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Toward understanding and manipulating the microbiome to treat intestinal disease

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The human intestinal tract is inhabited by approximately 100 trillion bacteria, comprised of at least 3000 distinct species, which are collectively referred to as the gut microbiota. Studies of animals maintained without a microbiota demonstrate that this diverse microbial community plays an important role in both metabolism and immunity. Moreover, the microbiota is increasingly being appreciated as being a central “environmental factor” in numerous diseases states in which alterations in gut microbiota composition are associated with a variety of chronic inflammatory diseases including inflammatory bowel disease, metabolic syndrome and cancer. Investigations into the relationships between such altered bacterial populations indicate that manipulation of the gut microbiota may represent a novel means by which to treat these increasingly common inflammatory diseases. Herein, we review some of the key areas of progress in this endeavor over the last year.

A number of previous studies have demonstrated that changes in the composition of the microbiota correlate with development of deleterious inflammatory and metabolic phenotypes that are transferable to germ-free mice via microbiota transplant. While such observations have fostered a phenomenological appreciation of the influence of the entire microbiota, advances in analytical chemistry, especially nucleic acid sequencing technologies, have allowed interrogation of species-level contributions to the microbial ecosystem and overall host health. A striking example of this notion is the demonstration that a diet wherein all fats are derived from milk promotes dramatic expansion of *Bilophila wadsworthia*, a sulfite-reducing bacteria which owes its success to a milk fat-induced increase in biliary taurine¹. This bloom of a normally undetectable species was accompanied by an enhanced T_H1 immune response, induced colitis in mice deficient in IL-10 and conferred sensitivity to DSS colitis in WT. This is the first report of what are likely many specific diet-host-microbe interactions capable of effecting microbial dysbioses that result in interlinked metabolic and inflammatory changes. States of chronic inflammation, like colitis, have long been associated with incidence of cancer although mechanistic links remain poorly defined. While inflammation-associated oxidant production was presumed to be the major means of inducing DNA damage, the key driver of carcinogenesis, recent findings reveal such damage results not only from inflammation *per se* but is exacerbated by the presence of *E. coli* capable of expressing the enzyme polyketide synthase (PKS)². Such PKS+ *E. coli* increased in relative abundance following the initiation of inflammation and were associated with colon cancer in both humans and susceptible

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mouse models. This work underscores the multifactorial and interrelated nature of microbiota-mediated disease as inflammation promotes DNA damage both primarily and by facilitating the expansion of genotoxic bacteria.

The development of host/microbiota symbiosis is perhaps the most ancient, and most central, evolutionary process as proper management of the microbiota is essential for basic daily survival. Indeed, as the gut represents a roughly tennis court-sized interface with the outside world, immunity and metabolism are, of necessity, tightly intertwined. Loss of B cell-derived IgA results in a dysregulated microbiota that necessitates a compensatory immune response from intestinal epithelial cells³. This shift away from normal Gata4-mediated metabolic function in favor of enhanced interferon-inducible immune pathways results in undernourishment, specifically impaired lipid absorption, with gene expression resembling that seen in immunodeficient and HIV+ humans. The opposite scenario, that of malnutrition resulting in dysregulation of the microbiota, has also been mechanistically established. Mice lacking angiotensin 1 converting enzyme 2 (ACE2) display impaired absorption of neutral amino acids, including tryptophan, resulting in colitis and diarrhea that mimics the human disease pellagra⁴. Such malabsorption also resulted in microbial dysregulation *via* reduced antimicrobial peptide secretion in the small intestine, an effect mediated by mTOR and that could be rescued with supplementation of tryptophan or the metabolic end product nicotinamide. This work suggests that a variety of metabolic deficiencies feature, at least as a component, dysregulation of the microbiota and may thereby be ameliorated through careful manipulation of resident bacteria and/or immunity.

The treatment of overnutrition, however, is of greater interest to the developed world, which faces an epidemic of metabolic syndrome related to increased consumption and reduced physical activity. Metabolic syndrome is promoted by loss of inflammasome signaling as deletion of NLRPs 3 or 6 results in an altered microbiota capable of exacerbating the development of metabolic syndrome via increases in ligands for TLRs 4 and 9⁵. Specifically, increased levels of such ligands in portal circulation promote TNF α -mediated development of non-alcoholic fatty liver disease (NAFLD). Perhaps the most provocative aspect of this study is the observation that simply co-housing these immunocompromised mice with WT conferred the observed predispositions to NAFLD and obesity with the implication that an altered pathogenic microbiota may be somewhat analogous to an infectious disease. While respecting the important caveat that this study was performed over a relatively short time with coprophagic rodents, a similar process may be occurring in humans, especially in family groups that cohabitate for extended periods. Study of mice with an innate immune deficiency in the flagellin receptor toll-like receptor 5 (TLR5) indicates that, perhaps, the state of microbial flux itself promotes inflammation and dysmetabolism in general rather than the presence or absence of particular bacterial species or genes⁶. Some TLR5-deficient mice develop colitis while others develop low-grade inflammation and metabolic syndrome without colitis and, while overall penetrance is stable from generation to generation, the development of colitis occurs randomly. Weekly sampling of fecal bacteria revealed that, unlike WT mice, TLR5-deficient mice exhibited increased week to week changes in microbial populations and that such volatility was observed whether or not mice eventually developed colitis. This phenomenon, wherein the host must engender a

