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Executive Proceedings

September 22-23, 2014 | Joseph B. Martin Conference Center
Harvard Medical School | Boston, Massachusetts, USA

PROBIOTICS Throughout the *Lifespan*

GUT MICROBIOTA, PROBIOTICS AND THEIR IMPACT THROUGHOUT THE LIFESPAN

2014

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HARVARD MEDICAL SCHOOL
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PROBIOTICS Throughout the Life

GUT MICROBIOTA, PROBIOTICS AND THEIR IMPACT THROUGHOUT THE LIFESPAN

A note from

W. Allan Walker, MD

Dear Colleague,

Research on the microbiota, and specifically the gut microbiota, is advancing rapidly. This new frontier of science is unveiling an entirely new understanding of how individual systems in the body can be affected by the gut microbiota. In addition, we are finding that the world of microbes plays a critical role across systems throughout the lifespan. Better understanding the Microbiome provides an essential context for knowing how to incorporate probiotics into our diets to promote health and prevent disease.

We hosted a major symposium in Boston at the Harvard Medical School to look at "Gut Microbiota, Probiotics and Their Impact Throughout the Lifespan". Leading researchers from around the world were convened to discuss current and emerging science in four areas of the age spectrum:

I. Pregnancy;
II. Neonatal Period;
III. Adult Period; and
IV. Older Adults.

This Executive Proceedings provides a brief view of the topics covered in each of these areas. However, I would also encourage you to view the Webcast of the event on **http://nutrition.med.harvard.edu**.

There you will see all the details of each scientific presentation. In addition, you will be able to hear the stimulating discussions among the experts at the end of each session. Each presentation can be viewed separately for you convenience.

I would like to thank the Program Committee and the Program Moderators for their insights and significant contributions in making this inaugural symposium on gut microbiota and probiotics throughout the lifespan a success. We hope you find this information encouraging as well as useful.

My best regards,

W. Allan Walker, MD
Conrad Taff Professor of Nutrition
Professor of Pediatrics
Director, Division of Nutrition
Harvard Medical School
Mucosal Immunology and Biology Research Center
Massachusetts General Hospital

span 14

Manipulating the Microbiota: Beyond Traditional Probiotics and Fecal Transplant

Opening Keynote

R. Balfour Sartor, MD
Distinguished Professor of Medicine, Microbiology and Immunology
Director, UNC Multidisciplinary Center for IBD Research and Treatment
University of North Carolina

For centuries, bacteria were dubbed as enemies of the human body. They were thought to only cause disease and infections, and a sterile, bacteria-free world was the safest. Today, scientists know that the human body is teeming with bacteria, many of which are vital for survival, health, and keeping the less desirable microbes at bay. In every person, there is a unique balance between beneficial and detrimental bacteria. As researchers move toward understanding this balance better, they're entering a new frontier: altering the microbiome.

Being able to therapeutically manipulate the microbiome in a targeted way will allow clinicians to restore the balance between beneficial and detrimental bacteria without wiping out all microbes. The options for how to do this are still growing, but fall into a few main categories: selective antibiotics which can kill only certain bacteria, probiotics that introduce microbes into the gut, prebiotics or dietary changes that encourage particular microbes to flourish over others, and microbial transplantations.

Each of these therapies is already known to be incredibly beneficial in certain situations. Take pouchitis, an inflammation that can develop after a surgery that creates a small pouch in the gut to help treat ulcerative colitis. Antibiotics are very effective at treating flare-ups of pouchitis. But recent studies have also shown that, in cases of recurrent pouchitis, doses of probiotics can prevent future recurrences. While every patient in a control group relapsed after 4 months, more than 85 percent were still inflammation-free after 8 months when they took probiotics.

At this point, scientists know a lot about how some probiotics work. In addition to simply populating the gut with a particular microbe or microbial mix, many can activate immune signaling the gut, or change the permeability of the gut lining. *Clostridium IV* and *XIVa*, for instance, stimulate regulatory T cells, *Bacteroides fragilis* produces polysaccharide A, a molecule that communicates with the immune system, and *Faecalibacterium prausnitzii* inhibit TNF cytokines, involved in inflammation.

Aside from these properties of known microorganisms, scientists are also realizing a new direction they can take for probiotics: the development of genetically modified bacteria. What a bacteria does, they know, is actually more important than what it is. *Lactococcus* or *E. ecoli* engineered to produce cytokines or inhibitors may allow the fine-tuning of probiotics. And the growing use of so-called "-omics" technologies are allowing a greater look at exactly what functions of microbes are the most beneficial. By looking at what genes are present in the microbiome, what genes are active, what proteins are produced, and what metabolic products are produced, scientists can home in on the ideal functional balance of the microbiome. Then, they can move toward engineering bacteria, prebiotics, or understanding what dietary or environmental influences angle microbiota toward this functional role.

Fecal transplant, a procedure in which fecal matter is transferred from a donor to a patient to repopulate their colon, has already begun to prove its utility in treating diseases including ulcerative colitis, C. difficile infection, and Crohn's disease. But questions remain about its staying-power and the optimal protocols for a transplant. And like probiotics, the ultimate goal with fecal transplants is to be able to create individualized, reprpducibe therapies. For fecal transplant, this might mean moving away from donors, who provide a random mix of microbes. Instead, researchers hope they can learn what components of this mixture are most important for which patients, and create tailored microbial communities in the lab that can then be transplanted.

The future of microbiome-based therapies for disease is based on this development of personalized approaches; since no two people have an identical microbiome to begin with. When such unique therapies arise, we'll be able to move toward curing inflammatory diseases and maintaining long-term remission by restoring the balance of microbes in the body.

Pregnancy

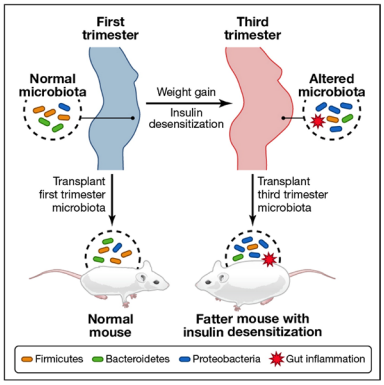
Keynote: "The Microbiota During Pregnancy"

Omry Koren, PhD
Assistant Professor
Faculty of Medicine
Bar Ilan University, Israel

During the nine months of pregnancy, a woman's body undergoes dramatic changes that effect every organ in her body and alter numerous physiological processes, from digestion to immunity. So it's no surprise that the microbiota—not only in a woman's gut, but in her mouth and vagina, and even inside the womb—also change during this time. Over just the past four years, research has begun to illuminate the shifts in the microbiome during pregnancy and reveal exciting links between microbiota and the health of both a woman and her unborn child. As scientists piece together more of this story, it's becoming clearer that probiotics might be able to influence the course of a pregnancy, prevent complications, and give a baby the best odds of a healthy start to life.

