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Updating on gut microbiota and its relationship with the occurrence of necrotizing enterocolitis

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ABSTRACT

Necrotizing enterocolitis (NEC) remains a leading cause of morbidity and mortality, affecting primarily preterm neonates. The pathogenesis of this intestinal disease appears to be linked to the disruption or delay of bacterial colonization, termed gut dysbiosis. Intestinal immaturity, antibiotic use and hospital microbial environment are the main triggers of this pathological process. Conversely, gut symbiosis is made possible by the presence of beneficial and commensal bacterial species that protect the immature gut from opportunistic pathogens overgrowth and inflammation. Herein, we review the relationships between gut microbiota and NEC in preterm neonates. We also discuss the role of specific microorganisms belonging to the commensal microbiota, highlighting the possibility for a toxigenic mechanism involved in NEC pathogenesis. We conclude on the importance of interventions aimed at providing or restoring beneficial bacteria populations, in view to efficiently preventing or treating NEC.

Contents

Introduction

Necrotizing enterocolitis (NEC) is a life threatening intestinal disease affecting primarily preterm neonates that represent 5–13% of all infants [1]. The prevalence of NEC can reach 12% in preterm neonates with very low birth weight (i.e. 500–1000 g) [2], and related mortality ranges from 20% to 50% [3]. Despite decades of research, the pathogenesis of NEC is poorly understood. It remains an emerging cause of morbidity and mortality. For instance, in the USA, preterm neonates mortality from most causes has decreased across the period from 2000 to 2011, whereas the incidence of death from NEC has increased [4]. Moreover, nearly 25% of the neonates recovering from NEC have long-term poor neurodevelopmental outcomes [5].

Recent research expanded knowledge about the assembly for the human infant gut microbiome. Among healthy term neonates, early gut colonization patterns are driven largely by delivery mode

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and feeding patterns [6,7]. Moreover, in neonates, the gut microbiota varies substantially, beeing more susceptible to environmental factors than the adult microbiota [8]. Preterm neonates, characterized by an immature intestinal and immune system, are commonly exposed to antibiotics and are raised in a hospital environment that is host to a low diversity of microbes under excessive hygiene conditions [9]. These factors promote the development of gut dysbiosis characterized by decreased overall initial bacterial diversity and richness, delayed colonization by beneficial commensal species, including *Bifidobacterium* species [10].

Several lines of evidence suggest a role of specific bacteria and gut microbiota patterns in the pathogenesis of NEC. Indeed, outbreaks of NEC have long been described, although the reported causal agents greatly differed [11]. Moreover, *pneumatosis intestinalis* that is one of the specific radiological signs of NEC, likely represents submucosal gas produced by bacterial fermentation [5]. Most notably, NEC could not be reproduced in germ-free animal and rarely occurs until at least 8–10 days postpartum in preterm neonates, after strict anaerobic bacterial populations establishment [5]. Recently, Hourigan et al. described the serial microbiome changes in twins discordant for NEC, who shared similar intrauterine and early environmental exposures. Both preterm neonates had distinctly different microbiome compositions from the first stool samples collected. In the twin who developed NEC there was a decrease in bacterial diversity and an increase in Proteobacteria a week before developing any clinical symptoms [12].

In this review, we will update information on the role of the intestinal microbiota in NEC. Based on current research evidence, we define the characteristics of the NEC-related gut dysbiosis. We highlight the role of specific microorganisms associated with NEC and discuss the potential role of biotoxins involved in the pathogenesis of NEC. Finally, we emphasize the importance of interventions aimed at providing or restoring symbiotic commensal bacteria populations, in view to efficiently prevent or treat NEC.

Initial gut microbiota establishment

Recent data prompt reconsideration of the dogma postulating that fetal gut contains no bacteria. Indeed, several studies have identified bacterial DNA sequences in freshly produced meconium [13,14]. DiGiulio et al. identified from the amniotic cavity a great diversity of bacteria, including previously uncharacterized taxa [15]. Bearfield et al. showed that bacteria in the maternal mouth could reach the amniotic fluid *via* the bloodstream, specifically in the presence of gingivitis during pregnancy [16].

After birth, the neonate's gut bacterial population rapidly expands, especially during the first week of life. The first bacteria that colonize normal infants are mainly aerobes or facultative anaerobes such as *Enterococcus* spp., *Streptococcus* spp. and *Staphylococcus* spp., Enterobacteriaceae, and Lactobacilli [7,17]. These initial colonizers consume oxygen, thereby creating reductive conditions [18].

