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EDITORIAL

Decoding Hidden Messages: Can Fecal Host Transcriptomics Open Pathways to Understanding Environmental Enteropathy?

In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Yu et al[1] showed how human RNA could be isolated from feces and interrogated. They applied this technology to feces from 259 children in rural Malawi to show that host messenger RNA (mRNA) transcripts encoding immune and epithelial cell adhesion proteins associate with environmental enteric dysfunction (EED), defined as the percentage of lactulose excretion (%L), as a measure of barrier dysfunction, growth impairment, or both. The transcripts identified include those associated with broad immunologic responses (T-cell chemokines, immunoglobulin Fc fragments, interferon-induced proteins, neutrophil, and B-cell activators), mediators that dampen cell responses to growth hormones, and reduced expression of mucin, epidermal growth factor receptor, and mucous layer kinase. This ability to noninvasively assess intestinal mRNA provides an intriguing approach to the difficult problem of understanding the dynamic interplay occurring in the relatively inaccessible small-bowel mucosa between environmental microbes and nutrients and the human host. Such interactions profoundly affect the delicate absorptive barrier and inflammatory responses of the rapidly renewing small-bowel mucosa and can determine healthy child growth and cognitive development. Certainly many will be concerned that the %L is a controversial and imperfect assessment of environmental enteric dysfunction, because it does not include direct assessments of active absorptive function or local or systemic inflammation. Importantly, however, the investigators analyzed which associations with %L also were linked to growth impairment over the 3 months after stool collection. These included CLEC7, FCGR2, FCGR3, IFITM1, IFITM2, LYN, MNDA, and SELL.

This technically impressive achievement was predicated upon the enrichment of a relatively small pool of eukaryotic-specific transcriptomes amid the far greater pool of bacterial RNA in fecal samples. How then can human fecal transcriptomics enhance our understanding of the etiology and pathogenesis of EED? First, these findings reinforce our conceptual framework that intestinal inflammation is coupled with increased gut permeability. These data also provide upstream corroboration of protein markers of EED, such as neopterin (a macrophage or dendritic cell protein that is produced upon stimulation with interferon-γ from activated T cells) and myeloperoxidase (a marker of neutrophil activation), which correlate with α-antitrypsin as a marker of gut permeability in other investigations.[2] The work also shows how one can analyze host pathways to elucidate mechanisms of health and disease in a manner that was not previously possible. For example, the investigators showed decreases in mucin expression and mucous layer kinase transcripts that correlate with increases in immune activation. Perhaps surprisingly, relatively few changes were seen in epithelial cell growth or restitution pathways, and most of the transcripts associated with EED were not specific to the small intestine, where EED is thought to primarily localize. It is possible that this reflects degradation of mRNA in sloughed epithelial cells, particularly those from the proximal small intestine that have had to survive the harsh conditions of the distal small intestine and colon, and are more subject to degradation or functional modulations than inflammatory cells. Whether the new technique does bias toward distal small-bowel or colonic epithelial mRNA will require further study. This may be facilitated by the development of markers to provide bowel region specificity when analyzing fecal samples.

Despite potential technical limitations, the investigators were able to perform a robust analysis of gene expression. They then used this to develop a framework for understanding the pathogenesis of EED as a condition arising from decreased mucus production, allowing more intimate association between microbes and the mucosa and promoting chronic inflammation. Whether mucin down-regulation is the cause (traceable perhaps to variation in mucin-modulating microbiota such as *Akkermansia* or pathogens or both, select nutritional deficiencies, or even epigenetic methylation of CpG islands in specific regions of *MUC2* promoter that down-regulate *MUC2* expression,[3–5] or a consequence (secondary to potential tumor necrosis factor-α–induced inhibitory effects on mucin transcription along with goblet cell depletion[6]) of chronic inflammation currently is unknown. Furthermore, whether broad inflammatory activation signifies an appropriate attempt to repair a damaged mucosa, as the investigators suggested, or reflects a dysregulated mucosal response to resident and/or pathogenic microbes as may occur in conditions of immunocompromise, specific nutrient deficiency,[5] or inflammatory bowel diseases, also is unclear. Future sequential measurements of fecal host transcriptomes layered on other innovative methods such as multipathogen molecular fecal diagnostics, microbiomics, and metabonomics (which show perturbed choline and tryptophan metabolism associated with undernutrition and metabolic adaptations for catch-up growth)[7] should provide a more complete overview of the global biological system helping to resolve key questions about EED pathogenesis.

Nevertheless, this work describes an intriguing noninvasive method to interrogate the host responses to microbial and nutritional challenges. In this analysis of young children in impoverished areas, the data also were used to develop hypotheses about EED pathogenesis and potential approaches and biomarkers for assessing innovative

interventions to improve growth and development, not to mention potential long-term metabolic and cognitive consequences.

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