When researchers first began looking at the communities of bacteria in the guts of pregnant women, a few things stood out: for one, while the gut microbiome of most women was similar during the first trimester, it varied wildly between individuals by the third trimester. And while the first trimester gut makeup resembled that of healthy adult women, the third trimester microbiome looked more like that seen in people with inflammatory bowel disease or metabolic syndrome. More proteobacteria and actiobacteria inhabit these guts, opportunistic pathogens thrive, and levels of inflammatory cytokines rise.

Phenotype Effect: Microbiota Transfer



Why would the microbiome of a pregnant woman resemble that of people with these diseases? Are the altered microbial communities a cause or effect of other pregnancy symptoms? Omry Koren and other scientists are still trying to figure it out, but studies in mice have started giving them some hints. When the researchers transferred stool samples from women in their third trimester to mice, the mice gained weight, became less sensitive to insulin, and had greater inflammation throughout their bodies. Could the right probiotics help dampen these changes? There are no definitely experiments yet showing that's the case, but Koren suspects it's true. Studies out of other labs have already suggested that some probiotics can treat pregnancy-associated constipation and decrease a woman's risk of developing gestational diabetes.

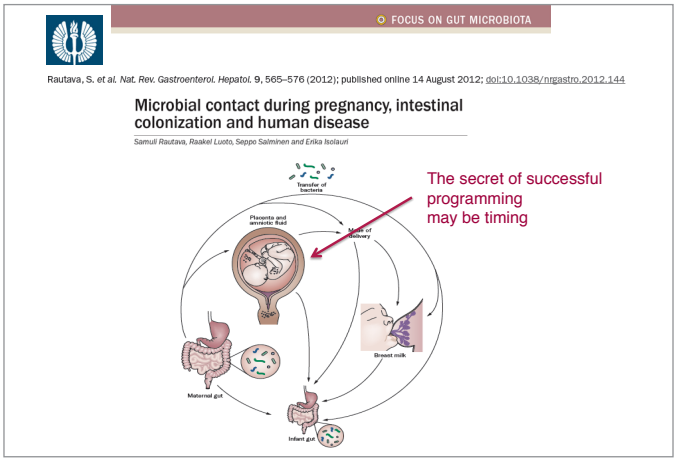
When it comes to pregnancy, though, it's not just a woman's own health that is influenced by microbiota. Since 1900, researchers have been living by the dogma that babies are living microbe-free in a totally sterile womb until they're colonized at birth. But now, Koren and others are beginning to challenge this idea. Researchers have recently discovered that the placenta—bridging a woman's bloodstream with that of her baby's—is full of microbes. Whether these communities of bacteria could relate to a woman's risk of conditions like pre-eclampsia it yet to be seen.

As scientists like Koren continue to probe questions like this, they'll move closer to understanding how clinicians might use probiotics to maintain a healthy pregnancy, prevent pre-term labor, and perhaps even cure common pregnancy problems like morning sickness.

Probiotic Use During Pregnancy for Protection Against Childhood Diseases

Around the world, the prevalence of childhood obesity, metabolic disease, and allergies are all on fast slopes up. The increases have been attributable to complex changes in young children's diet and exercise, exposure to environmental toxins, and social pressures. But one thing could put the numbers on a downswing: giving probiotics to pregnant women and young children.

Pregnancy is among the most critical stage for shaping a child's gut and immune system. And the health, diet, and probiotic use of a mother during this time—as well as when she's breastfeeding—is key to her baby's future for decades to come. It offers a window of opportunity for therapeutics to alter the microbiome.



When a mother has allergies, pediatrician Erika Isolauri has discovered, she can reduce the odds of passing them along to her child by controlling weight gain during pregnancy, breast-feeding exclusively for at least two and a half months, and taking a probiotic. The link between these

Erika Isolauri, MD, D. Med. Sc
Professor of Pediatrics,
University of Turku, Finland
Chief Physician, Department of Paediatrics, Turku University Hospital, Finland

behaviors and allergies: the microbiome. Infants who develop allergies have less diverse communities of microbiota in their guts, and a mother's health during pregnancy and breastfeeding directly shapes these communities.

Similarly, whether or not a child becomes overweight or obese at a young age is influenced—among other factors—by the microbes that their mother passes along them, as well as the microbes that colonize their bodies over the first two years of life. Babies born to mothers who gained excess weight during pregnancy have different types of bacteria inhabiting their intestinal tracts—more bacteroides and staphylococcus and less bifidobacterium, for example—compared to babies born to lower-weight women. And babies born to women with diabetes have a similar microbiome composition to adults with diabetes, even though they themselves don't have the disease at birth.

With all this in mind, Isolauri has launched a series of clinical trials studying how allergies, metabolic disease, and obesity in childhood can be thwarted by shaping a mother's microbiome before or immediately after she gives birth. The secret of programming a child's microbiome, Isolauri has shown, is in the timing: probiotics given during pregnancy and early in life can reduce birth size, lower a mother's glucose levels, reduce allergy risk, and decrease the odds of early childhood obesity. Studies in mice have echoed what Isolauri has shown in human trials: antibiotics or probiotics during pregnancy or shortly after birth alter total fat mass in mice.

Diet and Microbiotic Exposure During Pregnancy and Immune Protection Against Allergic Manifestations

Erika von Mutius, MD, MSc
Professor of Pediatrics
Head of Asthmas and Allergy Department
Dr. von Hauner Children's Hospital, Germany

In rural villages across Germany, there's a group of children who seem to be protected from the normal rates of asthma, allergies, and itchy skin. For more than two decades, pediatrician Erika von Mutius has been studying these children, uncovering what makes them different. The top three factors, she's found: their exposure to farm animals, straw hay, and raw milk.

The research began when von Mutius and her colleagues discovered that 6 through 12 year olds who had grown up on farms had less asthma and hay fever than their classmates. They began delving into the children's lives, launching a prospective study that recruited 1,100 women during pregnancy and then followed their children after birth, tracking behaviors and testing for allergies.

