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Decreased Intestinal Microbiome Diversity in Pediatric Sepsis: A Conceptual Framework for Intestinal Dysbiosis to Influence Immunometabolic Function

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OBJECTIVES: The intestinal microbiome can modulate immune function through production of microbial-derived short-chain fatty acids. We explored whether intestinal dysbiosis in children with sepsis leads to changes in microbial-derived short-chain fatty acids in plasma and stool that are associated with immunometabolic dysfunction in peripheral blood mononuclear cells.

DESIGN: Prospective observational pilot study.

SETTING: Single academic PICU.

PATIENTS: Forty-three children with sepsis/septic shock and 44 healthy controls.

MEASUREMENTS AND MAIN RESULTS: Stool and plasma samples were serially collected for sepsis patients; stool was collected once for controls. The intestinal microbiome was assessed using 16S ribosomal RNA sequencing and alpha- and beta-diversity were determined. We measured short-chain fatty acids using liquid chromatography, peripheral blood mononuclear cell mitochondrial respiration using high-resolution respirometry, and immune function using ex vivo lipopolysaccharide-stimulated whole blood tumor necrosis factor-α. Sepsis patients exhibited reduced microbial diversity compared with healthy controls, with lower alpha- and beta-diversity. Reduced microbial diversity among sepsis patients (mainly from lower abundance of commensal obligate anaerobes) was associated with increased acetic and propionic acid and decreased butyric, isobutyric, and caproic acid. Decreased levels of plasma butyric acid were further associated with lower peripheral blood mononuclear cell mitochondrial respiration, which in turn, was associated with lower lipopolysaccharide-stimulated tumor necrosis factor-α. However, neither intestinal dysbiosis nor specific patterns of short-chain fatty acids were associated with lipopolysaccharide-stimulated tumor necrosis factor-α.

CONCLUSIONS: Intestinal dysbiosis was associated with altered short-chain fatty acid metabolites in children with sepsis, but these findings were not linked directly to mitochondrial or immunologic changes. More detailed mechanistic studies are needed to test the role of microbial-derived short-chain fatty acids in the progression of sepsis.

KEY WORDS: child; dysbiosis; microbiome; mitochondria; sepsis; short-chain fatty acid

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The intestinal microbiome has a broad impact on immune homeostasis. A normal commensal microbiota actively helps to balance pro- and anti-inflammatory signals, whereas germ-free animals exhibit an increased susceptibility to inflammatory diseases (1). Furthermore, different classes of intestinal bacterial have been shown to induce distinct immunological phenotypes (1, 2). Altered intestinal microbial diversity (termed "dysbiosis") caused by illness, antibiotics, perturbed nutrition, and other factors has been linked to immune paralysis, inflammation, organ injury, and death (1–4). However, how dysbiosis impairs immune function during sepsis is not known (5).

One mechanism through which the intestinal microbiome may modulate immune function is an alteration in microbial-derived short-chain fatty acids (SCFAs) (3, 6). Dietary carbohydrates that go undigested in the upper gastrointestinal tract transit to the colon where they can be degraded by the microbiota (7). SCFAs are products of microbial carbohydrate fermentation that distribute systemically via the blood and lymph. Prior studies demonstrate that SCFAs, such as acetate, butyrate, and propionate, can alter immune cell responsiveness and affect mitochondrial respiration and cellular adenosine triphosphate (ATP) production (2, 3, 8, 9). For example, a reduction in butyrate that occurs when *Bacteroides* and *Clostridia* are lost from the intestinal microbiome can lower cellular bioenergetic capacity by reducing mitochondrial biogenesis (8). Although several studies have shown mitochondrial dysfunction in immune cells in adult and pediatric sepsis (10–16), there are few data investigating a potential link between intestinal dysbiosis, microbial-derived SCFAs, and immunometabolic dysfunction in humans.

In this study, we sought to determine if changes within the intestinal microbiome are associated with specific stool and plasma SCFA patterns and whether intestinal dysbiosis or SCFAs are associated with immunometabolic changes in peripheral blood mononuclear cells (PBMCs) in pediatric sepsis. We hypothesized that 1) reduced microbial diversity (i.e., intestinal dysbiosis) relative to nonseptic controls would be linked to distinct patterns of stool and plasma SCFA levels and 2) intestinal dysbiosis and/or distinct patterns of SCFAs would be associated with low PBMC mitochondrial respiration and immune paralysis.

MATERIALS AND METHODS

Study Design and Population

We performed a prospective observational pilot study in conjunction with a study of mitochondrial dysfunction in pediatric sepsis in a single academic PICU. For this analysis, patients 3 to less than 18 years old treated for severe sepsis/septic shock between January 2017 and June 2018 were eligible. Sepsis and septic shock were defined using consensus pediatric criteria (**Supplemental Digital Content**, http://links.lww.com/CCX/A535) (17). Patients less than 3 years old were excluded because of previously reported age-related differences in the microbiome from very young children (18). Additional exclusion criteria were sepsis for greater than 24 hours, WBC count less than $0.5 \times 10^3/\mu L$, primary mitochondriopathy, and unrepaired cyanotic heart disease. This study was approved by the Children's Hospital of Philadelphia Institutional Review Board (IRB Number 16-012691) and separate written informed consent for this study was obtained after consent was provided for the parent study. A control group of healthy children was enrolled through an unrelated study with separate IRB approval and informed consent to share data.

