The Journal of Infectious Diseases

SUPPLEMENT ARTICLE

Necrotizing Enterocolitis and the Microbiome: Current Status and Future Directions

Robert Thänert,[1,2,a] Eric C. Keen,[1,2,a] Gautam Dantas,[1,2,3,4] Barbara B. Warner,[5] and Phillip I. Tarr[3,5]

[1]The Edison Family Center for Genome Sciences and Systems Biology, Washington University School of Medicine in St Louis, St Louis, Missouri, USA, [2]Department of Pathology and Immunology, Washington University School of Medicine in St Louis, St Louis, Missouri, USA, [3]Department of Molecular Microbiology, Washington University School of Medicine in St Louis, St Louis, Missouri, USA, [4]Department of Biomedical Engineering, Washington University in St Louis, St Louis, Missouri, USA, and [5]Department of Pediatrics, Washington University School of Medicine in St Louis, St Louis, Missouri, USA

Decades of research have failed to define the pathophysiology of necrotizing enterocolitis (NEC), a devastating pediatric gastrointestinal disorder of preterm infants. However, evidence suggests that host-microbiota interactions, in which microbial dysbiosis is followed by loss of barrier integrity, inflammation, and necrosis, are central to NEC development. Thus, greater knowledge of the preterm infant microbiome could accelerate attempts to diagnose, treat, and prevent NEC. In this article, we summarize clinical characteristics of and risk factors for NEC, the structure of the pre-event NEC microbiome, how this community interfaces with host immunology, and microbiome-based approaches that might prevent or lessen the severity of NEC in this very vulnerable population.

Keywords. metagenomics; microbiome; microbiota; necrotizing enterocolitis; preterm birth; TLR4.

Necrotizing enterocolitis (NEC) is one of the most catastrophic disorders in all of gastroenterology and a major contributor to morbidity and mortality in infants born preterm. NEC presents suddenly, mostly in the first 2 months of life; the treatment of severe cases (surgical resection of the affected gut) and the range of case fatality rates (15%–40%) has not changed in decades [1, 2].

In recent years, massively parallel sequencing of stool from longitudinal cohort studies of preterm infants has greatly expanded our understanding of the pre-NEC microbiome. Case-control studies, in which stools are collected prospectively from dozens of neonates, have established associations of particular taxa with the subsequent development of NEC. Current data strongly suggest that NEC is driven by aberrant host-microbial community interactions rather than by any single organism within this community. This association is broadly analogous to what is observed in inflammatory bowel disease, in which dysbiotic, low-diversity microbiota interacting with host tissues, immunity, and risk alleles, results in tissue injury. Here, we review our current concept of the NEC microbiome, highlight how technology is transforming the field, and emphasize the need to refine our understanding of clinically actionable microbiome signatures that predict disease risk before onset.

APPROACH

We identified relevant literature using the search terms *necrotizing enterocolitis* with and without *microbiome* or *microbiota, microbiota development,* and *preterm microbiome* in PubMed and Google Scholar. We excluded non–English-language publications and studies that focus on NEC in children born at term. We emphasized studies in which sequencing is used to define the pre-event gut microbiome.

CLINICAL ASPECTS OF NEC

NEC is a necroinflammatory gastrointestinal disease, largely in preterm infants, with significant morbidity and mortality rates. NEC occurs only after birth, during an interval in which bacterial communities rapidly populate the newborn gut. Nonspecific early signs of NEC include feeding intolerance, abdominal distention, and/or bloody stools [3], which often progress rapidly to intestinal perforation and systemic hypotension requiring immediate medical and surgical intervention [3, 4]. The Bell scoring system is widely used to describe NEC severity and guide treatment; most studies consider Bell stage II and III, with typical clinical and radiographic (pneumatosis, portal venous air) findings [5], to represent bona fide NEC.

Treatment of NEC escalates with disease severity and ranges from abdominal decompression by suction, bowel rest, broad-spectrum intravenous antibiotics, and total parenteral nutrition in the mildest cases, to exploratory laparotomy and bowel resection in severely affected infants [3, 5]. Surgical intervention is lifesaving in only about 50% of cases in which it is attempted [5], but even if resection is successful, the resulting short bowel syndrome can cause lifelong complications. NEC

[a]R. T. and E. C. K. contributed equally to this work.

