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UNCOVERING THE COMMUNICATIONS THAT MODULATE HOST PHYSIOLOGY IN HOST-MICROBE RELATIONSHIPS

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SUMMARY

Alterations to the microbiome have been associated with numerous aspects of health and disease. Application of microbiome studies to the improvement of human health will require identifying under which contexts the components of the microbiome have a causal influence on health and disease, identifying the smallest consortia of taxa capable of recapitulating the phenotypic variation observed by the whole community, and identifying the molecular communication signals between the consortia and the host to enable targeted interventions to improve health. Here we summarize research progress on experimental platforms and designs to move from causation, to causative organism, to molecular communications.

INTRODUCTION

Human microbiome studies suggest the microbiota can serve as a biomarker of disease progression, severity, and treatment efficacy (*Faith*, et al., 2013; *Karlsson* et al., 2013; *Scher* et al., 2013; *Gevers* et al., 2014; *Subramanian* et al., 2014; *Taur* et al., 2014). Further research and refinement will determine if microbiome-based biomarker assays are sufficiently precise, sensitive, and cost effective for clinical practice. In parallel, there is a need to determine in which diseases the microbiome influences the onset and progression of pathogenesis in a causative manner and to identify the microbial members or community structures with a causative role in disease susceptibility as well as any environmental or host factors that operate in parallel or in concert with the microbiota to modulate disease risk and severity (*de Vos* and *de Vos*, 2012; *Ahern* et al., 2014). The ultimate goal is to determine through what molecular language microbes communicate these beneficial and deleterious effects and the mechanisms by which these communications influence health and disease so that we may intervene to promote health.

One common source of host-microbe communication is the rich intestinal metabolic diversity derived from the diet, the host, and the gut microbiota. These metabolites are in constant flux from the metabolic processing and modifications by both the host and the microbiota. Given that each person has a unique collection of roughly one-hundred organisms harboured in their gut microbiota, unique dietary preferences driven by their personal tastes and cultural influences, and their own genome's unique potential to absorb and utilize metabolic input, interpersonal metabolite diversity represents a large potential influence on disease susceptibility with rich

opportunities for synergism. Advances in microbiome and gnotobiotic methods have uncovered a few examples of these synergisms in model systems (*Hansen* and *Sartor*, 2007; *Ivanov* et al., 2009; *Faith* et al., 2014; *Woting* et al., 2014; *Chassaing* et al., 2015). As we move forward, advances in metabolomics, microbiome tools, microbial culturing, and human microbiome study design will foster greater complexity in model systems and improved identification of key microbial components and metabolites influencing health.

THE INFLUENCE OF INTERPERSONAL MICROBIOME VARIABILITY ON HEALTH

Quantifying the influence of interpersonal microbiome variability on health remains a challenge. Given the interacting components of diet, host genotype, and microbiota, an essential factor in quantifying the microbiome's influence is to design experiments that control for diet and host genotype. Gnotobiotic animals colonized with the gut microbiota sampled from individual humans represent one such system (*Turnbaugh* et al., 2009). These "humanized" microbiota mice have the benefit of allowing different human microbiota to be tested for their differential influences in the context of a diverse array of gnotobiotic animal models (mostly rat and mouse) where both diet and genotype can be controlled. Sixty to seventy percent of the genus-level taxa in a human microbiota colonize a gnotobiotic animal, and both 16S rRNA amplicon sequencing and metagenomics methods indicate that mice colonized with a human microbiota exhibit significantly greater similarity to their human donor's microbiota than to mice colonized with the microbiota of the human donor's sibling or unrelated human donors (*Turnbaugh* et al., 2009; *Goodman* et al., 2011; *Ridaura* et al., 2013).

Colonization of germ-free animals with human gut microbes leads to numerous alterations in host physiology including increases in colonic lamina propria regulatory T cells, increases in adiposity, and alterations in intestinal metabolite profiles (*Atarashi* et al., 2013; *Faith* et al., 2014). Importantly, in the context of understanding human disease, mouse models can display differential phenotypic responses when colonized with the microbiota of different human donors. For example in *Ridaura* et al. (2013), gnotobiotic mice were colonized with the gut microbiota of one of four different twin pairs discordant for obesity. Two weeks later, the animals colonized with the gut microbiota from one of the four obese human donors had significantly increased total body and fat mass relative to mice colonized with their discordant lean sibling. In addition, co-housing animals harbouring an obese donor's microbiota with animals harbouring a lean donor's microbiota prevented the adiposity phenotypes. Interestingly, these observations where diet dependent and did not occur under the metabolic conditions fostered by consumption of a high fat, low fruit and vegetable diet. Differential responses to interpersonal variability in human gut microbiota composition have also been observed in the context of irritable bowel syndrome (IBS) where the transfer of faecal microbiota from IBS patients, characterized by hypersensitivity to colorectal distension, to gnotobiotic rats led to an increase in abdominal

contractions in response to colorectal distensions relative to rats colonized with non-hypersensitive healthy volunteers (*Crouzet* et al., 2013). In addition, early evidence suggest that germ-free mice after colonization with the faecal microbiota of humans with diarrhoea dominant IBS (IBS-D) phenocopy features of their microbiota donor, with animals receiving the IBS-D donor having significantly faster transit time than those receiving the microbiota of healthy controls (*Bercik* et al., 2012).

