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Review

Noninvasive molecular fingerprinting of host–microbiome interactions in neonates ☆

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ABSTRACT

The early postnatal period is a critical window for intestinal and immune maturation. Intestinal development and microbiome diversity and composition differ between breast- (BF) and formula-fed (FF) infants. Mechanistic examination into host–microbe relationships in healthy infants has been hindered by ethical constraints surrounding tissue biopsies. Thus, a statistically rigorous analytical framework to simultaneously examine both host and microbial responses to dietary/environmental factors using exfoliated intestinal epithelial cells was developed. Differential expression of ~1200 genes, including genes regulating intestinal proliferation, differentiation and barrier function, was observed between BF and FF term infants. Canonical correlation analysis uncovered a relationship between microbiome virulence genes and host immunity and defense genes. Lastly, exfoliated cells from preterm and term infants were compared. Pathways associated with immune cell function and inflammation were up-regulated in preterm, whereas cell growth-related genes were up-regulated in the term infants. Thus, coordinate measurement of the transcriptomes of exfoliated epithelial cells and microbiome allows inquiry into mutualistic host–microbe interactions in the infant, which can be used to prospectively study gut development or, retrospectively, to identify potential triggers of disease in banked samples.

1. Introduction

Intestinal epithelial cells and the commensal microbiota are in close and intimate contact. Studies emanating from germ free and gnotobiotic animals have provided conclusive evidence of the critical role of the intestinal microbiota in regulating gut development and gene expression [1,2], mucosal and systemic immunity [3], the enteric nervous system [4], gut brain axis [5] and host metabolism [6,7]. Recent studies have dispelled the concept that amniotic fluid and meconium are sterile under normal conditions [8]. Meconium, which is formed primarily by ingestion of amniotic fluid by the fetus in utero, also contains exfoliated intestinal cells and mucus. The meconium microbiome is influenced by maternal factors, including clinical conditions [9] and probiotic use [10], and may impact child health outcomes [8,9,11]. Thus, host–microbe interactions and education of the neonatal immune system begin in the womb [8].

Immediately after delivery, the human infant acquires a much more complex microbiota, whose composition is influenced by an interplay between genetic and environmental factors [12], of which nutrition is a key component [13]. At the same time, the gastrointestinal tract undergoes rapid structural and functional adaptation, which differs between breast-fed (BF) and formula-fed (FF) infants [14,15]. Although human milk contains growth factors and bioactive proteins and lipids that may directly promote the growth of the gastrointestinal tract [16,17], we speculated that dissimilarities in the composition of the microbiota between breast- and

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formula-fed infants [17,18] could also be contributing to the enhanced gut development observed by mode of nutrition [14,15].

Our long-term goal is to determine the role of host–microbe interactions within the neonatal intestine on infant development and to define how these cross-functional communications are affected by diet. Among the components of human milk that shape the composition of the microbiota are the human milk oligosaccharides (HMO). The HMO are comprised of a mixture of up to 200 complex oligosaccharides that constitute the third most predominant component of human milk [19]. The HMO content and composition is influenced by the mothers' genetics (FUT-2 secretor status and Lewis blood group) [20], preterm delivery [21] and, to a lesser degree, the stage of lactation, where sialic acid containing HMO decline, while fucosylated HMO increase or stay constant over the course of lactation [19]. The potential physiological roles of HMO for the developing infant is far reaching in that their multifunctional actions range from regulation of intestinal cell proliferation, functional differentiation and apoptosis [22,23], gene expression [24], immune function [25–27], pathogen protection [28,29], and prebiotic activities, including serving as substrates for fermentation [30,31] and promoting growth of specific bifidobacteria [32], bacteroides [33] and *Lactobacillus* [34] species (reviewed in [35]).