Correspondence: Phillip I. Tarr, Department of Pediatrics, Washington University School of Medicine in St Louis, Campus Box 8208, 1 Children's Pl, St Louis MO 63110 (tarr@wustl.edu).

The Journal of Infectious Diseases® 2021;223(S3):S257–63

DOI: 10.1093/infdis/jiaa604

is also accompanied by systemic inflammation and damage to extraintestinal organs including the brain, which hinder neurodevelopment among infants who survive the initial gut injury [6].

NEC RISK FACTORS

The pathophysiology of NEC is incompletely understood, and reliable strategies for its early detection have not emerged. Therefore, attempts to understand and predict NEC currently revolve around studying risk factors for its development. Preterm birth remains the single greatest risk factor for developing NEC [5]. There is an inverse gestational risk, with infants born after the briefest gestations having both the highest incidence and highest mortality rate. In very low birthweight infants (<1.5 kg at birth), NEC incidence ranges between 5% and 13% [1, 2]. Prolonged antibiotic use in the first week of life and feeding of formula in lieu of maternal milk are additionally and consistently associated with subsequent development of NEC [7]. Congenital and especially cardiac defects, transfusions, indomethacin treatment of patent ductus arteriosus, and gastric acid suppression, are less consistently associated with NEC occurrence [5].

Multiomic analyses of clinical samples from human cohort studies, combined with experimental evidence from animal models, suggest that NEC has multifactorial causes. Immune immaturity, underdeveloped gut function (particularly motility and barrier integrity), and aberrant microbial colonization all likely contribute to intestinal injury, excessive inflammatory responses, and NEC development [3, 5]. At the time NEC is manifest, infants have increased concentrations of circulating proinflammatory cytokines, including tumor necrosis factor α and interleukin 8, 12, and 18 [8]. Host pathways invoked in NEC pathogenesis include those associated with activation of Toll-like receptors (TLRs). These receptors and their associated signaling pathways play critical roles balancing inflammatory responses to bacteria and homeostasis, including tissue repair and maintenance of barrier integrity. TLR4 and its downstream pathways has received particular attention (reviewed in [9]), because bacterial lipopolysaccharide binds TLR4 and activates downstream signaling [10].

Moreover, NEC has been associated with variants of human genes (eg, *NFKB1* and *SIGIRR*) whose products are engaged in signaling cascades downstream of TLR4 activation [11, 12]. SIGIRR inhibits lipopolysaccharide-mediated effects on TLR4. Excessive TLR4 activation causes epithelial cell death, reduces mucosal restructuring, and constricts mesenteric vessels, contributing to local ischemia that is also typical of NEC [10]. Intriguingly, pharmacological inhibition and genetic knockout of TLR4 in mouse models protect against early life intestinal injury, providing further evidence for this transmembrane protein's importance in NEC pathogenesis [10].

GUT MICROBES AND NEC RISK IN PRETERM INFANTS

Despite years of attempts, no single species or subspecies has emerged as the cause of NEC. However, multiple lines of evidence suggest that the gut microbiome plays a major role in NEC development. First, NEC does not occur in utero, an interval during which the gut harbors few, if any, viable bacteria. Second, as noted above, risk factors that increase the likelihood that NEC will develop (antibiotics, formula feeding, and possibly acid suppression) affect gut bacterial communities. Third, animal models of early life gut injury suggest that intestinal immune immaturity plays an important role in tissue injury, and that microbial modulation can alter this outcome [13].

The concept that gut community perturbations are the most substantial risk factor for NEC has been enabled by massively parallel sequencing technology, where a single nucleic acid extraction from a specimen is sequenced and microbial content profiled (Table 1). Community profiling first demonstrated that the earliest gut communities of healthy, term infants are dominated by *Bifidobacterium*, *Bacteroides*, *Escherichia* and *Parabacteroides* [19, 23]. After initial colonization, the gut microbiota rapidly gains diversity, undergoing individualized developmental trajectories that are structured by environmental factors including diet, cohabitation, and antibiotic exposure [19, 23].

