Dirty Diaper Contents May Inform Researchers of Infant Intestinal Development and Disease Susceptibility

At birth, the intestinal tract of the human infant is functionally immature and sterile. Accordingly, the early neonatal period is a critical phase for both intestinal digestive development as well as establishment (colonization) of intestinal microorganisms (microbiota).

The newborn is required to discriminate between food and microbial antigens that should be "tolerated", e.g., milk proteins and non-pathogenic bacteria vs. pathogens to which the infant should mount an immune response. The intestine is lined by epithelial cells that process nutrients and provide the first line of defense against food antigens and pathogens. Because colonization of the intestine with non-pathogenic (commensal) microbiota is vital for infant health, it is important to understand how epithelial cells and the microbial ecosystem are modulated by diet and disease.

Breast milk is considered the optimal form of nutrition for the human infant; however, most babies in the U.S. are either not breastfed or only receive breast milk for a short period of time. Formulas provide adequate nutrition, but differ in nutrient composition from breast milk and do not contain the bioactive components that are present in breast milk. How these various components influence the infant's intestinal development, microbiota and immune responses over the long term is largely unknown. Therefore, our ongoing efforts are directed at understanding the regulation of neonatal development by components present in human milk and infant formula. Unfortunately, due to the lack of tissue biopsies, no investigators have performed a global analysis of the genes expressed (turned on or off) in the developing intestine in infants.

Approximately one-sixth to one-third of normal adult intestinal epithelial cells are shed (exfoliated) daily. This corresponds to the daily exfoliation of approximately 10 trillion cells. We are

the first investigative team to propose that the ability to use epithelial cells shed from the intestine, instead of biopsy or autopsy material, would be highly advantageous to document the impact of nutrition on the continuum of intestinal and microbiota development and maturation in the newborn.

By bringing together scientists in the fields of nutrition, microbiology, pediatrics, computational modeling and statistics, we developed novel non-invasive high throughput gene sequencing techniques to simultaneously examine both intestinal gene expression and microbial composition in the same stool samples. The technique involves isolating genetic material from both intestinal epithelial cells and microbes shed in the infants' stools, and comparing the extent to which genes are expressed in both the host and microbiome in breastfed versus formula-fed babies.

This breakthrough technology, for the first time, will provide insight into both host and microbial responses to dietary components in the early neonatal period. Ultimately, the integration of information from the infant and the microbiome will be used to identify important regulatory pathways of the gut microbiome affecting intestinal development in the first few months of life.

The information gained by this integrative approach has many potential applications. By understanding how the intestine of a healthy infant develops, we can establish biomarkers that can be compared to disease states where intestinal development is compromised. For example, food allergies and inflammatory bowel diseases arise when the intestinal immune system does not learn to tolerate food proteins or commensal bacteria.

Using this novel methodology, we have recently demonstrated that the gene expressed most often in breastfed infants is involved in the cell's response to oxygen deprivation. Lack of oxygen is a factor in the development of necrotizing enterocolitis (NEC), a type of gangrene of the intestine that can be fatal in premature babies. Specifically, NEC is a leading

cause of disease and death in neonatal intensive care units, with a reported 2,500 cases occurring annually in the U.S. and a mortality rate of 26 percent. In addition, this is a particular problem in developing countries where high rates of prematurity increase the overall prevalence of NEC. An abnormal pattern of bacterial colonization in preterm infants may contribute to the pathogenesis of NEC. Hence, it is imperative to understand the adaptive responses of the preterm intestine so that specific nutritional practices can be employed in order to optimize intestinal development.

Another potential use of this methodology is to screen for potential additives to infant formula. For example, it could be determined whether the addition of a specific nutrient or bioactive component (such as a pre- or probiotic) could shift the gene expression pattern of the formula-fed infant closer to that of a breastfed infant.

The use of *non-invasive stool-based tests* will become critical tools in tailoring diet and practices that modulate epithelial (host) cells and microbiota to promote intestinal development and health of the growing infant. The advantages of this tool for intestinal disease screening are 1) it is noninvasive, only requiring a stool sample from the subject; 2) it is sensitive, able to detect alterations in host and microbiome gene expression, and differentiates between factors that promote versus protect against feed intolerance and NEC; and 3) it serves as a tool not only to identify high risk patients in order to encourage preventative actions, but also to assist in better customizing patient therapies by identifying those babies who are more likely to respond to drug and/or diet intervention.

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