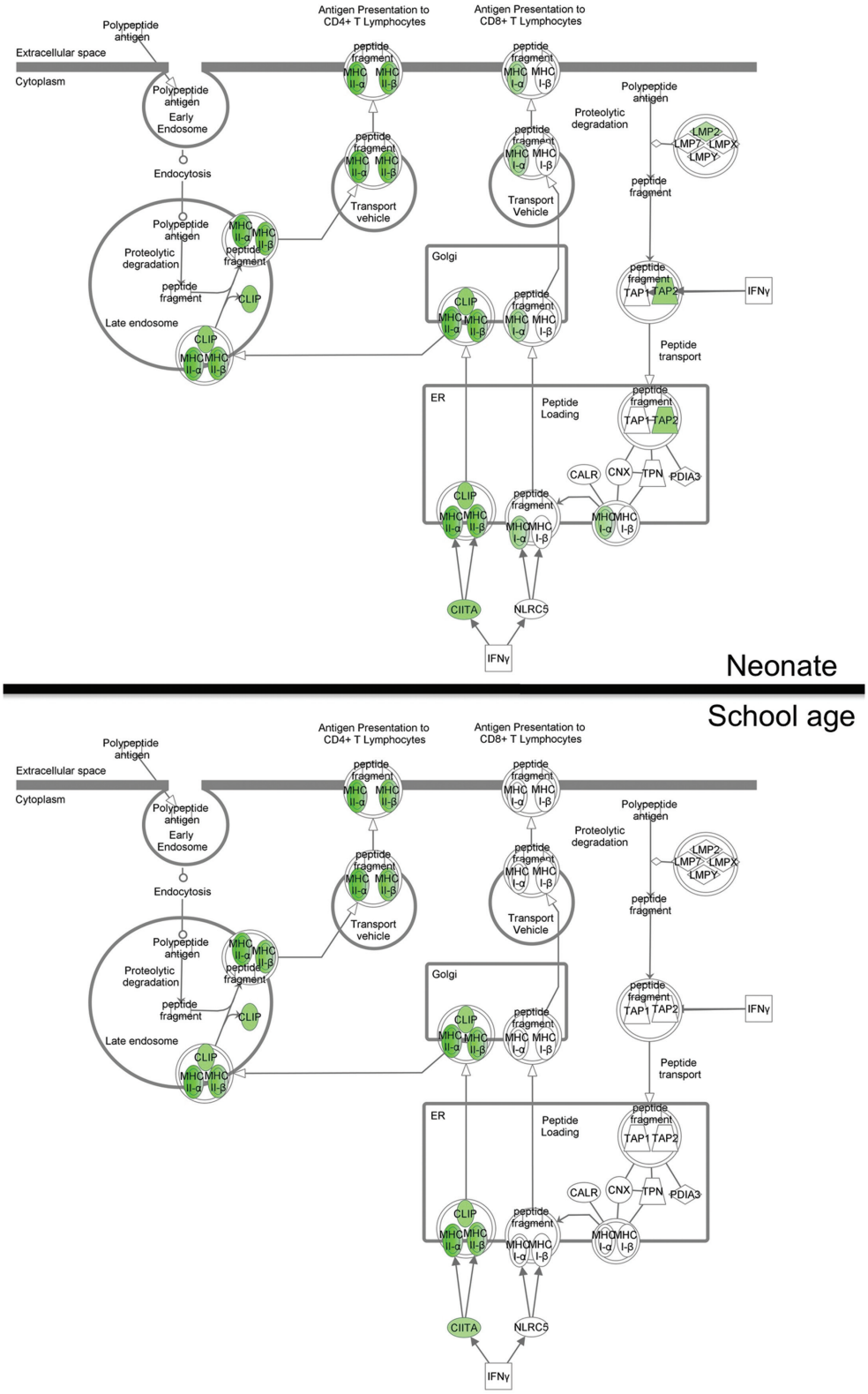

Figure 5. Differential regulation of genes in the antigen presentation pathway in neonates and school-age children with septic shock versus age-matched controls. An illustrative example of the downregulated pathway shown in Figure 3 is demonstrated. Greater intensity of green color represents greater degree of downregulation in gene expression.

three other developmental-age groups, neonates exhibited predominantly downregulated responses when compared with age-matched controls. These alterations represented downregulated pathways related to adaptive immunity. It is well known that baseline neonatal adaptive immune responses are distinct from those seen in more mature populations. These alterations in cellular response have been suggested to permit avoidance of perpetual hyperinflammation through regulation of T-cell responses and increased T-cell apoptosis (26).