Although the use of humanized microbiota gnotobiotic models is still in its infancy, early evidence across a range of models suggest these studies will enable a broader understanding of the potential for interpersonal variation in microbiota composition to generate phenotypic variation in gnotobiotic animals receiving the donor microbiotas. It is also critical to understand the role of interpersonal variation in microbiota composition directly in humans. While safety and practical concerns prevent identical experimental paradigms in humans, faecal microbiota transplantation (FMT) whereby an individual with a particular disease has the faecal microbiota of a healthy donor infused into their intestine, provides the unique opportunity to gain a better understanding of the role of specific donor microbiota in humans. FMT has repeatedly proven to be a highly effective treatment for recurrent *Clostridium difficile* infections with a cure rate of >90%. Given this efficacy and the high rate of association of alterations in the microbiota to different diseases, there are numerous clinical trials being designed and underway for other clinical indications. The second largest current application of FMT is in clinical trials of Ulcerative Colitis (UC) where a few case reports and clinical trials have already been published (*Angelberger* et al., 2013; *Moayyedi* et al., 2015; *Rossen* et al., 2015). From these early results, it is clear that if FMT significantly improves UC, the magnitude and efficacy of the intervention is far less than in recurrent *C. difficile*. Nonetheless there are early hints that donor selection may be essential to efficacy (*Moayyedi* et al., 2015), which suggests that simply taking a "healthy" individual's microbiota as a donor to a patient with a particular disease is likely overly simplistic. If this variability in donor microbiota efficacy holds true in UC and other indications it provides the rare opportunity to understand overlapping microbiotas (the donor and the subset of the donor that engrafts in the recipient) in the context of humans with different genotypes and environmental histories and enables the quantification of interpersonal microbiota features such as the prevalence of effective donors for different indications.

IDENTIFYING MICROBIAL EFFECTOR STRAINS

Given the diversity of responses of different rodent models and phenotypes to interpersonal variation in gut microbiota composition, a subsequent challenge is to identify the microbial consortia or individual taxa within each community that are responsible for the observed differences. Gnotobiotic animals colonized with defined consortia provide a means to identify such taxa (*Faith* et al., 2010). These models systems have enabled the identification of bacterial strains and consortia that modulate regulatory T cells (*Round* and *Mazmanian*, 2010; *Geuking* et al., 2011; *Atarashi* et al., 2013; *Faith* et al., 2014), Th17 cells (*Ivanov* et al., 2009), tumorigenesis (*Arthur* et al., 2012), and

colitis (*Hansen* and *Sartor*, 2007; *Eun* et al., 2014). More recently, advances in high throughput microbial culturing systems (*Goodman* et al., 2011; *Lagier* et al., 2012) have enabled the isolation, archiving, and reuse of personalized culture collections, from individual human donors, whose membership contains the majority of genus level taxa from the original community (*Goodman* et al., 2011). Coupling such systems with high throughput combinatorial gnotobiotic screening experiments (*Ahern* et al., 2014; *Faith* et al., 2014) or targeted in vitro assays (*Romano* et al., 2015) provides the opportunity to identify the specific strains driving variation in phenotypic diversity between animals colonized with the microbiota of different humans. Such experiments and follow-up mechanistic studies will determine if variability in pathogenesis is driven by the same taxa or mechanisms in different donors, the extent to which the whole community context is necessary for full pathology manifestation, and could identify key beneficial organisms for addition in future FMT clinical trials and pathogenic organisms to target for removal.

Although animal models provide a valuable resource to understand host-microbe interactions and move towards mechanistic underpinnings, at this still early stage in our knowledge of the human microbiome, it is unclear if the transfer of phenotypic variation from humans to mice is mediated by the same strains and mechanisms in rodents as in the original human community (assuming the microbiota contributes in a causative way to a given phenotype in humans). It is clearly a possibility, even in cases where the microbiota contributes to phenotypic variation or disease pathogenesis in a casual way, that the community members responsible for these changes only do so in the context of a specific host where their molecular communications with the host are interpreted in a host-dependent manner. Therefore it is essential that, in addition to advances in gnotobiotic models, we push forwards technologies and study designs to move human microbiota studies towards more finely grained quantitation of the microbial consortia that explain disease risk. One such method to quantify the risk associated certain taxa harnesses the unique data of FMT. For indications where FMT is effective, but only in a subset of individuals, analysing the strain-specific differences in microbiota engraftment in responders and non-responders provides the possibility to identify key taxa that must be removed or added for a successful therapy. The drastic heterogeneity between individuals' microbiota creates a formidable challenge to this type or research, as the recipient microbiota will vary drastically - and likely completely at the strain-level (*Faith* et al., 2013, 2015) - from person to person. However, FMT studies that use one donor microbiota to treat several individuals provide at least a consistent donor microbiota to generate safety profiles and to begin quantifying the molecular details of faecal microbiota transplantation and how strain-specific differences in engraftment modulate FMT efficacy.