In comparison, the gut microbiota of preterm infants is compositionally distinct and less diverse than that of term-born infants [20, 25]. Its constituents are mainly influenced by postmenstrual age (gestational age at birth plus day of life) of the infant, and has a characteristic progression, though with considerable interday variability [17]. Immediately after birth, the gut bacterial community is dominated briefly by bacilli, which are soon outnumbered by Gammaproteobacteria, including *Klebsiella*, *Escherichia*, and other Enterobacteriaceae. Gammaproteobacteria predominance steadily cedes to obligate anaerobic populations (Clostridia and Negativicutes) in the absence of NEC.

This transition occurs more rapidly in infants who are born after longer gestations, while anaerobic colonization is delayed to later days of life among those born after the shortest gestation. This is noteworthy given the timing of NEC development: the shorter the gestation, the later in life NEC develops [27]. After hospital discharge, the preterm gut microbiota rapidly gains diversity, and by 2 years of life, these communities are taxonomically indistinguishable from those of term infants [18, 25, 28]. Nevertheless, there is evidence of subtle microbiota "scars" of preterm birth that persist after taxonomic recovery, including long-term gut carriage of multidrug-resistant Enterobacteriaceae [20].

Gut bacterial diversity as a risk factor for NEC was first proposed 19 years ago [29] and has been confirmed in several studies since [30–32], though with some exceptions [33, 34].

Table 1. Studies of Gut Microbiota Assembly in Term and Preterm Infants Without Necrotizing Enterocolitis[a]

Study (Year); Sequencing Technology	Participants and Specimens, No.	Conclusions
Palmer et al (2007) [14]; 16S rRNA	14 Term infants, 26 specimens	Intestinal microbiota trajectories are highly individual; environmental exposure shapes gut microbiota trajectories
Koenig et al (2011) [15]; 16S rRNA and WGS	1 Term infant, 60 specimens	Discrete steps of bacterial succession are structured by diet and health
Eggesbø et al (2011) [16]; 16S rRNA	85 Term infant, 24 specimens	Gammaproteobacteria and bifidobacteria dominate the intestinal microbiome throughout the first month of life
La Rosa et al (2014) [17]; 16S rRNA	58 Preterm infants, 922 specimens	Community population is a function of postmenstrual age; interday instability in structure
Stewart et al (2015) [18]; 16S rRNA	29 Preterm infants, 57 specimens	Preterm infant gut microbiome develops a complexity comparable to that in term infants after NICU discharge
Bäckhed et al (2015) [19]; WGS	98 Term infants, 294 specimens	Species shifts represent nonrandom transitions in infants' guts; cessation of breast milk rapidly matures intestinal microbiome
Gibson et al (2016) [20]; WGS	84 Preterm infants, 401 specimens	Antibiotics most commonly administered in the NICU, have nonuniform effects on the microbiota; distinct antibiotic treatments enrich for specific antimicrobial resistance genes
Yassour et al (2016) [21]; 16S rRNA and WGS	39 Term infants, 1069 specimens	Antibiotic exposure reduces both species and strain-level diversity in the developing gut microbiome and transitionally increases the antibiotic resistance gene burden
Bokulich et al (2016) [22]; 16S rRNA	43 Term infants, 578 specimens	Antibiotic exposures, cesarean section, and formula feeding delay microbiome development and alter microbiota diversity
Stewart et al (2018) [23]; 16S rRNA and WGS	903 Term infants, 12 005 specimens	Gut microbiota progress through developmental, transitional and stable phases over the first 4 years of life, shaped by birth mode, diet, and environmental exposure
Baumann-Dudenhoeffer et al (2018) [24]; WGS	60 Near-term infants, 402 specimens	Distinct early-life microbiome signatures is correlated with breastfeeding, formula ingredients, and maternal gestational weight gain; commensal microbiota gene content adjusts to counterbalance components relatively lacking in human milk
Gasparrini et al (2019) [25]; WGS	41 Preterm and 17 near-term infants, 437 specimens	Early life antibiotic exposure is associated with an enriched intestinal resistome, prolonged carriage of multidrug-resistant Enterobacteriaceae, and distinct patterns of intestinal microbiome assembly

Abbreviation: NICU, neonatal intensive care unit; rRNA, ribosomal RNA; WGS, whole-genome sequencing.

[a]Adapted from Warner and Tarr [26].