The differences, studies have revealed, began during pregnancy. When women spent time around farm animals while pregnant, their babies were born with more regulatory T cells—a type of immune cell—in their cord blood. A few years later, these same babies had experienced fewer incidents of pruritus, itchy, allergic skin rashes. But the behavior of children after birth also made a difference, the researchers showed. Babies that began consuming raw, unboiled farm milk before age one were less likely to develop allergies, as tested by skin prick tests.

Von Mutius explored what parts of the raw milk were associated with this drop in allergy rates, and homed in on the whey, the liquid fraction of milk that remains after its been strained or curdled. The proteins in the whey are destroyed by the process of pasteurizing milk at high temperatures, explaining why unboiled milk, but not other milk, causes the association. And other dietary habits, von Mutius's group has shown, also help decrease the risk of asthma and allergies. When children are introduced to more diverse foods during their first year of life, they have less allergies—to both foods and other substances—later in life.

The team has yet to conclusively show the mechanism by which farm life, raw milk, and diversity of foods helps prevent allergies, but they hypothesize that the lifestyle leads to broader exposures to bacteria and fungi during childhood. There's likely a cocktail of exposures which shape a child's microbiome. Researchers are now beginning studies on the farm-raised cohort to back this idea up, by sequencing microbiome samples collected from the children. The lessons they learn could one day lead to a probiotic that captures those microbial exposures for children who don't eat, breathe, and sleep the farm lifestyle.

Neonatal Period

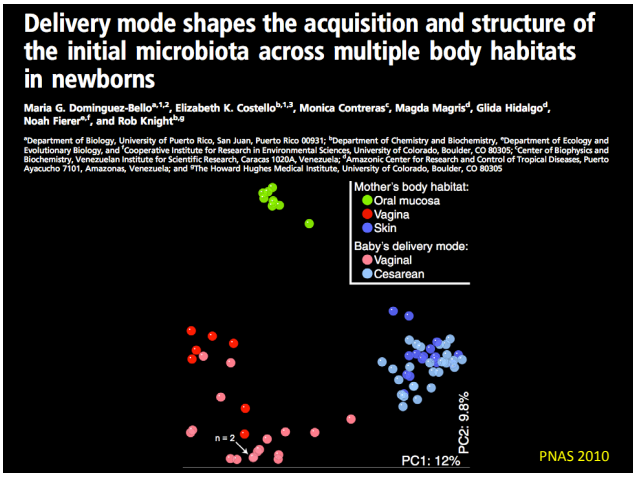
Keynote: "Development of Human Infant Intestinal Microbiota"

David A. Relman, MD

Thomas C. and Joan M. Merigan Professor
Departments of Medicine and of Microbiology and Immunology
Stanford University

From the moment a baby is born, their microbiome begins to be shaped not only by their mother's microbiome—as it was throughout pregnancy—but by the rest of the world around them. Whether they eat breastmilk or formula, whether they have to stay in intensive care after birth, and whether they take a course of antibiotics in those early days all affect which microbes take up residency in their bodies.

When immunologist David Relman first analyzed the microbiome of newborns, he found that the communities of microbes in their fecal samples sometimes resembled their mother's gut microbes, sometimes most closely mimicked the vaginal microbiota, and other times was similar to the microbial makeup of breastmilk. But whatever the composition, the community structure underwent a rapid turnover in terms of both types and numbers of organisms over the first weeks of life. In all the newborns, the number and density of microbes, as well as diversity of species, increased at a steady and predictable pace. The findings suggest that there's something intrinstic about the infant gut that makes it an open ecological system—allowing organisms to come and go and stay at this defined rate.



The question now facing researchers like Relman is what impacts this early rate of change, and how to alter it. Over the past few years, microbiologists trying to tackle this question have turned to methods developed by physicists and meteorologists—who study how other types of disturbances affect the stability of systems. The new approaches view the stability of the microbiome as a landscape with hills and valleys—the deeper a community is within a valley, the harder it is to shift to another state. But outside influences—from a change in diet to a course of drugs—can lower these hills, encouraging transitions between states.

With this type of model in mind, Relman and others have begun detailed analyses of children's microbiomes over time, observing how and when they shift between states. The infant gut, Relman has discovered, takes up to 21 days to completely mature from an unstable environment to a more predictable and adult-like community. Within this timeframe, there are distinct and obvious shifts at the genus and species level every few days. But even between those large fluctuations in population makeup, looking more closely reveals less obvious changes: shifts at the level of microbial strains, for instance, even when genus and species compositions appear to stay steady.

As a whole, the data so far on infants suggests a window of opportunity—or a window of susceptibility—in which a newborn's gut microbiome is particularly open to change. This offers the real possibility that probiotics during this time frame may help protect against adverse disturbances, or promote healthy microbiome development—particularly in premature or hospitalized babies prone to problems.

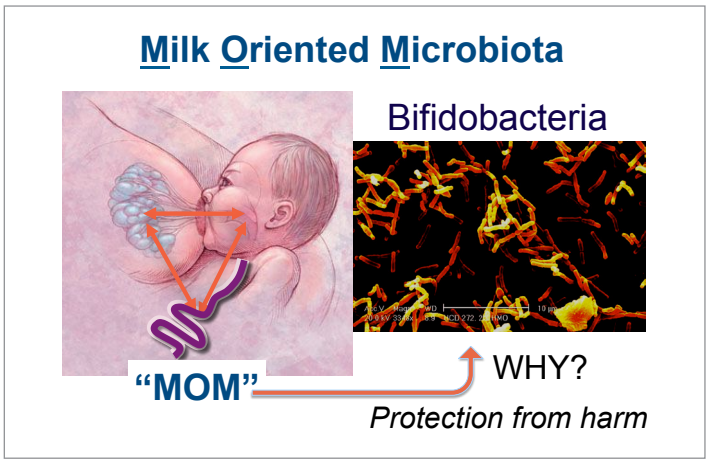
Establishment of a Milk-Oriented Microbiota in Infants: New Insight into Probiotics and Prebiotics

David A. Mills, PhD
Peter J. Shields Endowed Chair in Dairy Food Science
Department of Viticulture and Enology
Department of Food Science and Technology
University of California, Davis

Of all the things humans consume around the world, there's only one that's evolved for 200 million years specifically to provide optimal nutrition: human breastmilk. Each compound in milk has been selected for as key to infant nutrition, and any extra components have been selected against, culled out so a mother doesn't lose unnecessary energy. It's because of this storied past that scientists have turned to breastmilk to learn how to shape the newborn microbiome.