This is the first report describing downregulation of adaptive immunity-related genes during septic shock in neonates compared with age-matched controls. The predominance of downregulated adaptive immune pathways in neonates could be interpreted to support why adaptive immune responses were not critical for survival in an animal model of neonatal polymicrobial sepsis (27). In distinct contrast, the absence or dysfunction of the adaptive immune system has a profound negative impact on adult survival in preclinical models (28) and in humans (29,30). As these data illustrate, the contribution of adaptive immunity for protection and response against septic shock, and in particular which components may be protective, is unclear in neonates and requires further investigation.

Many attempts have been made to improve immune function in neonates and reduce the incidence and burden of infection (31). The failure of these interventions in large randomized trials likely reflects underappreciated differences in the functional capacity of the neonatal host response (32). To successfully modify immune function and improve infection outcomes in human neonates, as has been done in neonatal animal models (27,33,34), consideration of the unique immuno-developmental stage of the neonate must be taken into account.

We acknowledge there are several potential confounding factors in this study. First, there were fewer neonatal patients compared with the other age groups. To address this potential confounder and the associated risk of over-fitting the data, we set the primary filter to require a ≥two-fold increase in gene expression. We then used a stringent statistical test by setting a false discovery rate at 1% (equivalent to a *P* value of 0.01).

Table 5. Expression of leukocyte subset signature probes across the four developmental-age groups of patients with septic shock.

	Neutrophil probes	Lymphocyte probes	Monocyte probes
n	38	50	28
Neonate	28	24	14
Infant	30	21	15
Toddler	30	21	15
School-age	32	17	16

Data are number of signature probes present (see text for presence criteria).

Second, the neonatal group had a higher mortality rate and a higher PRISM score, thus raising the possibility that the differences in gene expression reflect a poorer physiologic state, rather than differences reflecting developmental age. To address this potential confounder, we calculated the median time (d) to death for all nonsurvivors. There was no significant difference in median days to death (interquartile range) across the four developmental-age groups: neonate = 2 (1.5–5); infant = 7 (5–14); toddler = 2 (1–9); and school-age = 2 (1–10). We also extracted the 1,823 gene probes differentially regulated between the neonate group and controls (Table 4) and compared these genes between the neonate survivors and nonsurvivors. None of the 1,823 gene probes were differentially regulated between the survivors and nonsurvivors (ANOVA with a false discovery rate of 1%).

Third, neonates had a significantly higher proportion of infections due to gram-positive bacteria compared with the school-age group. This observation raises the possibility that the differences in gene expression described above reflect a pathogen class effect rather than an effect of developmental age. An analysis (same sequential expression and statistical filters as described for the previous analyses) was performed to compare expression data from all patients with gram-negative infection (n = 46) to all patients with gram-positive infections (n = 47) and revealed only 11 differentially regulated probes (Supplementary Table 2).

Fourth, there were a variety of significant differences between the four developmental-age groups with respect to peripheral differential white blood cell counts. Because we used whole blood–derived RNA, it is possible that the differential gene expression patterns described above reflect differences in peripheral white blood cell counts rather than an effect of developmental age. To address this, we analyzed our data for the presence of previously published "signature probe sets" for neutrophils (38 probes), lymphocytes (50 probes) and monocytes (28 probes), respectively (35,36). We used the following criteria to assess the presence of the signature probe sets: ≥300 raw expression values in a least one-half of the subjects in each developmental age category. Table 5 demonstrates that the signature probe sets were present to a similar degree across the four developmental-age groups. These data indicate that the relative contributions of the three major leukocyte subsets to the whole blood transcriptome expression patterns were not substantially different across the four developmental-age groups.

Although we cannot fully correct for all potential confounders, the above analyses indicate that the differences in gene expression reported in this study reflect, at least in part, a direct influence of developmental age. We recognize that whole-blood transcriptome alterations corresponding to specific immune path-

ways do not yield specific pathophysiology. However, these data do offer insight into the complex, multifactorial heterogeneous host response to sepsis and allow identification of critical differences between age groups.

Children are not small adults, and, in the host response to sepsis, we show that neonates are not small children. Age-specific neonatal and pediatric studies of the host response to sepsis are critically necessary to permit identification of novel, developmentally appropriate translational opportunities that might lead to improved sepsis outcomes.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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