Several factors, including the mode of delivery, play an important role in the initial colonization of fetal gut. Neonates born vaginally typically are seeded with maternal vaginal microbiota, including *Lactobacillus* and *Prevotella* species, whereas neonates born by cesarean delivery are colonized by bacteria belonging to the skin microbiota, including Staphylococci and *Corynebacterium* spp. [19,20]. After the first 3–5 years of life, there is a convergence to a relatively stable and common profile of bacterial communities similar to the adult gut microbiota [7]. However, the high interindividual variability makes it difficult to define a 'normal' dynamic of colonization [21]. Indeed, geographical provenance, environment, dietary habits represent major factors contributing to the human gut microbiota's diversity [22].

Overall, establishing a core microbiota of diverse commensal and beneficial bacterial species is essential to the host as it provides competition with the pathogenic microbes. This gut symbiosis plays also a key role in nutrient digestion and metabolism, vitamin synthesis, immune tolerance, intestinal mucosa's maturation, and brain development [21].

Gut dysbiosis and necrotizing enterocolitis

Disruption of the gut microbiota development and homeostasis, termed gut dysbiosis has been associated with development of multiple intestinal disorders, including NEC [23]. This pathological process is characterized by a lack of beneficial commensal microbes, a low diversity of bacteria allowing the overgrowth of pathogenic bacteria inducing an excessive inflammatory response [24] (Fig. 1). While prematurity and gut dysbiosis are considered as pivotal in the pathogenesis of NEC, these are also associated with many other factors like antibiotic and anti-acid exposure, the neonatal intensive care environment, the type of feeding, the altered metabolic and immunological dysfunctions [21].

The recent advent of genomic analysis including high throughput 16S rRNA pyrosequencing supported the expanding of the knowledge on microbiome changes before and during the development of NEC. For instance, in a study by Wang et al. comparing 10 preterm neonates with NEC and 10 matched controls, fecal samples from those with NEC had a lower bacterial diversity and clustered separately from those without NEC, a predominance of γ-Proteobacteria in NEC was also reported [25]. Mai et al. compared the developing gut microbiome of nine preterm neonates before the occurrence of NEC with nine unaffected control patients using 16S rRNA sequencing and found a surge of Proteobacteria in the samples collected 1 week to less than 72 h in those who developed NEC [26]. Morrow et al. identified two distinct forms of dysbiosis (Firmicutes dominance during postnatal days 4–9 or Enterobacteriaceae dominance during postnatal days 10–16) associated with NEC together with a lack of *Propionibacterium* spp. and a lower diversity [27]. Similar findings were seen by Torrazza et al. in a longitudinal prospective study with 18 NEC cases and 35 control patients, with an increase in Proteobacteria two weeks and of Actinobacteria one week before the diagnosis of NEC [28]. They also demonstrated lower numbers of Bifidobacteria counts and Bacteroidetes proportions in the weeks before NEC diagnosis. Moreover, Claud et al. reported that preterm neonates with NEC had overgrowth of Proteobacteria at the expense of Firmicutes and, in addition, a decrease in the abundance of lactose fermenters from the family Veillonellaceae [29]. Notably, these changes occurred several weeks prior to NEC. In another study, Warner et al. prospectively collected stool samples in a case-control study and observed a relative abundance of γ-Proteobacteria (i.e., Gram-negative facultative bacilli) and relative paucity of strict anaerobic bacteria (especially Negativicutes) preceding NEC in preterm neonates born at less than 27 weeks' gestation [30]. More recently, Ward et al. in their large-scale deep shotgun metagenomic sequence analysis of the early intestinal microbiome of 144 preterm and 22 term infants, observed a loss of diversity associated with an increased relative abundance of *Escherichia coli* (correlated with antibiotic consumption), and a lower relative abundance of *Veillonella* spp. in preterm neonates who developed NEC [31].

These data support the view that an increase in Proteobacteria may trigger a hyperinflammatory response linked with the occurrence of NEC [24]. This condition may also promote an abnormal colonization by strict anaerobes. However, many preterm neonates experience a « bloom » in Proteobacteria without developing NEC [32]. Thus, Proteobacteria dysbiosis alone does not fully explain the pathogenesis of NEC. Moreover, a recent shotgun metagenomic