Breastmilk consists of water, lactose, lipids, proteins, and oligosaccharides, and delivers not only nutrients but also immune protection—novel antimicrobial peptides are still being discovered in milk. And among the ingredients that have microbiologists most excited are glycans—carbohydrate moieties that can stand on their own or be parts of larger glycolipids or glycoproteins. These molecules interact with microbes in the gut in a huge variety of ways, from providing symbionts with a food source to binding pathogens to flush them out of the digestive tract.

Glycans are also what sets breastmilk apart from baby formulas and have been cited as the reason breastfed infants have guts more enriched in *bifidobacterium*. While formula contains a handful of glycans, it doesn't deliver the huge diversity of glycans that milk does, and *bifidobacterium* are known to thrive on the mixture of carbohydrate molecules in a mother's milk. It's these roles of glycans that has researchers probing them as model prebiotics—molecules that aren't themselves microbes, like probiotics, but help shape microbial communities in their own right.



Food scientist David Mills has studied this concept by focusing on the natural variation in one particular glycan that, even among breastfed infants, exists. Because of natural genetic differences, some 20 percent of mothers have a non-functional fucosyltrasferase 2 gene, and don't fucosylate glycans in a particular way. Mills has studied how the lack of 2'-fucosylated glycans in the breastmilk of these women's children changes their microbiomes and has discovered striking differences: the infants lack some species of *bifidobacteria*, especially in the first days of life, and have more E. coli and Streptococcus bacteria than other babies. Whether there's a health effect of these differences isn't clear, but it backs up the general idea of prebiotics: they can shape the composition of the microbiome.

But the knowledge gained by studying the microbial effects of breastmilk also sheds light on probiotics. Milk research, for instance, has underscored the importance of *bifidobacterium*, so clinical trials have begun testing the effect of giving premature infants doses of *bifidobacterium infantis*—a strain known to thrive on breastmilk. When the infants are given the probiotic in combination with breastfeeding, the bacteria colonize and persist—a phenomenon rarely seen in adult probiotic studies.

Probiotic Studies in NEC

W. Allan Walker, MD
Conrad Taff Professor of Nutrition
Professor of Pediatrics
Director, Division of Nutrition
Harvard Medical School
Mucosal Immunology and Biology Research Center
Massachusetts General Hospital

Among babies born prematurely weighing less than 1500 grams, almost ten percent go on to develop necrotizing enterocolitis (NEC), a serious intestinal disease that causes abdominal distention, vomiting, diarrhea, and can lead to the necrosis (death) of sections of the bowel and long-term disease. Research on NEC has found that it's driven by a combination of immature intestinal cells, undeveloped immune responses, and an imbalance in gut microbes. The timing and symptoms of the condition suggest that probiotics, given during an optimal window after birth, could help prevent NEC by encouraging the maturation of both the microbiome and the intestines.

When researchers, including pediatrician Allan Walker, have characterized the cells of the gut epithelium in full-term compared to pre-term infants, they've seen major differences. The pre-mature intestines, they've discovered, don't respond to commensal bacteria in the same way as mature intestines, instead launching an inflammatory response again even non-pathogenic microbes. And while inflammatory responses in adult enterocytes are self-limiting, eventually shutting off, those in the pre-term baby lack the negative feedback that shut off such a response, letting it continue for longer.

In terms of the microbiomes of the infants who go on to develop NEC, there are also differences. These immature infants have less species diversity, more pathogens, more proteobacteria, and have received more doses of antibiotics, on average, compared to babies that don't develop NEC.

Around the world, scientists have begun to test the utility of probiotics on helping these immature guts develop, shifting the microbiome toward a healthy state, and preventing NEC. Numerous small studies have found benefits to different probiotics-of-choice in decreasing overall NEC rates as well as the rate of death from the disease. But, together, the studies don't yet offer enough proof for major pediatric associations and societies to recommend their routine use in the neonatal intensive care unit, since each study has been relatively small and relied on a different protocol. A large, multi-center, randomized controlled trial is needed to form recommendation, many argue.

Rather than relying on live probiotics, Walker's group has tested the benefits of isolating secretions from *Bifidobacteria infantis*—one microbe used in probiotic studies—on premature infants. The secretions, the researchers have shown, increase the number of negative regulators of immunity in the neonatal gut, controlling inflammation and encouraging gut maturation. Along with clinical trials of probiotics, this basic research aims to uncover the window of opportunity for protecting the tiniest of babies from gastrointestinal problems.

The Perinatal Microbiome: Implications for Health and Disease

It's becoming clear that when it comes to the importance of the microbiome in health and disease, there's no single time point or influence that captures all of the effects of microbes. For babies, the development of the healthy microbiome is shaped by factors in utero, mode and timing of birth, feeding and dietary differences, and treatment by drugs after birth. And each of those, in term, can be related.

Scientists have a growing appreciation and understanding for the microbes that are present before birth, in both the placenta and amniotic fluid of the uterus, and research is beginning to point toward the idea that these communities could influence premature birth. In one study, researchers analyzed the amniotic fluid from 46 pregnancies and then followed their outcome. Women who had more microbes detectable only by PCR—a chemical detection method rather than culturing of the bacteria—had elevated inflammatory markers in their amniotic fluid and were more likely to deliver a baby early, as well as to have a baby that developed sepsis, a body-wide infection.

While many think the inflammation that drives pre-term labor is caused by the placenta, neonatologist Josef Neu is beginning to have another theory: the early deliveries are caused by inflammation in a fetus's intestines when they swallow amniotic fluid that has the wrong balance of microbes. Neu and his colleagues have studied the microbial composition of meconium—a baby's first stool that's considered a good marker of what they were exposed to in utero—with other microbial communities.

Josef Neu, MD
Professor of Pediatrics
Division of Neonatology
University of Florida

While the meconium has few similarities to skin, oral, and vaginal microbiome, it shared 60 percent of its microbes with amniotic fluid.

Similarly, research has revealed that the diversity index of microbes in a baby's meconium are influenced by many factors: whether a mother took antibiotics during pregnancy, the gestational age at birth of a baby, and the intent to feed breastmilk or formula.

Outside the womb, these influences don't end. Studies are still probing the differences between babies delivered vaginally and those born through a Cesarean section, the effects of breastmilk, and the role that care in neonatal intensive care units—including antibiotic prescriptions—may have. Each factor has been linked to the makeup of the gut microbiome—particularly the prevalence of proteobacteria, which includes many pathogens as well as microbes linked in adults to inflammatory bowel disease.