compensatory response in order to attempt to control the composition of the microbiota, may be reflective of human IBDs, which is promoted by disparate innate immune deficiencies but in which at least half of overall disease risk is dictated by non-genetic (i.e. environmental) factors. Further, in these mice, transient colonization by normally undetectable species, in this case a human Crohn's disease-associated *E. coli* strain, promotes chronic inflammation and colitis that persists well after clearance, suggesting that uncontrolled volatility may promote inflammation simply by allowing deleterious species the opportunity to interact with the host. Studies in humans, though surely enlightening, would require long term repeat sampling of patients beginning well before the development of IBD.

Given that the prevalence of IBDs and the obesity epidemic have occurred very recently in evolutionary terms, the contribution of host genetics to such population-level microbial change is most likely relatively minor. Rather, societal and technological progress has reoriented the host/microbiota axis, as evidenced by increases in obesity and IBD that correlate with rising income in the developing world. One possible culprit is the widespread use of antibiotics, both by humans and by large scale farming operations, which have routinely used low doses of antibiotics to stimulate weight gain for over 50 years. Young mice exposed to single or combination antibiotic therapy exhibiting lasting increases in adiposity and accelerated bone mineral deposition and that, while total bacterial numbers remained similar to untreated mice, the composition of the microbiota was altered substantially⁷. Notably, this study found expansion of the phylum Firmicutes and contraction of Bacteroidetes, a shift that generally accompanies weight gain in a variety of models. Further, bacterial expression of genes related to carbohydrate metabolism was altered, resulting in increased energy extraction and hepatic lipogenesis.

Interestingly, changes in the microbiota that have been shown to promote adiposity may have a normal physiological role, to increase energy extracted from food throughout pregnancy and lactation⁸. Indeed, by delivery, pregnant women develop many of the hallmarks of metabolic syndrome: adiposity, insulin resistance, increased bacterial load with reduced diversity, and an altered ratio of Firmicutes/Bacteroidetes. Further, the microbiota of a third trimester human induced a similar metabolic phenotype when transferred to germ-free mice. These data suggest that metabolic syndrome mimics or even coopts evolved mechanisms designed to increase energy extraction during the most metabolically demanding phases of reproduction.

Despite our relatively rudimentary understanding of the specific mechanisms behind the development of metabolic syndrome, treatment avenues do exist. A well-controlled clinical trial designed to manipulate the microbiota in order to treat a central hallmark of metabolic syndrome, insulin resistance, achieved a significant degree of success⁹. Small intestinal infusion of gut bacteria from lean donors increased sensitivity to insulin within 6 weeks without changes in diet, hormone profile or total number of bacteria. In contrast, control subjects given their own microbiota showed no improvement. Consistent with experimental work, an increase in microbial diversity, with a notable expansion of butyrate-producing bacteria, was observed. Thus, while still in very early stages, the development of techniques that replace or augment the host's microbiota hold significant promise for the treatment of

metabolic disease and could possibly be extended to treat any number of gut-linked disorders including IBDs, atherosclerosis and nutritional deficiencies. Indeed, increasingly fine techniques that allow interrogation and manipulation of the microbiota may presage next-generation treatment regimes that are both personalized and capable of acting through existing, natural pathways.

In conclusion, research performed over the last year has begun to reveal some of the specific molecular mechanisms by which both diet and host immunity determine microbiota composition. Moreover, researchers have made significant headway in interrogating how specific microbiota species and families contribute to a broad array of intestinal disease state. A central theme emerging from these studies is that host defense and metabolisms are highly intertwined with innate immunity and inflammation serving as a central interface of these central life-sustaining biological processes. This last year has also seen substantial progress in the identification and examination of societal factors, like increased antibiotic use, that have eroded the established distinction between commensal and pathogen and rather established a nuanced relationship between host and symbiont as well as proof that interventional therapy allows an avenue by which to reassert host control over the microbiota. Given the increasing appreciation of the relevance of gut bacteria to host health, methods of establishing and maintaining such control should continue to occupy researchers and hold great potential for advances in treatment of a variety of heretofore intractable chronic intestinal diseases.

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