Data Collection

Data were collected about patient characteristics, organ dysfunction, therapies, and vital status. Severity of illness was determined by the Pediatric Risk of Mortality III (19), Pediatric Logistic Organ Dysfunction (20), and inotrope (21) scores.

Sample Collection

For sepsis patients, stool samples were collected following a bowel movement as close to study enrollment as available and then every 48 hours (±24 hr) for 10 days or until hospital discharge. Immediately after collection, stool was stored on ice at 4°C, with subsequent transfer to –80°C within 48 hours, for batched analyses. A days 1–2 blood sample of 7–9 mL was collected as soon as possible after enrollment, with additional blood collected between study days 3–6 (day 3 preferred, but collected at least 2 d after first sample) and again between days 8–14. For healthy controls, a single stool sample was collected for sequencing of the intestinal microbiome. Stool and plasma SCFAs and

blood for mitochondrial and immune function measurements were only available from sepsis patients.

Microbiome Sequencing

Patients stooled into a specimen collector, from which stool was collected using two separate nylon-flocked dry swabs (Copan Diagnostics, Murrieta, CA) while avoiding urine contamination to the extent possible. Microbial DNA was isolated from stool using the DNeasy PowerSoil Kit (Qiagen, Germantown, MD), followed by 16S ribosomal RNA (rRNA) gene sequencing by the Children's Hospital of Philadelphia Microbiome Center as detailed in the Supplemental Digital Content (http://links.lww.com/CCX/A535). The composition of the intestinal microbiome was described using alpha- and beta-diversity (4).

Short-Chain Fatty Acids

Approximately 50–150 mg of stool was aliquoted into two microcentrifuge tubes and stored at –80°C for batched analysis of stool SCFAs. One to 2 mL of blood was collected in an EDTA vacutainer and, within 30 minutes of collection, was centrifuged at 3,000 g for 15 minutes at 4°C. The supernatant was then stored at –80°C for batched analysis of plasma SCFAs. SCFAs were quantified as detailed in the Supplemental Digital Content (http://links.lww.com/CCX/A535). For measurements below the level of assay detection, we imputed a value of 1 μmol/g for stool SFCA and 5 μM for plasma SCFA (the lower limit of detection) to facilitate inclusion of these low levels into the analyses.

Mitochondrial Respiration

Mitochondrial respiration was measured in fresh PBMCs isolated by density gradient centrifugation (13, 14). The rate of oxygen consumption was measured in $2–4 \times 10^6$ intact PBMCs at 37°C using a high-resolution oxygraph (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria) as previously described (13, 14) (further details in Supplementary Digital Content, http://links.lww.com/CCX/A535). We directly measured baseline (routine) respiration, proton leak after inhibition of ATP synthase (LEAK), and maximal uncoupled respiration through the electron transport system (ETS_{max}), with subtraction of non-mitochondrial respiration from all parameters. Respiration supporting mitochondrial ATP synthesis (ATP-linked respiration) was calculated as routine minus LEAK. Spare respiratory capacity (SRC), calculated as ETS_{max} minus routine respiration, is the mitochondrial bioenergetic reserve available for cells to produce ATP in response to a stress-induced increase in metabolic demand (22).

Immune Function

Ex vivo lipopolysaccharide (LPS)-stimulated whole blood tumor necrosis factor (TNF)-α was measured by mixing 50 μL heparinized whole blood with 500 μL (250 pg) of phenol-extracted LPS from *Salmonella enterica abortus equi* (Sigma-Aldrich, St. Louis, MO, L5886) within 60–90 minutes of blood collection, as previously described (23). The sample was then incubated for four hours at 37°C, following which the sample was centrifuged at 1,000 g for 5 minutes. The resulting supernatant was stored at –80°C for batched analysis. TNF-α was measured using a commercially available enzyme-linked immunosorbent assay kit (Invitrogen KHC3011C, Waltham, MA).

Statistical Analysis

Analyses were performed using R 3.6.2 (R Foundation, Vienna, Austria) and STATA (StataCorp Version 12.1, College Station, TX). Descriptive data were compared using Wilcoxon rank-sum or Fisher exact tests, respectively. Taxa distribution for sepsis and control patients are presented as a heatmap with alpha-diversity shown as box plots and beta-diversity as 2D principal coordinate analysis (PCoA) plots. We used linear mixed-effects modeling, with patient as a random effect to account for repeated measurements, to compare alpha-diversity between sepsis patients and healthy controls and, separately, to determine the association of alpha-diversity with stool and plasma SCFA levels. We examined the association of beta-diversity with SCFA patterns on PCoA plots and quantified the association using a permutational multivariate analysis of variance test as implemented by the function "adonis" in the vegan R package 2.5-6 (R Foundation). We then examined if the abundance of individual dominant taxa was associated with SCFAs and tested the association of both microbial diversity and SCFA levels with PBMC mitochondrial respiration and immune function using linear mixed modeling with patient as a random effect. Statistical significance was defined as

p value of less than 0.05 after correction for multiple comparisons using the Benjamini-Hochberg false discovery rate method.