Adult Period

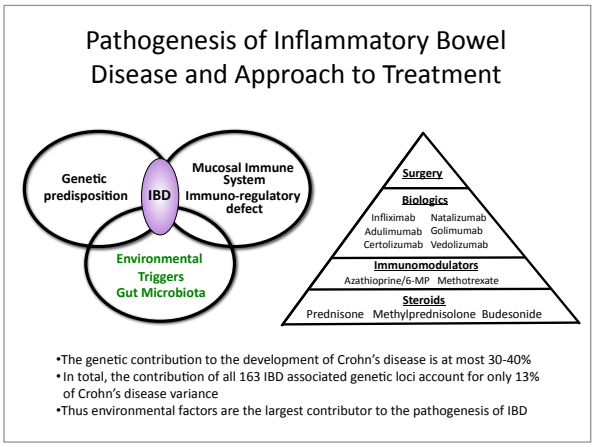
Keynote: "Diet and the Gut Microbiota"

Gary D. Wu, MD

Ferdinand G. Weisbrod Professor of Gastroenterology
Associate Chief of Research, Division of Gastroenterology
Associate Director, Center for Molecular Studies in Digestive and Liver Disease
Perelman School of Medicine, University of Pennsylvania

Every human on the planet lives in an ongoing equilibrium with the population of microbes that reside inside the lumen of their gut. The body produces substances that shape the composition of the gut and, in turn, the microbiota impact inflammatory pathways, produce metabolites, and play a role in the pathogenesis of diseases from cancer to diabetes.

Diseases that gastroenterologists like Gary Wu are interested in understanding—inflammatory bowel disease (IBD) and Crohn's disease—seem to be particularly linked to the gut microbiomes of patients. Currently known genetic mutations explain only 13 percent of cases of Crohn's and 30 percent of IBD, suggesting that environmental factors have a large contribution as well. Yet treatment strategies don't focus on altering this environment, and are instead largely aimed at treating symptoms. Clinician-scientists like Wu want to change that, and learn how to re-engineer the environment of the gut to treat disease.



In many parts of the world, defined formula diets—easy-to-follow liquid meals—are a first-line treatment for Crohn's disease and have shown success in controlling flare-ups. But the mechanism by which they work has been unclear—are they providing key nutrition, or excluding something that caused a flare-up? Research is now looking at how these dietary regimens affect the gut microbiome to shed light on the question.

In another example of a disease potentially linked to the microbiome, people with chronic kidney disease are prone to cardiovascular disease. Scientists believe that the link may revolve around a molecule called trimethylamine which is produced by gut microbes and elevated in chronic kidney disease.

In both of these cases, the link between the microbiota and disease is likely due to the production of metabolites by gut microbes—the metabolites then moves through the body causing symptoms. To understand the connection between the metabolites in a person's body—also called the metabolome—and their diet, Wu has launched comprehensive studies comparing the metabolomes of vegans and omnivores, groups that have drastically different diets. Of 363 metabolites, around a third, he discovered, have statistically significant differences in levels between the two groups. And he can now predict, with a 94 percent accuracy whether someone is a vegan or an omnivore based on these metabolites.

In that same study, however, some data points were less different than expected—the actual microbiota of the groups didn't vary much, and there wasn't a significant difference in fecal short chain fatty acids between vegans and omnivores, as there is between people who live in different parts of the world. The similarities suggest that there are other factors in the western world—even between groups with different diets—that control the microbiome. It points the way toward the next wave of research that gets at these factors, by combining data on not only diet, but genetics, infections, living conditions and antibiotic use.

Microbial Determination of Mucosal Homeostasis vs Chronic Intestinal Inflammation

R. Balfour Sartor, MD

Distinguished Professor of Medicine,
Microbiology and Immunology
Director, UNC Multidisciplinary Center
for IBD Research and Treatment
University of North Carolina

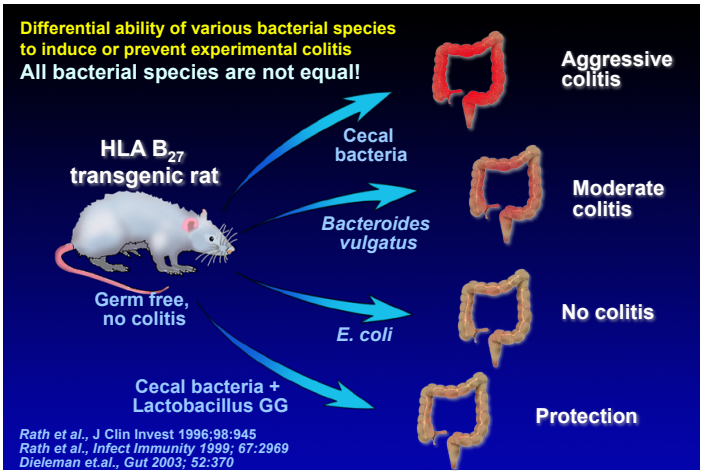
The more researchers delve into the details of the link between human health and the microbiome, the more complexity they uncover. While it's tempting to lump bacteria into two groups—those that are harmful to the human body, and those that are beneficial to the body—it's quickly becoming clear that things aren't that simple. The same microbiota, in two different settings, can have two different effects. So what dictates that setting? An organism's genetics, health history, diet, antibiotic use, and environmental exposures, to name a few.

The protective effects of bacteria—when they exist—are mediated largely through the immune system. Bacteria that live commensally with the human body produce antigens that react with specialized immune cells in the gut to educate and activate the immune system without causing inflammation. Regulatory T cells are produced that keep this healthy balance between the microbiome and the gut.

Balfour Saltor and other immunologists want to know how and why this peace is sometimes broken. What happens to cause colitis, an inflammation of the colon? Several groups of researchers have now shown that butyrate, a short chain fatty acid produced by some microbes, is key to this transition. Butyrate and other similar fatty acids help stimulate the generation of T cells. In animals who have taken doses of antibiotics, extra butyrate will boost T cell numbers. Specific bacteria, Sartor and his colleagues have discovered, stimulate the immune system through their metabolites. Bacteroides fragilis, for instance, produces polysaccharide A, and clostridium groups IV and XIVA stimulate regulatory T cells.

So what happens to these bacteria in disease? Analyses of patients with Crohn's disease revealed that they have less Clostridium groups IV and XIVA than usual, an overgrowth of proteobacteria, and decreased microbial diversity overall.

