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Tipping the Balance in Microbiome Standoffs

The Human Microbiome May Dispose Us toward Health or Illness On The Basis Of Tiny Shifts in Microbial Subpopulations

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The human body is immersed in the microbial world; our microbiota takes care of us. Yet the microbiota is complex, and much remains unknown about its composition and function.

Moreover, microbiota are subject to subtle shifts. These are associated with a multitude of human diseases, raising the possibility that favoring or disfavoring specific microbiota members may preserve or restore health.

In 2008, the human microbiome became the subject of two major initiatives—the Human Microbiome Project (launched by the National Institutes of Health) and the Metagenomics Project of the Human Intestinal Tract (launched by the European Commission). Although these initiatives have facilitated research, microbiomics is still an emerging field.

Whether the reported dysbioses for metabolic or other pathological states associated with a dysfunctional gastrointestinal (GI) microbiome are cause or an outcome is not clear. “The GI microbiome is an ecosystem,” points out Mark Heiman, Ph.D., vice president of research and CSO, MicroBiome Therapeutics. “Biodiversity is a critical aspect of its function.”

Due to redundancy, additions or losses of species with similar roles tend to have only small effects on microbiome function; yet domination by a few species may influence function significantly.

Shifting Microbiomes with Prebiotics

Diet impacts the species that make up the GI microbiome. The more diverse the diet, the more diverse the microbiome—and the better it can adapt to perturbations. Each microbiotic species transforms energy, which arrives in the form of undigested and partially digested food, and is processed into new forms, molecules that may serve as signals for the body’s regulatory systems.

Patients with some disorders, such as metabolic syndrome and prediabetes, have a GI microbiome that contains significantly less species diversity than is reported in healthy individuals. Disease states that reflect low-diversity and hence vulnerable microbiomes have attracted the notice of MicroBiome Therapeutics, a company that

intends to expand microbiome diversity by using key ingredients derived from certain foods, termed GI microbiome modulators (GIMMs).

GIMMs can be used together with approved drugs to enhance efficacy, reduce side-effects, improve safety, or address related comorbidities. They are designed to help correct dysbiosis by supporting the beneficial microflora and GI environment needed to provide important missing functions and resolve dysfunction in the absence of other dietary changes.

According to Dr. Heiman, a clinical trial demonstrated that positive changes induced by GIMMs could last at least two weeks and potentially longer; however, this finding needs to be confirmed in subsequent studies. NM504, a GIMM for prediabetes, was tested in combination with metformin in a pilot trial that showed improved glucose regulation. It also reduced metformin-induced GI side-effects in type 2 diabetic patients who were known to have metformin intolerance.

Using "Good" Bugs as Drugs

Ecobiotic drugs, a new therapeutic modality based on communities of beneficial microbes, help restore the complex underlying ecology and function of a healthy human microbiota. In many disease states, overabundance of gram-negative bacteria called gammaproteobacteria, such as *Escherichia coli*, indicate a dysbiosis.

"Ecobiotics are small ecologies of bacteria that can change the microbiome or provide a missing function," explains David Cook, Ph.D., CSO, Seres Health. "We believe the only way that you can impact and change the state of the microbiome ecology is by also using ecologies of multiple other organisms."

SER-109, a spore ecology for the prevention of recurrent *Clostridium difficile* infection, received FDA breakthrough drug designation on the strength of its Phase IB/II clinical results. A randomized placebo-controlled double-blinded Phase II trial started in May 2015.

One to three days after their course of antibiotics is complete, patients take a single four-capsule SER-109 dose, which contains about 108 purified spores derived from healthy stool donation. These spores germinate and regrow in the GI tract, where they compete with *C. difficile* and restore diversity.

A second platform is based on a synthetic ecobiotic, made entirely in vitro, which has a reduced complexity. In this approach, which involves computational methods, synthetic ecologies are designed, tested, and optimized using organisms obtained from the company's proprietary strain library, then a cell bank is established. SER-262, focused on treating the microbiome to prevent recurrence following a primary *C. difficile* episode, starts clinical trials in mid-2016.

Since no one has manufactured drugs from organisms that are commensal anaerobes for human use, the company faces unique challenges. To meet them, the company has developed novel assays for potency, quantitation, viability, stability, purity, and characterization.

"We developed a lot of technology to grow the organisms, and to get them to sporulate in vitro in an FDA-compliant setting," adds Dr. Cook. "If you want to demonstrate there are no contaminants, even at a low level, from the environment in a composition of 50 spores, one-of-a-kind assays are needed to say with regulatory certainty that the requirement is met."

Engineering Bacteriocins for Picking Off "Bad" Bacteria

The GI microbiome complexity is apparent by the number and heterogeneity of bacterial species and strains. Specific pathogenic bacteria, such as *C. difficile*, the number-one nosocomial infection, cause certain GI diseases whereas pathobionts, asymptomatic bacteria in the gut, cause disorders in other places. Metabolic diseases are more complex and may be caused by multiple organisms.

A means of grappling with this complexity has been developed by AvidBiotics, a company that has assembled a portfolio of therapeutic proteins designed to selectively destroy pathogenic bacteria and other target cells. The proteins are based on a complex protein structure that has long existed in nature, a high-molecular-weight bacteriocin.

Bacteriocins are pure proteins and thus cannot replicate, and are used by bacteria to inhibit the growth of other bacteria. AvidBiotics genetically engineers bacteriocins to target them to almost any bacterium. The versatile platforms can be directed at gram-negative or gram-positive bacteria, and are currently designed to go after pathogens and pathobionts.