RESULTS

Patients

Forty-four patients with sepsis were enrolled. One enrolled patient was determined not to have met all eligibility criteria prior to study procedures, leaving 43 patients with sepsis available for analysis (**eFig. 1**, http://links.lww.com/CCX/A536). Blood was collected from 41 (95%), including a days 1–2 sample from 41, days 3–6 from 35, and days 8–14 from 23 patients. Stool samples were collected from 32 (74%) of the sepsis patients, including at least one sample between days 1–2 from 19, between days 3–6 from 18, and between days 8–14 from 14 patients (**eTable 1**, http://links.lww.com/CCX/A544). Missing blood samples were due to clinical improvement with removal of blood-drawing access (no patients died prior to day 14), whereas lack of stool availability was the primary reason for missing stool samples. Stool samples from 44 healthy children were included in the analysis of the intestinal microbiome as a control group. Patient characteristics are shown in **eTable 2** (http://links.lww.com/CCX/A545).

Intestinal Dysbiosis

The intestinal microbiota of pediatric sepsis patients differed substantially from the profile of healthy controls, particularly after days 1–2 (**eFig. 2*a***, http://links.lww.com/CCX/A537). The microbiome of sepsis patients came to be dominated by taxa such as *Enterobacteriaceae*, *Streptococcus*, and *Veillonella*, while more typical taxa observed in healthy control samples, such as *Bacteroides* and *Faecalibacterium*, were depleted or missing in a number of pediatric sepsis patients, particularly after days 1–2. This pattern is characteristic of intestinal dysbiosis, which has been previously associated with inflammation or antibiotic exposure.

Because exposure to antibiotics might have lowered the total bacterial abundance in sepsis patients relative to healthy controls, we sought to estimate the bacterial load in our sample set. We used the post-polymerase chain reaction (PCR) concentration of 16S rRNA gene amplicons, measured during the sequencing protocol, as an approximate measure of total bacteria in each sample in order to identify large-scale differences between groups. The total concentration of PCR products was lower in samples from the sepsis group relative to healthy controls ($p = 0.004$; **eFig. 2*b***, http://links.lww.com/CCX/A537). Analysis with mixed-effects models indicated that prominent bacterial taxa differed in abundance by several orders of magnitude after correction by post-PCR DNA concentration (**eFig. 2*c***, http://links.lww.com/CCX/A537). In particular, the mean abundance of *Klebsiella* and *Enterobacteriaceae* was increased in sepsis (**eFig. 3**, http://links.lww.com/CCX/A538).

Overall, sepsis patients demonstrated significantly lower alpha-diversity compared with healthy controls that was evident among all stool samples from sepsis patients (**Fig. 1**, ***A*** and ***B***) and when analyzed over time (**Fig. 1**, ***E*** and ***F***). In addition, the dispersion or variation among bacterial communities was greatly increased in sepsis samples relative to healthy controls, as indicated by a change in unweighted and weighted beta-diversity (**Fig. 1**, ***C***, ***D***, ***G***, and ***H***). In addition, sepsis samples exhibited a longer distance from the healthy control centroid at all timepoints, indicating a persistent departure from a healthy microbiota configuration (**eFig. 4**, http://links.lww.com/CCX/A539).

Stool and Plasma SCFAs

Plasma SCFAs were available from 39 of 41 (95%) and stool SCFAs were available from 29 of 32 (91%) of sepsis patients with blood and stool collected, respectively. Plasma and stool SCFA values in sepsis patients by study day are shown in **eFigure 5** (http://links.lww.com/CCX/A535). There was poor correlation between stool and plasma levels of all SCFAs (data not shown). Decreased alpha-diversity among sepsis patients was associated with increased stool acetic (**Fig. 2*A***) and propionic acid (**Fig. 2*B***) but not with plasma levels of these SCFAs (**Fig. 2**, ***C*** and ***D***). Decreased alpha-diversity was also associated with lower levels of both stool and plasma isobutyric acid, but the association was primarily driven by two sepsis patients with outlier values (data not shown). Beta-diversity, measured with UniFrac distance, was associated with plasma butyric acid and stool butyric, acetic, and propionic acid (**eFig. 6**, http://links.lww.com/CCX/A541). Although associations were estimated using methods robust to outliers, we noted a high level of variation in SCFA