However, when considering diversity, it is important to note that neonatal gut microbial populations are highly noncomplex: only 4 bacterial classes represent >90% of the preterm infant stool. Hence, there are limits to the degrees of freedom available for populations to differ. In other words, overrepresentation or underrepresentation of a single taxon obligates reciprocal changes in the proportions of a highly constrained number of other taxa. In these situations, it is difficult to ascribe a host phenotype to a variation in diversity as opposed to the expansion or contraction of a single taxon.

It remains unclear whether pre-onset microbiome diversity is truly a risk factor for NEC, or whether discrepancies in patient cohorts and procedures, such as the use of different 16S ribosomal RNA sequencing primers in different studies, have effectively confounded a genuine biological interaction. However, the most replicable finding across preterm infant cohorts is that NEC is associated with pre-event enrichment of Proteobacteria, particularly Enterobacteriaceae, and with corresponding underrepresentation of Firmicutes and Bacteroidetes [26, 35] (Table 2). This same result has been repeatedly observed, though various genera within Enterobacteriaceae (*Klebsiella*, *Escherichia*, and *Enterobacter*) are implicated in different cohorts [32, 34, 36, 37]. While Proteobacteria are overrepresented in NEC infants immediately before onset, obligate anaerobes, specifically *Veillonella*, were significantly associated with control status in one of the largest longitudinal studies of NEC microbial risk performed to date [31].

Although overrepresentation of Enterobacteriaceae is the most commonly reported microbiome signature of NEC, this value has limited predictive value for the many neonates whose infant gut microbiota is dominated by that family from birth. Thus, discovery of more refined microbiome signatures of NEC is a top priority. More recent efforts have begun to leverage technology with higher taxonomic and genomic resolution. For example, Olm et al used whole-genome shotgun sequencing, not 16S ribosomal RNA sequencing, to extensively evaluate metagenomic features from a prospective cohort of NEC cases and controls, of which a small subset was collected before to NEC onset [37]. This approach enables assessment of numerous genome-resolved features, including individual genes, bacterial strains and plasmids, viruses, and eukaryotes, and even growth rates for their relative association with NEC.

Using these high-resolution data, Olm et al built a machine learning classifier, which identified bacterial replication rate,

Klebsiella abundance, and genes encoding fimbriae and several secondary metabolites as the best predictors of NEC [37]. Despite the unprecedented integration of genome features, the resulting classifier achieved a median accuracy of 64%, only 14% better than random chance. Future classifiers may be improved by additional data, such as metatransciptomics, and by including more pre-onset samples from NEC cases and matched controls.

HOW ABERRANT HOST-MICROBIOTA INTERACTIONS MIGHT DRIVE NEC

The postulated association between Proteobacteria and NEC before onset is particularly intriguing in light of these bacteria's interactions with the gut's innate immune system. As the dominant gram-negative bacterial group in the preterm infant gut microbiota, Proteobacteria are chief candidates for stimulating proinflammatory immune responses via TLR4 signaling [35]. This lends support to the hypothesis that NEC results from microbiota dysbiosis and overstimulation of TLR4, resulting in massive inflammation, loss of barrier integrity, local ischemia, and tissue death, as described above [39]. Nevertheless, blooms of Proteobacteria are insufficient to cause this cascade, because NEC does not exclusively occur in infants in whom Enterobacteriaceae dominate the gut microbiota. Conversely, infants whose gut microbial communities are dominated by Enterobacteriaceae do not always develop NEC.

Exploration of the role of immunoglobulin A (IgA) in NEC pathophysiology offers new and compelling insights by synergizing host and microbial biology. In older children and adults, IgA is secreted in large quantities by intestinal B cells, where it binds epithelium-associated (and thus potentially invasive) bacteria in a proximity-dependent manner. For roughly the first 40 days of life, however, maternal breast milk is the primary source of IgA.

Gopalakrishna et al [38] applied IgSeq, in which IgA-bound and IgA-unbound bacteria are sorted by flow cytometry and then 16S sequenced, to a longitudinal NEC case-control cohort with samples from this interval. Consistent with previous reports, the authors observed a relative enrichment of Enterobacteriaceae and reduction in obligate anaerobes before NEC onset. However, the association of NEC development and an increase in the relative abundance of IgA-unbound Enterobacteriaceae was even stronger. Although the absolute abundance of Enterobacteriaceae did not differ statistically between case patients and controls, the proportion of Enterobacteriaceaei bound by IgA was lower in neonates who later developed NEC. Remarkably, in a mouse model of disease, milk from IgA-deficient (*Rag1*$^{-/-}$ or *Igha*$^{-/-}$) dams did not protect from experimentally induced NEC in, in contrast to milk from wild-type dams. Although the number of specimens analyzed was modest (<100 samples) and the underlying mechanisms remain unclear, this study supports a protective role for maternal IgA that at least partly explains why formula feeding increases the risk of NEC.