Simply changing the abundance of these bacteria in mouse models, however, isn't a predictable way to induce colitis. In one mouse model, certain strains of bacteria cause colon inflammation while others don't. But switch to a mouse with a different genetic background, and suddenly neither—or both—bacteria will promote colitis. Similarly, even when two bacteria cause colitis in the same mouse, they may not cause identical symptoms: one may cause right-sided colitis, while the other causes disease on the left. The results emphasize the interplay between colonizing bacteria and genetics that is likely unique in every human patient. It's unlikely, Sartor's research suggests, that a single approach to altering the microbiota will work for every patient. Instead, probiotics and microbiome-based therapies will have to veer in the direction of personalized medicine to be effective.



Microbial Interventions for Obesity and Metabolic Syndrome

Among obese humans, about two-thirds are metabolically healthy—despite their weight, their insulin sensitivity remains within the normal range. But the other third have insulin resistance. A growing number of studies are showing that the difference comes down to the gut microbiome. Associative studies have shown correlations between microbiota and type 2 diabetes. When physician Max Nieuwdorp tested the stool samples of patients in one clinic, for instance, he found that two species of microbes were predictive of whether someone was insulin resistant. Tests for these microbes, the results suggest, could be used to predict the risk of diabetes in patients with reduced insulin sensitivity.

Now, researchers are beginning to move from these associative studies to clinical trials and mouse studies testing whether altering the microbiota through fecal transplants can treat insulin resistance or infections. Some research is still case studies—Nieuwdorf cites the instance of a patient who'd been bedridden with diarrhea, caused by a *Clostridium difficile* infection, for more than a year. When he and his colleagues gave her a fecal transplant from a healthy donor, she recovered quickly, able to walk out of the hospital a few days later.

But other studies are much larger. In one randomized, controlled trial of patients with the early stages of type 2 diabetes, people who received fecal transplants from healthy donors had a rise in insulin sensitivity that lasted for months. In another study, *C. difficile* patients had increased diversity of microbes in their guts more than three months after a fecal transplant. Interestingly, the most successful cases in each study had one thing in common: the fecal transplant donors. It hints that a few crucial strains of bacteria, only carried by some people, may be key to the treatments.

Max Nieuwdorp, MD, PhD

Associate Professor, Internal Medicine
Head of Experimental Vascular Medicine
Department, Academic Medical Center
University of Amsterdam, Netherlands
Visiting Professor, Gothenborg University, Sweden

One of these bacteria, Nieuwdorf's research has shown, is likely *Eubacterium hallii*, a bacterial species that produces butyrate in both the small intestine and colon. Treating mice with doses of *E. hallii*, Nieudorf has found, improves their insulin sensitivity, makes them burn more energy, and increases the levels of secondary bile acids they produce—thought to be important to glucose metabolism. *E. hallii*, however, is likely one of many strains that are important to treating patients.

For now, researchers are confident that microbiota are altered in obesity and that, sometimes, this alteration is what drives insulin resistance. Shifting the gut to a more healthy state, many believe, can reverse these changes and treat or prevent insulin resistance.

Microbiota and Brain Gut Axis

Emeran A. Mayer, MD
Professor, Department of Medicine, Physiology and Psychiatry
Co-Director, CURE: Digestive Disease Research Center
David Geffen School of Medicine, University of California, Los Angeles
Executive Director, Oppenheimer Family Center for Neurobiology of Stress

If research linking the microbiome with conditions like diabetes and Crohn's disease is still in its early stages, research on the brain-gut axis is in its infancy. Historically, many brain disorders were treated with changes to diet or probiotics, but until a few years ago, concrete research in this area remained a fringe topic. Now, though, evidence is beginning to grow that the body's microbes and the function of the brain are intrinsically linked with a two-way street.

Researchers know now that the microbiome produces substances—like immune molecules—that link back to the brain. In lab mice, experiments have shown that antibiotics or probiotics can alter not only the microbiomes of animals, but their levels of stress, social behaviors, and responses to behavioral tests. Likewise, studies in monkeys have shown that stress alters the composition of the gut microbiome, predisposing the stressed-out animals to infections. The limitations of these studies, of course, is that it can be difficult to extrapolate from behaviors in animals to human feelings and emotions, to begin to draw conclusions about how the microbiome could play role in diseases from Alzheimer's to depression.

However, new methods in neuroscience, allowing clinician-scientists like Emeran Mayer to map the networks of the brain, are starting to push the field forward. Now, it's not only animal behaviors that can be used as readouts, but brain neurochemistry as well. Mayer, for instance, has discovered that when people ingest probiotics for an extended period of time, the brain's activation during tests in the lab changes. When subjects who have taken a control or a probiotic mixture are asked to match faces with emotions, the activity of the brain network involved differs between the groups.

It's clear that substances produced by the microbiota—from metabolites to quorum-sensing molecules and fatty acids—have the potential to bind to cells of the nervous system. But whether this occurs, and where and when, has yet to be shown. And whether the changes to the brain seen after probiotic use are linked to well-being, happiness, or disease states, also needs more study. But researchers are beginning to open the book on the brain-gut axis, and hypothesize that the microbiome may be linked to a broad variety of brain diseases and disorders.

Microbiome-gut-brain Interactions in an Autism Mouse Model

Elaine Y. Hsiao, PhD
Research Assistant Professor
Division of Biology & Biological Engineering
California Institute of Technology

As studies on the microbiome become more conspicuous across multiple disciplines of science, more researchers find themselves asking whether their own areas of interest might relate to microbiota. When Elaine Hsiao began studying autism, she didn't know that she'd be among those pulled down the path of the microbiome. Now, she's leading the way in uncovering links between autism spectrum disorders and microbes in the gut.

The research began from the observation that people diagnosed with autism also often have immune dysfunction and gastrointestinal symptoms. Moreover, a series of epidemiological studies from around the world—and spanning different centuries—all found that infections during pregnancy boost the chance of a child being born with autism. In each case, it didn't seem to be the exact pathogen that mattered, but the activation of the maternal immune system during pregnancy—even an autoimmune disease in the absence of a real infection can also increase the autism risk.

Based on the results, Hsiao's lab developed a new mouse model of autism by activating a pregnant mouse's immune system with RNA during gestation. The offspring have telltale autism signs: repetitive behaviors, impaired social interactions, and decreased communication. They also show some of the classic signs of autism in the brain, with decreased numbers of certain cell types. But that wasn't all that Hsiao saw. The mice also had immune abnormalities early in life, including fewer regulatory T cells than usual. Hsiao and her colleagues wondered whether the immune problems were causative of the core behavioral features of autism.