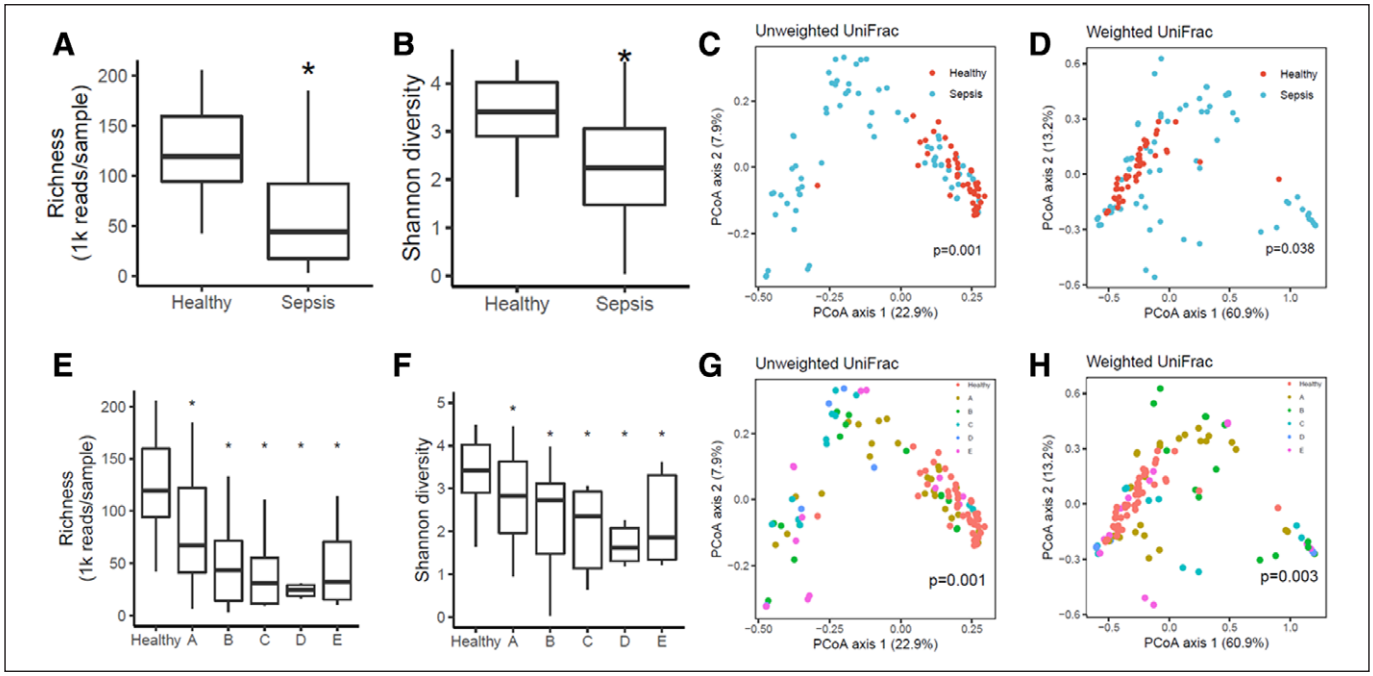

Figure 1. Alpha- and beta-diversity of the intestinal microbiome in sepsis and control patients. Alpha-diversity represented as the difference in the (**A**) operational taxonomic unit (OTU) count, or number of different species in a sample ("richness") and (**B**) Shannon diversity that indicates both richness and relative abundance of difference species between healthy controls and all sepsis stool samples, irrespective of study day collected. For alpha-diversity, lower values reflect reduced microbial diversity indicative of intestinal dysbiosis. Data are presented in box plot analysis with the central line indicating the median and boxes indicating the interquartile range. Beta-diversity presented as 2D principal coordinate analysis (PCoA) plots for (**C**) unweighted UniFrac accounting only for differences in species "richness" and (**D**) weighted UniFrac accounting for both richness and relative abundance of difference species with healthy controls (*orange dots*) tightly clustered and sepsis patients (*blue dots*) more diffusely spread. Only a portion of sepsis samples overlapped with healthy controls, whereas most were distant from the healthy controls along the first principal coordinate axis. Alpha-diversity by (**E**) OTU count and (**F**) Shannon index and beta-diversity by (**G**) unweighted UniFrac and (**H**) weighted UniFrac are also shown with sepsis samples separated by time of collection (A, study days 1–2; B, days 3–4; C, days 5–6; D, days 7–8; and E, days 9–10). A time-dependent decrease in richness (**E**) and Shannon diversity (**F**) in the gut microbiota of sepsis patients and a difference in the microbiota profile between timepoints based on analysis of unweighted (**G**) and weighted (**H**) UniFrac distance were evident. *$p < 0.01$ compared with controls, with all analyses corrected for repeated measures and multiple comparisons.

levels with many SCFA concentrations near the minimum level of detection.