MICROBIOTA-DIRECTED TREATMENT OF NEC

As discussed above, an increasing body of evidence connects microbial dysbiosis to development of NEC. Consequently, microbiota-directed therapies have been proposed to prevent NEC. General concepts for microbiota-directed therapies include (1) nutritional supplementation, (2) avoidance of interventions that are likely to promote dysbiosis (eg, antibiotics), (3) probiotics, prebiotics, and synbiotics, and (4) fecal microbiota transplants.

Human breast milk components, including oligosaccharides, lactoferrin, secretory IgA, and antioxidants and growth factors, have been suggested to reduce an infant's risk of developing NEC [5]. Consequently, donor breast milk is now widely given

Table 2. High-Throughput Sequencing Studies Characterizing the Gut Microbiota of Infants Who Develop Necrotizing Enterocolitis[a]

Study (Year); Sequencing Technology	Participants With NEC (of Total), No.	Specimens, No.	Factors Associated With NEC Risk Conclusions
Morrow et al (2013) [33]; 16S rRNA	11 of 32	58	Low community diversity and abundance of Firmicutes or Proteobacteria
Torrazza et al (2013) [34]; 16S rRNA	18 of 53	119	Abundance of Proteobacteria or Actinobacteria
Brower-Sinning et al (2014) [30]; 16S rRNA	18 of 19	26	High abundance of anaerobes and low community diversity in intestinal tissues
Warner et al (2016) [31]; 16S rRNA	46 of 120	2720	Gammaproteobacteria and lack of diversity; Negativicutes associated with NEC protection
Ward et al (2016) [36]; WGS	16 of 165	262	Uropathogenic *Escherichia coli* strain types
Dobbler et al (2017) [32]; 16S rRNA and WGS	11 of 40	132	Enterobacteriaceae and low community diversity
Olm et al (2019) [37]; WGS	34 of 160	1163	*Klebsiella* abundance and genes encoding fimbriae and secondary metabolites
Gopalakrishna et al (2019) [38]; WGS	10 of 23	98	High abundance of Enterobacteriaceae (particularly IgA-unbound Enterobacteriaceae) and reduced anaerobes

Abbreviation: IgA, immunoglobulin A; NEC, necrotizing enterocolitis; rRNA, ribosomal RNA; WGS, whole-genome sequencing.

[a]Adapted from Warner and Tarr [26].

to preterm infants when their mother's milk is unavailable [5]. Experimental evidence suggests that human breast milk acts by controlling expansion of detrimental microbes and by attenuating TLR4 signaling [38]. Dietary supplementation with donor milk or specific human milk components, including arginine, reduces NEC incidence in animal models [40], but human milk, especially milk from an infant's own mother, continue to offer the best opportunity to reduce the risk of NEC [41].

Antibiotics are also correlated with NEC development, with the most frequent association being prolonged administration during the first week of life [42–44]. When used in appropriate situations, antibiotics are among the most valued interventions available to neonatologists. However, as more is learned about unintended adverse effects of these agents, including but not limited to increased risk of NEC development, it is prudent to develop mechanisms to reduce antibiotic administration in all situations in which it offers little or no benefit.

Probiotics, defined here as "living micro-organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition [45]," frequently receive interest as interventions to favorably alter gut microbial communities and prevent NEC. Probiotics could "educate" the developing immune system, outcompete detrimental microbes, and support intestinal barrier function [46], attributes that theoretically would prevent NEC. Studies vary by probiotic, dose and duration, and results.

Two high-quality, large, double-blind randomized controlled trials exemplify the challenges in interpreting the literature: in one Australian/New Zealand consortium, a combination of *Bifidobacterium infantis*, *Streptococcus thermophilus*, and *Bifidobacterium lactis* reduced the NEC rate in preterm infants (birth weights <1500 g) from 4.4% to 2% [47]. However, the NEC rate in the controls in this study was low, the result was only modestly significant, and the benefit was confined to infants with birth weight <1000 g. In a multicenter study from the United Kingdom, *Bifidobacterium breve* [48] administration did not lower NEC incidence.