To test the hypothesis, they treated the mice with a probiotic—*Bacteroides fragilis*—after birth. Some behaviors, they found, improved, as well as the levels of T cells. And while not all autism features disappeared, the researchers also found that not all immune abnormalities were gone, suggesting that alone, *B. fragilis* was only correcting some of the problem caused by the immune activation during pregnancy.

Studying their mouse model more closely, the team went on to isolate one particular metabolite that they believe plays a role in the effect of *B. fragilis*. Of course, that doesn't mean it explains the full autism-gut link. But when levels of 4EPS in the gut are altered, some behaviors change. It's likely that isolating other *B. fragilis* metabolites, as well as those from other bacteria all together, will continue to fill in holes in the story of how the immune system related to autism.

Older Adult

Keynote: "Microbiota in Older Adults – Changes, Associations, and Intervention Prospects"

Paul W. O'Toole, PhD
Professor of Microbial Genomics
School of Microbiology & Alimentary Pharmabiotic Centre
University College Cork, Ireland

Throughout the lifespan, changes in diet, health, and environment are constantly reshaping the human microbiome. As people reach their elderly years, these factors often shift more than at any other time since infancy. Chronic diseases may appear, long-term residential care may become necessary, and changes in diet may accompany this. The plethora of changes offer a fertile ground for studying the links between the microbiome and health. Which health factors affect the microbiota of the gut and which microbiome changes predispose people to health risks?

Eldermet, a large cohort of elderly patients established in Ireland in 2008, has offered some answers to these questions. Over 500 subjects over the age of 65 have been profiled at the microbiome level and followed clinically. Among the most striking observations to come out of the initial Eldermet study was the fact that people living in long-term care homes had different microbes in their guts compared to people living in the community. Overall, the elderly population had microbiomes dominated by Firmicutes and Bacteroides, with much less Actinobacteria than younger adults. But those in care facilities had fewer genes responsible for short chain fatty acid production, and a slightly different balance of microbes.

When microbiologist Paul O'Toole and his colleagues looked at other data from the Eldermet cohort, they found a reason for this segregation: diet. Subjects living in the community tended to eat higher levels of fiber, fruit, cereal, and low-fat dairy; those in long-term residential care consumed more high-fat dairy, red meat, and high glycemic index foods.

Other differences also began to stand out as O'Toole's group analyzed the Eldermet cohort. The elderly group living in long-term stays had less diverse and less stable microbiome that seemed less resistant to external perturbation. They were also more likely to have health struggles related to blood pressure, frailty, independent living, and inflammation. Moreover, when the scientists analyzed the presence of methanogens—archaea in the microbiome that produce methane—they found that eldely living in long-term care had more of one type of methanogen, called Mx1, while healthier and more independent individuals had more Mx2-3.

Trying to prove causation using this data—concluding, for instance, that diet is responsible for these microbiome shifts which are, in turn, responsible for health outcomes—is difficult. But one hint came when researchers realized some community-dwelling elderly had microbiomes more resembling those in long-term care. And indeed, when they looked at their diets, they resemble those usually seen in the residential care group, with less fiber, fruit, and low-fat dairy.

Now, scientists are beginning to try to get at causation in another way: developing interventions in the elderly. One multicenter dietary intervention including 1,250 European subjects is using supplementation to mimic Mediterranean diets. The results are still forthcoming. Other research is focused on growing mixtures of bacteria that specifically mimic the bacteria missing from the frail, long-term care, elderly population's microbiomes.

Probiotics and the Elderly Microbiome – Is it ever too late to change?

Patricia L. Hibberd, MD, PhD
Professor, Department of Pediatrics
Harvard Medical School
Chief, Division of Global Health
Department of Pediatrics
Massachusetts General Hospital

Clinician-scientist Patricia Hibberd is all too aware of the challenges of studying the microbiome from a regulatory perspective. Hibberd wanted to study the effect of a *Lactobacillus* probiotic on the response of elderly people to the influenza vaccine. But, after the National Institutes of Health agreed to fund her project, the US Food and Drug Administration intervened. Hibberd would need to show the safety of the *Lactobacillus* in healthy individuals, without a flu vaccine, before she would be allowed to test the probiotic in a randomized, controlled trial.

To make the most of the setback, Hibberd decided to track the microbiomes of the elderly individuals enrolled in the safety study. Every participant received Lactobacillus for 28 days, then were followed for an additional 28 days, during which time they were surveyed with a daily diary, phone calls, study visits, and microbiome sampling. The results of her study are forthcoming.

Prevention of Recurrent *Clostridium difficile* Infection

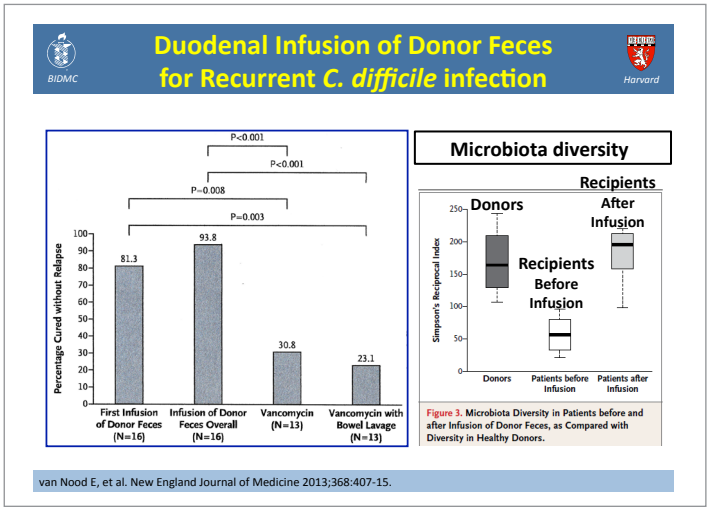
Ciarán P. Kelly, MD
Professor of Medicine
Harvard Medical School
Director, Gastroenterology Fellowship Training
Medical Director, Celiac Center
Beth Israel Deaconess Medical Center

One of the most dreaded outcomes of a course of antibiotics in the hospital is a new infection; colonization of the gut by *Clostridium difficile*. A *C. difficile* infection can be symptomless if it's a non-toxin-producing strain, or if someone's immune system keeps the toxins at bay, but about half of people who contract a toxin-producing strain get disease, characterized by severe diarrhea and inflammation of the colon. Over the past decades, the number of cases of *C. difficile* have jumped more than three-fold in the US, and age-adjusted death rates have also leapt up.