"There are two ways to determine causality of a suspected bacterial species for a disease or disorder," advises David Martin, M.D., CEO, AvidBiotics. "Add the suspected bacterium that may cause the phenotype, or subtract the suspected bacterium and see if the phenotype alters. You need a sniper that takes out one suspected target; not a grenade."

Avidocins and purocins are engineered R-type bacteriocins, targetable and tailorable bactericidal proteins that have been shown to remove a targeted bacterial strain from the GI tract in animal models without collateral damage to healthy and critical other microbiota members. To validate the model, the company is focusing on known pathogenic enteric bacteria.

Molecules that are responsible for virulence or fitness and that occur on the surface of the bacterium are targeted. The bacterium becomes resistant by losing the surface target, and then is no longer virulent or fit. Avidocin-CD, an engineered bacteriocin that kills the hypervirulent or epidemic strains of *C. difficile*, targets the major S-layer protein on the surface. Resistant organisms lose expression of that protein, and have been shown to be avirulent in animal models.

These bacteriocin snipers avoid unintended collateral damage, but different shots using different targeting mechanisms are needed to kill 80–90% of all *C. difficile* offenders.

Targeting Bacterial Enzymes

The launch of irinotecan, a DNA topoisomerase inhibitor, revolutionized the treatment of colon cancer. Although irinotecan represented a major advancement, patients either died or had to reduce therapy due to dose-limiting side-effects caused by symbiotic bacterial β-glucuronidases that reactivate irinotecan in the gut.

A crystal-structure-based approach to understanding and recognizing key differences between bacterial and mammalian enzymes' active sites has been developed by Symberix. Using this approach, the company has identified inhibitors that are 100- to more than 1,000-fold more effective against bacterial β-glucuronidase than human β-glucuronidase, which is an essential enzyme in humans.

Bacterial β-glucuronidase is involved in metabolic processing of a number of drugs besides irinotecan, such as nonsteroidal anti-inflammatory drugs, that also frequently cause ulcer, inflammation, and bleeding in the small intestine.

"It was serendipitous that the active site in the bacterial enzyme is different from the human enzyme," notes Ward Peterson, Ph.D., CEO, Symberix. "Because the active sites are different, we are able to inhibit the bacterial enzyme and not the human enzyme. I suspect that there are other similar examples of microbiota nonessential enzymes involved in human pathophysiology that can be selectively targeted with drugs that do not have antibiotic activity."

Inhibitors are intended to improve the outcomes of existing medications, the benefits of which are limited by their GI toxicities. Symberix' products will be developed as therapeutic adjuncts to improve patient compliance because of the prophylactic benefit.

"Our job is to turn this first druggable bacterial target into a new class of medicines—drugs that correct harmful bacterial activity without having antibiotic activity," informs Dr. Peterson. "We treat the gut microbiome as an important organ, and our inhibitors selectively target a portion of this organ without killing its vast population of beneficial bacteria."

"Unlike other innovative microbiome therapies in development, our therapies are well positioned for regulatory approval," asserts Dr. Peterson. "We believe that the existing FDA regulatory pathway is suitable for gaining approval for this new class of medicines."

Preventing "Ugly" Skin Problems

Acne, rosacea, eczema, and other dermatological conditions seem to involve unrestrained inflammatory responses to underlying imbalances in the epidermal microbiota. Some imbalances relate to ammonia-oxidizing bacteria (AOB), which were ubiquitous, ancestral, keystone skin commensals. Sensitivity to soaps, surfactants, and preservatives have depleted them; they are no longer found on human skin.

These AOB are being evaluated by AOBiome. The company's strain D23 came from the soil and was passaged on human skin. The stable bacterium quickly returns from its stasis storage form once given ammonia. A randomized placebo-controlled double-blinded study showed that D23 could repopulate the skin of the face and head and produce subjective cosmetic improvements if not eradicated by hygiene products.

“Instead of moving straight to drug development, we decided to release D23 as a cosmetic on a very limited basis just to see if there were any blind spots,” says Larry Weiss, M.D., CMO, AOBiome. “In July of 2015, we launched them under the Mother Dirt brand.”

In another study, one looking for appearance endpoints in people with acne-prone skin, there was significant improvement in some of the major acne endpoints, such as a reduction in sebum. AOB react with the unsaturated lipids found in sebum and form potent anti-inflammatory nitrolipids. If AOB are not present, these same unsaturated lipids will react with oxygen to become proinflammatory peroxide lipids.

Systemic inflammatory disorder may have cutaneous manifestations. For example, evidence indicates that half of the people with rosacea may have SIBO, a form of inflammatory bowel disease. Skin and systemic health should be redefined to include the health of their respective microbiota.

Mice or Men?

When asked what is the relevance of mouse microbiome research to humans, Jennifer Phelan, scientific product manager, germ-free models, Taconic Biosciences, is happy to respond.

“By associating a human microbiome into the gut of germ-free mice, we gain insight into how the human microbiome acts within a fully living organism,” she says. “The human gut flora is a highly complex interaction of bacteria. Placing a human microbiome into a mouse, while not a perfect replication of its natural environment, allows for complex interactions to occur in the mouse gut.”

Translational mouse models can be used in research projects to understand how the human microbiome responds to study-induced stimuli, such as changes in diet, the addition of drugs, or induction of a disease state, according to Phelan. Numerous parameters can be measured, including shifts in specific bacterial populations or changes in metabolites.

“By generating these mouse models, suppliers like Taconic Biosciences enable accelerated understanding of how the microbiome affects and is affected by disease in humans,” she continues. “Without these translational models, researchers’ ability to understand these dynamics and try new therapeutic strategies in response would be greatly diminished.”