To determine if SCFA levels were associated with specific changes within the intestinal microbiome despite a high degree of variation in overall bacterial community composition, we examined individual taxa. In particular, we found that loss of commensal anaerobes from the abundances observed among healthy controls, mainly from lower abundance of commensal obligate anaerobes among the *Bacteroidetes* and *Clostridia*, was associated with higher levels of stool acetic acid and lower levels of stool butyric, isobutyric, and caproic acids (**eTable 3**, http://links.lww.com/CCX/A546). However, we did not observe an association between post-PCR bacterial DNA abundance and stool or plasma SCFA concentrations (**eFig. 7**, http://links.lww.com/CCX/A542).

Mitochondrial Respiration

Beta-diversity was weakly associated with PBMC mitochondrial basal, ATP-linked, LEAK, and ETS_{max} respiration and SRC in sepsis patients (**eTable 4**, http://links.lww.com/CCX/A547). However, alpha-diversity was not associated mitochondrial respiration (eTable 4, http://links.lww.com/CCX/A547). Lower PBMC mitochondrial respiration was associated with decreased levels of plasma butyric acid (**Fig. 3*A*–*E***), but not with other microbial-derived SCFAs.

Immune Function

Reduced microbial diversity was not associated with ex vivo LPS-stimulated whole blood TNF-α on study days 1–2 or 3–6, but there was an inverse correlation on study days 8–14 (**eFig. 8**, http://links.lww.com/CCX/A543).

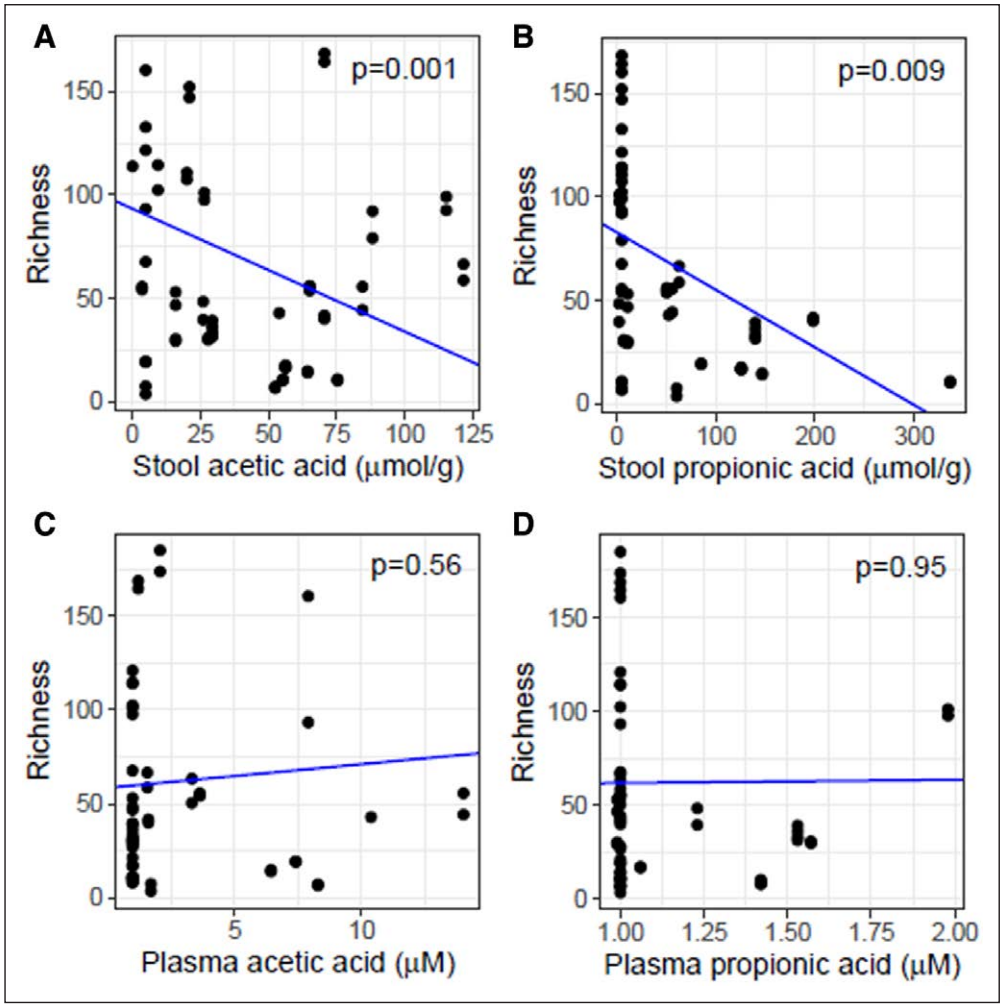

Figure 2. Alpha-diversity associated with short-chain fatty acids. Alpha-diversity indicated by operational taxonomic unit (OTU) count, or number of different species in a sample ("richness"), of the intestinal microbiome in sepsis patients was indirectly associated with stool acetic acid (**A**) and propionic acid (**B**). However, there was no association of alpha-diversity with plasma acetic acid (**C**) or propionic acid (**D**). Analyses are corrected for repeated measures and multiple comparisons.