Despite meta-analyses favorable to the use of probiotics to prevent NEC, we believe that the conclusions of many of the primary studies in which probiotics appear to prevent NEC are weakened by methodological and/or statistical concerns. We also note that the beneficial effects inferred from these meta-analyses do not apply to infants weighing <1000 g at birth, a group with the highest incidence of, and case fatality rate from, NEC. The challenges of producing high-quality evidence to test the efficacy of probiotics have been reviewed [49].

Finally, animal studies suggest the potential of inoculating the preterm digestive system with complex bacterial communities via fecal microbiota transplantation [50–52], echoing postulated benefits of probiotics on bacterial community structure and diversity, intestinal immunity, and tissue damage from proinflammatory TLR4 signaling. However, in view of challenges and safety concerns (selection of ideal donor microbial community, risk of pathobiont translocation from gut to bloodstream), it would be difficult to conduct a trial of fecal microbiota transplantation in the preterm population.

CONCLUSIONS

NEC remains a major unsolved challenge. Recent efforts and technological advances have dramatically improved our understanding of how the microbiome contributes to the pathophysiology of NEC, but key questions remain. The main challenge now for NEC microbiome research is translating results of large, associative case-control studies into mechanistic insight and clinically actionable targets. Many studies have identified general microbiota trends before NEC ensues, and common themes are overrepresentation of Gammaproteobacteria/Proteobacteria and, increasingly, underrepresentation of specific obligate anaerobes (Table 2). Findings of one study illustrate the potential of genome-resolved microbiome profiling for identifying species, functions, and genes associated with NEC [37]. No study to date, however, has combined high-resolution microbiome characterization with concurrent host profiling, a prerequisite for identifying causal relationships in the pathophysiology of NEC. We look forward to the integration of deep multiomic profiling of bacterial communities with robust characterization of host biology, using large longitudinal pre-onset sample collections, to develop classifiers that enable personalized risk assessment, early diagnosis, and timely intervention.

Notes

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

Financial support. This work is supported by the National Institute of General Medical Sciences, National Institutes of Health (NIH; grant R01 GM099538 to G. D.), the US Centers for Disease Control and Prevention (grant 200-2016-91955 to G. D.), the NIH (grant 5P30 DK052574 to P. I. T. through the Biobank Core of the Digestive Disease Research Core Center), the Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH (grant R01 HD092414 to G. D., B. B. W., and P. I. T.), the Children's Discovery Institute at St Louis Children's Hospital and Washington University School of Medicine in St Louis (B. B. W. and P. I. T.), the Deutsche Forschungsgemeinschaft (German Research Foundation; grant 402733540 to R. T.), and the National Science Foundation (graduate research fellowship DGE-1143945 to E. C. K.).

Supplement sponsorship. This work is part of a supplement sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC).