We hypothesize that nutrition is a central regulator of host–microbe interactions in early life. As noted above, the composition of the microbiota of BF and FF infants differs in terms of overall diversity as well as composition [12,13,18]. Epidemiological studies have demonstrated that human milk protects against common infectious diseases in infancy (otitis media, respiratory syncytial virus, urinary tract infection), necrotizing enterocolitis (NEC) in preterm infants as well as immune-mediated disorders in later childhood, including allergy, asthma, atopic dermatitis, inflammatory bowel disease, Celiac Disease, Type 1 and Type 2 diabetes mellitus, and leukemia (ALL and AML) [36]. Recently, Walker [37] proposed that a diverse balanced microbiota is necessary for the development of an appropriate innate and adaptive immune response. This is further supported by studies associating dysbiosis in early life with immune-mediated childhood disorders [38–40] and obesity [41,42]. Dysbioses can arise from common pediatric practices, including preterm delivery, formula feeding, cesarean section, and use of antibiotics [42,43] (Fig. 1). Interestingly, cesarean section [43] and antibiotic use [44] are independently associated with an increased susceptibility to immune-mediated disease, potentially through dysregulation of host immune homeostasis [44,45]. It is important to note that all of these practices are amenable to changes in clinical protocols, and, as such, should be a priority for pediatric practice.

Given the evidence that early life nutritional exposures program long-term health outcomes, potentially through host–microbe interactions, our research group set out to systematically integrate genomic data from both the infant (host mucosa) and gut microbiota in order to define host gene–diet interactions within the context of the structure and operations of gut microbial communities. Until recently, no investigators had comprehensively profiled intestinal gene expression during early postnatal development due to limited availability of intestinal tissue from healthy infants. Thus, the potential for exfoliated epithelial cells to provide a non-invasive readout of intestinal gene expression was investigated [46–48].

2. Use of exfoliated cells to assess host gene expression

Each day, ~1/3rd to 1/6th of normal adult epithelial cells are shed [49], which corresponds to ~10 billion (10^{10}) cells per day. Exfoliation of intestinal epithelial cells from the villus tips in the small intestine and crypt surface in the colon is ab active biochemical process linked to intestinal epithelial homeostasis [50]. Exfoliation typically induces anoikis, rather than apoptosis, which is a form of programmed cell death induced by anchorage-dependent cells detaching from the surrounding extracellular matrix. Detachment also induces autophagy, which is a survival mechanism to loss of nutrients [51]. The exfoliated cells enter into a quiescent state and appear to maintain viability for differing lengths of time depending on the sources of cells. For example, quiescent exfoliated epithelial cells without signs of apoptosis were recovered in gastric fluid aspirates obtained from preterm infants [52]. Furthermore, exfoliated quiescent epithelial cells can be cultured, evidenced by the ability to use exfoliated cells to forms lumens in 3-dimensional epithelial cell culture [47,48], suggesting that detachment-induced autophagy contributes to the viability of these cells.

This vast reservoir of host cells generated by exfoliation sparked interest from both basic and clinical translational investigators due to their potential utility to non-invasively assess cellular markers of gastrointestinal disease, predominantly colon cancer [53,54]. Subsequently, exfoliated epithelial cells had been used as sentinels of in vivo exposure to nutritional regimens [55,56] or as markers of disease states, including cancer in adults [57,58] and children with

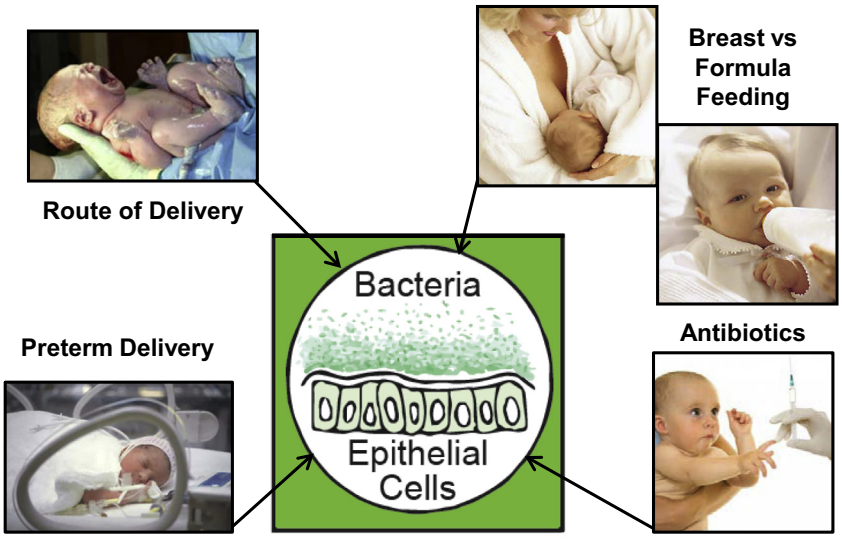

Fig. 1. Common pediatric practices that impact gut microbiota and host–microbe interactions.