There was no association of plasma or stool SCFAs with LPS-stimulated TNF-α on any study day (data not shown). However, PBMC mitochondrial respiration was associated with LPS-stimulated TNF-α, such that patients with lower levels of mitochondrial respiration also exhibited greater tolerance to ex vivo LPS stimulation as measured by lower TNF-α production (**Fig. 3*F*–*H***).

DISCUSSION

In this pilot study, intestinal dysbiosis was evident early in sepsis in children, with a further reduction in microbial diversity over time. Intestinal dysbiosis, with loss of commensal anaerobes, was associated with increased acetic and propionic acids and decreased butyric acid. In addition, lower levels of plasma butyric acid were associated with a decrease in PBMC mitochondrial respiration. Finally, decreased mitochondrial respiration was associated with a reduction in ex vivo LPS-stimulated whole blood TNF-α. Together, these preliminary findings led us to hypothesize a conceptual framework in which sepsis-induced intestinal dysbiosis may impact immunometabolic function through alterations in microbial-derived SCFAs in children with severe sepsis/septic shock (**Fig. 4**). However, the lack of associations of reduced microbial alpha-diversity with mitochondrial respiration and alpha-diversity or stool/plasma SCFAs with LPS-stimulated TNF-α argue against a direct effect of the intestinal microbiome on the immunological response to sepsis in children through the effects of microbial-derived SCFAs on mitochondrial function within immune cells.

Since critical illness was shown to alter the ecosystem of the body's microbiota (24), numerous studies have reported taxonomic changes in adults (25–27), neonates (28–30), and children (4) with sepsis. Similarly, we found a decrease in microbial diversity in children with sepsis compared with normal health that worsened over time, with a notable rise in the abundance of common pathogens (e.g., *Staphylococcus*, *Enterobacteriaceae*, and *Pseudomonas*). In addition, we observed a depletion of anaerobes associated with intestinal health, such as *Roseburia*, *Eubacterium*, and *Faecalibacterium*. Although our study was not intended