Potential conflicts of interest. P. I. T. is a member of the scientific advisory board of, a consultant to, and a holder of equity in MediBeacon, which is developing technology to measure gut permeability in humans. He is also a possible recipient of royalties based on a patent on this topic, and a consultant to Kallyope and Takeda Pharmaceuticals on childhood gastrointestinal disorders. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Stoll BJ, Hansen NI, Bell EF, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993-2012. JAMA **2015**; 314:1039–51.
2. Battersby C, Santhalingam T, Costeloe K, Modi N. Incidence of neonatal necrotising enterocolitis in high-income countries: a systematic review. Arch Dis Child Fetal Neonatal Ed **2018**; 103:F182–9.
3. Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med **201**; 364:255–64.
4. Lim JC, Golden JM, Ford HR. Pathogenesis of neonatal necrotizing enterocolitis. Pediatr Surg Int **2015**; 31:509–18.
5. Niño DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. Nat Rev Gastroenterol Hepatol **2016**; 13:590–600.
6. Hickey M, Georgieff M, Ramel S. Neurodevelopmental outcomes following necrotizing enterocolitis. Semin Fetal Neonatal Med **2018**; 23:426–32.
7. Rose AT, Patel RM. A critical analysis of risk factors for necrotizing enterocolitis. Semin Fetal Neonatal Med **2018**; 23:374–9.
8. Maheshwari A, Schelonka RL, Dimmitt RA, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Cytokines associated with necrotizing enterocolitis in extremely-low-birth-weight infants. Pediatr Res **2014**; 76:100–8.
9. Mihi B, Good M. Impact of Toll-like receptor 4 signaling in necrotizing enterocolitis: the state of the science. Clin Perinatol **2019**; 46:145–57.
10. Hackam DJ, Sodhi CP, Good M. New insights into necrotizing enterocolitis: from laboratory observation to personalized prevention and treatment. J Pediatr Surg **2019**; 54:398–404.
11. Sampath V, Le M, Lane L, et al The NFKB1 (g.-24519delATTG) variant is associated with necrotizing enterocolitis (NEC) in premature infants. J Surg Res **2011**; 169:e51–7.
12. Sampath V, Menden H, Helbling D, et al SIGIRR genetic variants in premature infants with necrotizing enterocolitis. Pediatrics **2015**; 135:e1530–4.
13. Tanner SM, Berryhill TF, Ellenburg JL, et al Pathogenesis of necrotizing enterocolitis: modeling the innate immune response. Am J Pathol **2015**; 185:4–16.
14. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. PLoS Biol **2007**; 5:1556–73.
15. Koenig JE, Spor A, Scalfone N, et al Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A **2011**; 108(suppl 1):4578–85.
16. Eggesbø M, Moen B, Peddada S, et al Development of gut microbiota in infants not exposed to medical interventions. APMIS **2011**; 119:17–35.
17. La Rosa PS, Warner BB, Zhou Y, et al Patterned progression of bacterial populations in the premature infant gut. Proc Natl Acad Sci U S A **2014**; 111:12522–7.
18. Stewart CJ, Skeath T, Nelson A, et al Preterm gut microbiota and metabolome following discharge from intensive care. Sci Rep **2015**; 5:1–9.
19. Bäckhed F, Roswall J, Peng Y, et al Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe **2015**; 17:690–703.
20. Gibson MK, Wang B, Ahmadi S, et al Developmental dynamics of the preterm infant gut microbiota and antibiotic resistome. Nat Microbiol **2016**; 1:16024.
21. Yassour M, Vatanen T, Siljander H, et al; DIABIMMUNE Study Group. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. Sci Transl Med **2016**; 8:343ra81.
22. Bokulich NA, Chung J, Battaglia T, et al Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med **2016**; 8:343ra82.
23. Stewart CJ, Ajami NJ, O'Brien JL, et al Temporal development of the gut microbiome in early childhood from the TEDDY study. Nature **2018**; 562:583–8.
24. Baumann-Dudenhoeffer AM, D'Souza AW, Tarr PI, Warner BB, Dantas G. Infant diet and maternal gestational weight gain predict early metabolic maturation of gut microbiomes. Nat Med **2018**; 24:1822–9.
25. Gasparrini AJ, Wang B, Sun X, et al Persistent metagenomic signatures of early-life hospitalization and antibiotic treatment in the infant gut microbiota and resistome. Nat Microbiol **2019**; 4:2285–97.
26. Warner BB, Tarr PI. Necrotizing enterocolitis and preterm infant gut bacteria. Semin Fetal Neonatal Med **2016**; 21:394–9.
27. Yee WH, Soraisham AS, Shah VS, Aziz K, Yoon W, Lee SK; Canadian Neonatal Network. Incidence and timing of