Improved design of human microbiota studies is also critical outside the realm of FMT. Current human studies often feature 16S rRNA amplicon sequencing. Although this method is effective in identifying high abundance organisms that are uniquely associated with a community or that are highly enriched in a pathogenic site (*Relman* et al., 1990, 1992), it lacks the resolution to identify unique strain variants of common species and genera. Disease associated changes in the microbiota to date have been dominated by descriptions of broad

community characteristics such as reductions in diversity and alterations in the abundance of different phyla. Although such descriptions can serve as weak biomarkers of disease, they also are sufficiently broad to encompass the microbiota of healthy individuals and lack sufficient detail to provide insight into disease aetiology or potential clinical interventions. Strain-level resolution techniques would provide the ability to identify unique strains from common species that are enriched or absent from individuals with disease. Early evidence suggests unrelated individuals do not share microbes at the strain level, thus strain level techniques would best be employed in family studies where strains are known to be shared between individuals (*Faith* et al., 2015) and perhaps in the context of a geographically isolated population where the total set of microbes in the community are perhaps more limited and freely shared between individuals. Such studies are currently limited by the precision, resolution and depth attainable by current microbiome methods, although improvements in metagenomics algorithms or experimentally-driven advances (*Beitel* et al., 2014) could enable studies such as using microbial inheritance patterns to quantify the risk associated with all of the taxa harboured in an individual's gut microbiota in the context of disease (*Faith* et al., 2015).

IDENTIFYING COMMUNICATION MECHANISMS OF MICROBIAL EFFECTOR STRAINS

To fully realize the potential of microbiome inspired health interventions, we must not only identify the organisms that modulate health but also the communication signals used to do so. Although this large challenge is virtually unexplored in the context of human health, animal studies have begun to identify the specific proteins or metabolites that mediate cross talk between the host immune system and microbes. For example, Peterson et al. characterized the influence of both strain-specific (*Peterson* et al., 2007) and species-specific IgA (*Peterson* et al., 2015) on the colonization and transcriptional expression of the abundant commensal organism *Bacteroides thetaiotaomicron*. *Yang* et al. (2014) characterized the T-cell antigen receptor repertoire of intestinal Th17 cells in animals colonized by segmented filamentous bacteria (SFB), a known potent inducer of Th17 cells in mice, showing both the localization of SFB specific T-cells and the potential of the immune system to maintain the specific Th17 response even in the context of co-colonization with a strong Th1 cell inducer (*Listeria monocytogenes*). Several groups have shown the potential for abundant microbially generated compounds like short chain fatty acids to increase regulatory T-cells (*Furusawa* et al., 2013; *Smith* et al., 2013). Importantly, metabolic output can vary between strains of the same species leading to strain-specific phenotypic outcomes such as the observation by *Fukuda* et al. (2011), that one of three tested strains of species *Bifidobacterium longum* were capable of preventing lethal infection with enterohaemorrhagic *Escherichia coli* O157:H7 in gnotobiotic mice. Similarly, *Romano* et al. (2015) found strain-specific variability in the potential of *Edwardsiella tarda* species to generate trimethylamine (a precursor of potential atherosclerotic compound trimethylamine-N-

oxide) from choline. Again these results highlight the importance of strain-specific variation in functional capacity to interact with and manipulate the host. While numerous individuals harbour *B. longum* and *E. tarda* species in their microbiota, a smaller fraction harbour specific strains with a given functional potential.

The above examples of microbe-host communication are mostly focused on targeted examples where some idea of the molecular signal is known by the nature of the interaction (T-cell or B-cell) or the abundance of the molecule and its use in prior clinical interventions (short-chain fatty acids). Nonetheless, platforms designed to identify effector strains, such as the combinatorial gnotobiotics strategies described above, can serve to narrow the search from the communications from a community of organisms with the host down to the smallest microbial consortium sufficient for phenotype modulation. In addition, we have previously shown these combinatorial gnotobiotic search strategies can be used to identify the specific microbial strains whose presence/absence best explains the observed variation in metabolite diversity (*Faith* et al., 2014). Identifying microbial effector strains in culture-dependent assays provides the potential to use *in vitro* cultures to perform gnotobiotic experiments where animals are fed a supply of crude extracts of one or more specific microbes, microbial supernatants from culture media processed by the target organisms, or some fractionated form of these proteins and metabolites to further refine the potential microbial effector signals that interact with the host to modulate health. Similar crude extract and fractionation studies could be performed in a culture independent manner in the context of animal experiments where some aspect of host health can be transmitted via the microbiota and also in the context of humanized microbiota experiments where different human microbial communities differentially modulate health in gnotobiotic animals.

CONCLUSION

Although much progress has been made in the characterization of the microbes in and on our body surfaces, we are still far from the day where we understand the molecular communication signals driving the phenotypic variation associated with interpersonal differences in microbiome composition. However, gnotobiotic animals combined with defined collections of specific microbial strains are aiding in the dissection of these communications and will be critical for understanding and designing the next generation of clinical studies to understand host-microbe communication systems in humans.

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