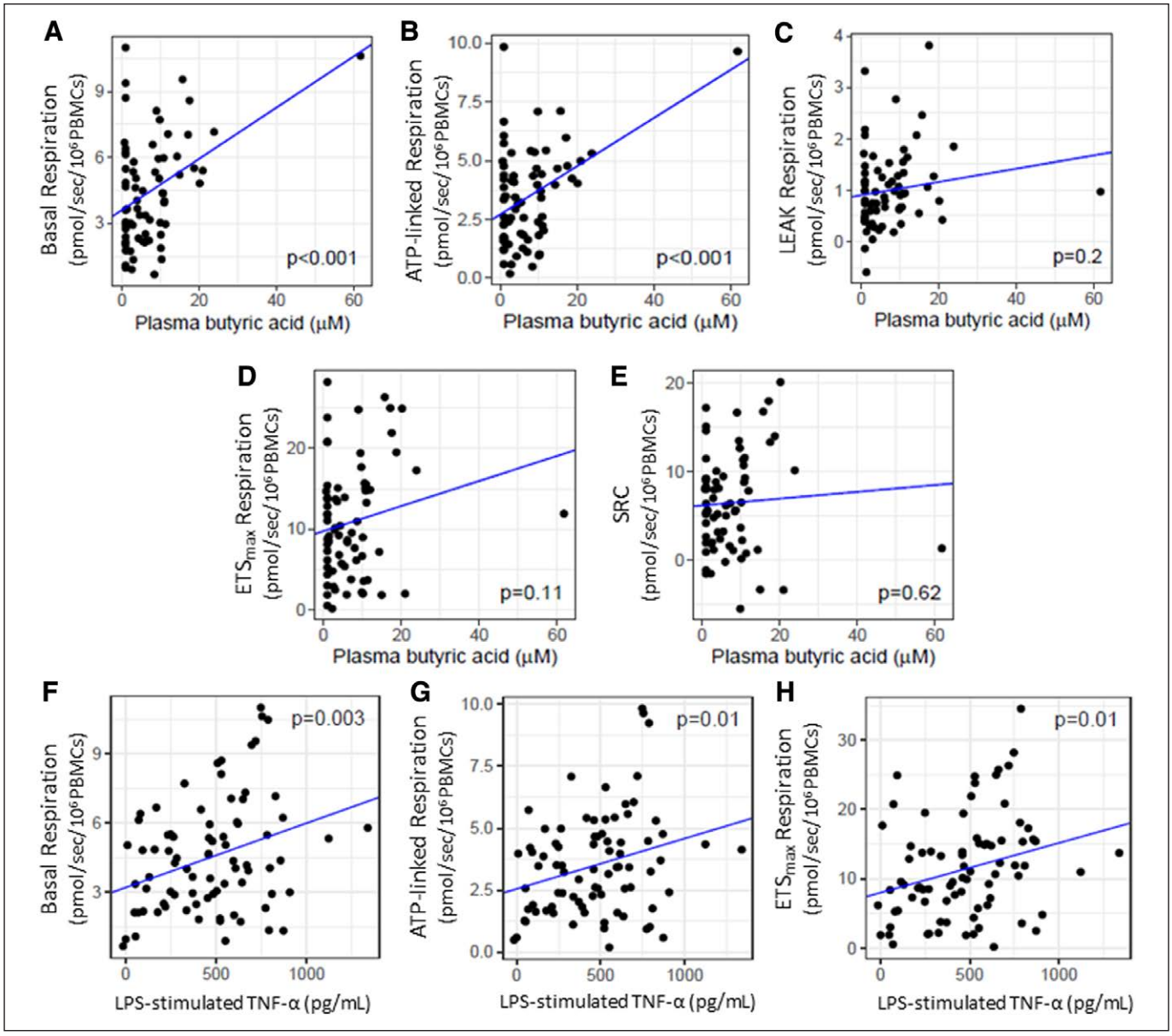

Figure 3. Association of peripheral blood mononuclear cell (PBMC) mitochondrial respiration with short-chain fatty acids and ex vivo lipopolysaccharide (LPS)-stimulated whole blood tumor necrosis factor (TNF)-α production. Lower levels of plasma butyric acid were associated with decreased basal (**A**) and adenosine triphosphate (ATP)-linked mitochondrial respiration (**B**) but not proton leak after inhibition of ATP synthase (LEAK) (**C**), maximal uncoupled respiration through the electron transport system (ETS_{max}) (**D**), or spare respiratory capacity (SRC) (**E**). Analyses are corrected for repeated measures and multiple comparisons. Basal (**F**), ATP-linked (**G**), and ETS_{max} (**H**) respiration were directly correlated with ex vivo LPS-stimulated whole blood TNF-α production in sepsis patients. All analyses are corrected for repeated measures and multiple comparisons.

to distinguish between the impact of sepsis itself from antibiotics, altered nutrition, or changes in perfusion on the intestinal microbiome, it is likely that reduction in microbial diversity we observed in this study was multifactorial.

Despite emerging data that variation in the microbiome can modify the immunologic response to and clinical outcomes from sepsis (31), the mechanisms underlying this potential link are not clear. In this study, we investigated the possibility that changes in SCFAs would follow loss of microbial diversity. Within sepsis patients, more severe alterations in the intestinal microbiome and loss of commensal anaerobes were associated with a pattern of SCFAs that included increased acetic and propionic acids and decreased butyric acid. SCFA production by the intestinal microbiome is

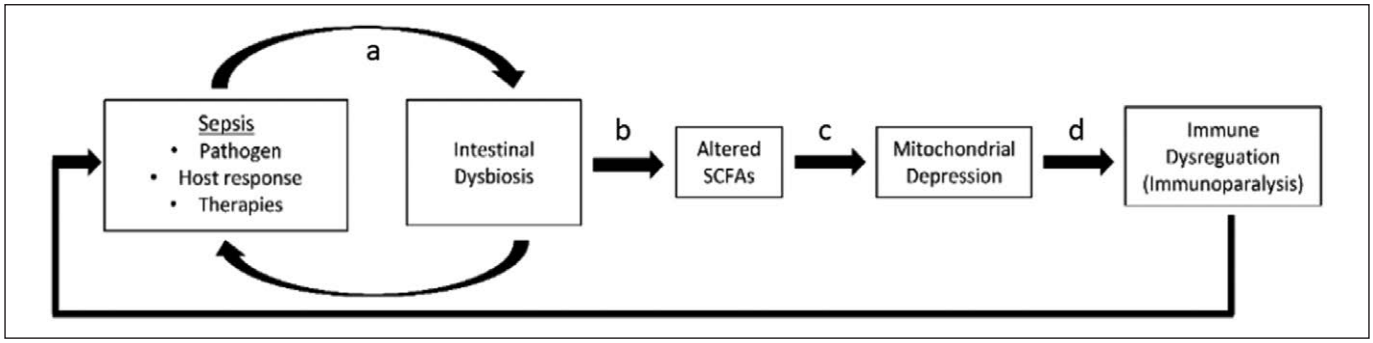

Figure 4. Conceptual model of the pathway from sepsis-induced intestinal dysbiosis to immune dysregulation in pediatric sepsis. In sepsis, the pathogen, host response, and therapies directly alter the composition of the intestinal microbiome. A loss of intestinal microbial diversity includes loss of commensal organisms and expansion of pathogenic species, which can influence the pathogen-host interaction. We hypothesize the loss of intestinal microbial diversity will alter the microbial-derived short-chain fatty acid milieu and contribute to mitochondrial-induced immune dysregulation to further influence the septic response. In this study, we demonstrated associations of: sepsis with intestinal dysbiosis (**A**), intestinal dysbiosis with select SCFAs (stool acetic acid and stool propionic acid [**B**]), low butyric acid with low mitochondrial respiration (**C**), and low mitochondrial respiration with diminished tolerance to ex vivo lipopolysaccharide stimulation as measured by lower tumor necrosis factor-α production (**D**).

regulated by the bacterial species composition, as well as by diet, substrate availability, and intestinal transit time (32). Therefore, we cannot attribute changes in SCFAs solely to changes in the microbiome. However, the loss of commensal anaerobes and general low levels of SCFAs in this study are consistent with a report from critically ill adults (25).