presentation of necrotizing enterocolitis in preterm infants. Pediatrics **2012**; 129:e298–304.
28. Moles L, Gómez M, Jiménez E, et al Preterm infant gut colonization in the neonatal ICU and complete restoration 2 years later. Clin Microbiol Infect **2015**; 21:936.e1–936.e10.
29. Claud EC, Walker WA. Hypothesis: inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. FASEB J **2001**; 15:1398–403.
30. Brower-Sinning R, Zhong D, Good M, et al Mucosa-associated bacterial diversity in necrotizing enterocolitis. PLoS One **2014**; 9:e105046.
31. Warner BB, Deych E, Zhou Y, et al Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. Lancet **2016**; 387:1928–36.
32. Dobbler PT, Procianoy RS, Mai V, et al Low microbial diversity and abnormal microbial succession is associated with necrotizing enterocolitis in preterm infants. Front Microbiol **2017**; 8:2243.
33. Morrow AL, Lagomarcino AJ, Schibler KR, et al Early microbial and metabolomic signatures predict later onset of necrotizing enterocolitis in preterm infants. Microbiome **2013**; 1:13.
34. Torrazza RM, Ukhanova M, Wang X, et al Intestinal microbial ecology and environmental factors affecting necrotizing enterocolitis. PLoS One **2013**; 8:e83304.
35. Pammi M, Cope J, Tarr PI, et al Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis. Microbiome **2017**; 5:31.
36. Ward DV, Scholz M, Zolfo M, et al Metagenomic sequencing with strain-level resolution implicates uropathogenic *E. coli* in necrotizing enterocolitis and mortality in preterm infants. Cell Rep **2016**; 14:2912–24.
37. Olm MR, Bhattacharya N, Crits-Christoph A, et al Necrotizing enterocolitis is preceded by increased gut bacterial replication, *Klebsiella,* and fimbriae-encoding bacteria. Sci Adv **2019**; 5:eaax5727.
38. Gopalakrishna KP, Macadangdang BR, Rogers MB, et al Maternal IgA protects against the development of necrotizing enterocolitis in preterm infants. Nat Med **2019**; 25:1110–5.
39. Hackam DJ, Sodhi CP. Toll-like receptor–mediated intestinal inflammatory imbalance in the pathogenesis of necrotizing enterocolitis. Cell Mol Gastroenterol **2018**; 6:229–38.e1.
40. Vincent D, Klinke M, Eschenburg G, et al NEC is likely a NETs dependent process and markers of NETosis are predictive of NEC in mice and humans. Sci Rep **2018**; 8:12612.
41. Ford SL, Lohmann P, Preidis GA, et al Improved feeding tolerance and growth are linked to increased gut microbial community diversity in very-low-birth-weight infants fed mother's own milk compared with donor breast milk. Am J Clin Nutr **2019**; 109:1088–97.
42. Alexander VN, Northrup V, Bizzarro MJ. Antibiotic exposure in the newborn intensive care unit and the risk of necrotizing enterocolitis. J Pediatr **2011**; 159:392–7.
43. Cotten CM, Taylor S, Stoll B, et al; NICHD Neonatal Research Network. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. Pediatrics **2009**; 123:58–66.
44. Kuppala VS, Meinzen-Derr J, Morrow AL, Schibler KR. Prolonged initial empirical antibiotic treatment is associated with adverse outcomes in premature infants. J Pediatr **2011**; 159:720–5.
45. Guarner F, Schaafsma GJ. Probiotics. Int J Food Microbiol **1998**; 39:237–8.
46. Patel RM, Underwood MA. Probiotics and necrotizing enterocolitis. Semin Pediatr Surg **2018**; 27:39–46.
47. Jacobs SE, Tobin JM, Opie GF, et al; ProPrems Study Group. Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. Pediatrics **2013**; 132:1055–62.
48. Costeloe K, Hardy P, Juszczak E, Wilks M, Millar MR; Probiotics in Preterm Infants Study Collaborative Group. *Bifidobacterium breve* BBG-001 in very preterm infants: a randomised controlled phase 3 trial. Lancet **2016**; 387:649–60.
49. Jarrett P, Meczner A, Costeloe K, Fleming P. Historical aspects of probiotic use to prevent necrotising enterocolitis in preterm babies. Early Hum Dev **2019**; 135:51–7.
50. Li X, Li X, Shang Q, et al Fecal microbiota transplantation (FMT) could reverse the severity of experimental necrotizing enterocolitis (NEC) via oxidative stress modulation. Free Radic Biol Med **2017**; 108:32–43.
51. Brunse A, Martin L, Rasmussen TS, et al Effect of fecal microbiota transplantation route of administration on gut colonization and host response in preterm pigs. ISME J **2019**; 13:720–33.
52. Prado C, Michels M, Ávila P, Burger H, Milioli MVM, Dal-Pizzol F. The protective effects of fecal microbiota transplantation in an experimental model of necrotizing enterocolitis. J Pediatr Surg **2019**; 54:1578–83.