Butyric acid is especially interesting given its established role in immune homeostasis (33). Prior studies of the microbiome in critical illness have similarly reported a loss of butyrate-producing obligate anaerobes, such as *Roseburia*, *Eubacterium*, and *Faecalibacterium* (4), with a decrease in detectable stool butyrate levels (25, 34). Furthermore, our observed association of low plasma butyrate with decreased PBMC mitochondrial respiration is consistent with prior studies of the effect of butyrate on mitochondrial function (35). For example, Gao et al (8) demonstrated that mice fed a low-butyrate diet exhibited lower mitochondrial function in adipocytes and skeletal muscle. In contrast, butyrate supplementation augmented mitochondrial respiration in lymphoblastoid cell lines from children with autism (36). Furthermore, in a rat model of sepsis, treatment with butyrate decreased inflammation, alleviated mucosal injury, and decreased mortality (37). The consistency of these findings supports further investigation into whether loss of microbial-derived butyrate is mechanistically linked to the immuno-inflammatory-metabolic response characteristic of sepsis and could be useful as a therapy.

We also found an association of increased stool acetic and propionic acid with loss of microbial diversity. Acetic acid is produced by select Gram-negative bacteria, and low levels have been associated with late-onset sepsis in preterm neonates (30). Propionic acid is produced by obligate anaerobes found within the normal microbiome (38), and low levels have been associated with increased inflammation in humans (25). Propionic acid is also a normal intermediate of human metabolism and consumed in foods (as a preservative). High levels of acetic and propionic acids can also inhibit the tricarboxylic cycle and impair mitochondrial oxidative phosphorylation (39, 40). However, in our study, neither acetic nor propionic acid carried through to an association with PBMC mitochondrial respiration.

The last step in our theoretical conceptual framework links mitochondrial dysfunction to immunoparalysis. Although we did find that lower rates of PBMC mitochondrial respiration were associated with a decrease in ex vivo LPS-stimulated TNF-α, we did not identify a statistical continuum between intestinal dysbiosis or a specific pattern of SCFAs and immune function among sepsis patients (Fig. 4). We focused on LPS-stimulated TNF-α because prior studies have demonstrated that mitochondrial function is downregulated in immune cells rendered tolerant to TNF-α production (15, 41). However, LPS-stimulated TNF-α may not be a sensitive indicator of the effects of dysbiosis or microbial-derived SCFAs on immunological function, as it is largely a feature monocyte (rather

than lymphocyte) activation. It would be important, therefore, to investigate the relationship between microbial-derived SCFAs in sepsis and immunometabolic function within additional cell compartments.

There are several limitations to this study. First, as this was a small pilot study, statistical power was limited to reach definitive conclusions. Second, the available control group with microbiome data was comprised of healthy children with a higher median age than the sepsis group, and a comparator group of critically ill children without sepsis was not accessible for this pilot study. Third, because stool samples were not available at all timepoints from all patients, direct comparisons of serial changes across patients were not possible. Thus, we chose to analyze all data available, irrespective of timepoint collected. However, we did account for repeated measurements within each patient. Fourth, we were unable to account for potential confounders, including differences in nutrition and antibiotic administration, due to the small sample size. It is likely that the alterations in intestinal microbiome and associated changes in SCFAs were related to the particular antibiotics used to treat these patients, such that generalizability to patients treated with different antibiotics is not known. Fifth, we measured both stool and plasma SCFAs because we believed that stool concentrations would more closely reflect microbial fermentation and plasma concentrations would more likely to reflect mitochondrial and immune function in PBMCs. Indeed, we did find that stool SCFAs (e.g., acetic and propionic acids) were more closely associated with dysbiosis, while plasma SCFAs (e.g., butyrate) were more closely associated with mitochondrial respiration. However, we cannot exclude the possibility that parenteral therapies influenced plasma SCFA levels (e.g., dilution from transfusion, acetate in fluids). Finally, although microbiomes were sequenced from a control group of health children, other study measures, including SCFAs, mitochondrial function, and immune function, were not collected from this comparison group.

CONCLUSIONS

We provide preliminary evidence that intestinal dysbiosis is linked to alterations in SCFA metabolites, of which decreased butyric acid was associated with lower PBMC mitochondrial respiration. Although we did not identify a statistical continuum between intestinal dysbiosis or a specific pattern of SCFAs with a commonly used measure of immune function, these initial findings do support more detailed mechanistic studies to test a possible role of microbial-derived SCFAs in the progression of sepsis and whether therapies targeting the microbiome can improve outcomes.

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