Once a person gets a *C. difficile* infection once, they have a one in four chance of another infection, as soon antibiotics aimed at the *C. difficile* are withdrawn. Once they've had multiple infections, that recurrence rate rises even more, to almost fifty percent.

Because of the ineffectiveness of repeated antibiotic use in these cases, researchers have turned to research on probiotics and fecal transplants as possible cures for C. difficile. The treatments are both based on the idea that factors in the gut microbiome have the ability to allow a *C. difficile* infection to flourish, or to keep it out. In fact, studies have shown that patients with recurrent *C. difficile* infections have less diverse microbiomes, and less Bacteroidetes microbes.

Trials on the use of probiotics in C. difficile, however, have led to contradicting results that have left the field struggling to come to a conclusion. In one study, patients with multiple episodes of *C. difficile* who received doses of *Saccharomyces boulardii* were almost as likely to have another infection. But other studies failed to confirm the results. As of today, no current national guidelines recommend the use of probiotics to treat of prevent *C. difficile* infections.



Fecal microbiota transplant (FMT) is another story, however. Studies have concluded that treatment with FMT dramatically reduces someone's chance of a recurrent *C. difficile* infection. Confident in these results, researchers like gastroenterologist Ciaran Kelly are moving to understand exactly what components of donor stool are important to the treatment. Isolating these particular strains of microbes could lead to a bacteriotherapy less burdened by regulation and logistical challenges than fecal transplants. Similarly, researchers are working to test whether infection with the non-toxin-producing strain of *C. difficile* can protect patients from colonization with the more dangerous strain. As these studies progress, and the use of FMT in *C. difficile* becomes more widespread, the development of new cocktails of microbes could make recurrent infection a thing of the past.

Future Directions

Final Overview Keynote

Jonathan Braun, MD, PhD
Professor
Chair, Department of Pathology and Laboratory Medicine
David Geffen School of Medicine
University of California, Los Angeles

Over the course of this conference, clinicians and scientists have spoken a lot about the microbiome, the idea that there are good microbes and problematic microbes and microbes related to development. But the microbiome is more spectral than can be captured in these talks. If you look at the intestinal microbiomes of 50 or 100 healthy people, you'll see a wide variety of microbial communities. Such observations point toward the importance of studying functions rather than compositions of the microbiome as we proceed toward a better understanding of how the microorganisms affect health.

It's clear that from the first days of life—and even before birth—the microbiota are changing and being shaped by their environment. But we're still learning how these changes are mediated throughout development; the microbiome will look quite different if you're growing up in Malawi than Boston, and researchers need to understand much better how these developmentally important microbiota are influenced by ones environment before they can move toward altering the microbiomes of neonates.

It's also clear that the microbial communities in one's body shift with disease but, again, scientists need to sift out cause and effect before moving toward therapies in this area. In one recent study, the microbiomes of children and adults newly diagnosed with Crohn's disease or ulcerative colitis were analyzed. There was a huge spectrum of diversities within these patients; it wasn't that some people were in one subgroup and some in another, but there was a complete gradation of differences.

So what does that mean? Is it that the composition isn't telling researchers what they need to know? Is it that some people's disease is more driven by genetics and environment and if they were removed from the study, a pattern would emerge among the rest of the cases that are more microbially-driven? For now, this is a question and a challenge. Computational approaches are beginning to emerge that can help pick out patterns of dysbiosis from disease cohorts and will, scientists hope, begin to answer questions like this.

The next challenge, though, it this idea that keeps coming up of moving from composition of the microbiome to function. Because function can change without composition changing, and we're not healthy or unhealthy strictly because of the microbes in our guts, but because of what they're doing and what they're producing. Moreover, microbes within the same genus can have drastically different functions, and so researchers need to move toward an ever more detailed definition of what's in a given microbiome.

The way that researchers have already begun to do this, it to look at categorization of the microbiome by the pathways that its metabolites fall into. When you chart the Crohn's disease patients this way, now, there is a larger difference between healthy and not healthy. Once scientist then tabulate what these metabolite pathways actually are, they can start to see how therapeutics might block or activate a given function.

There's another whole area, though, that is only beginning to come into focus in terms of microbiome research, but is likely going to have a big impact on the field: genetics. A microbiome is one thing that gets you further away or closer to the disease threshold, but, of course, genetics is another one. Now, one question is, what's the crosstalk between your genetics and your microbial composition? Mice studies have started to hint at this, analyzing the microbiomes of different strains of mice, but large human studies are yet to be published. The mice studies, though, confer the idea that your genetics is actually gardening your microbial community. And isolated examples in humans—whether someone has the gene to fucosylate carbohydrates—have suggested that the same is true in people; such genes can affect both microbiome functional states and disease propensity.

To move forward, and closer to effective therapeutics aimed at the microbiome, researchers need to do a few things: select a desirable microbiome, or range of microbiomes, as an endpoint, learn how to better take a snapshot of the microbiome with a blood test or other easy method, and deciding what the best method of modification is.

The Promise of Research on Gut Microbiota and Probiotics Throughout the Lifespan

More than a decade ago, scientists completed the first complete sequence of the human genome, revolutionizing personalized medicine and the study of genetic diseases. Today, they're faced with an even larger challenge: understanding the microbiome, the full set of microbes inhabiting every human's body. A single person's microbiome is thought to contain a hundred times more genes than their own genome and—while the forces that shape the human genome are well-understood—researchers have yet to fully understand what influences each person's unique microbiome. It's becoming clear, however, that the microbiome plays key roles in development, health, and disease throughout the lifespan. Getting a handle on what shapes the microbiome—and, in turn, how it affects health—at each stage of life is the first step toward being able to control the microbes and leverage the microbiome as a therapeutic strategy.

Pregnancy

For centuries, clinicians have described the uterus as a sterile, microbe-free environment, protecting a growing baby from the outside world until birth. Today, that view is increasingly outdated. Research has revealed that microbes from a mother's body sneak through the placenta and thrive in the amniotic fluid surrounding a fetus. The discovery upended the idea that someone's microbiome begins to be shaped once they enter the world, suggesting instead a new window within which a healthy—or disease-promoting—microbial balance can be established. Studies have now revealed that what a woman eats and where she spends her time during the nine months of a pregnancy can influence her microbiome and affect her baby's later risks of allergies, asthma, obesity, and metabolic disease. As more light is shed on these connections, researchers are beginning to wonder whether the right mixture of probiotics, given during pregnancy, could give a child's microbiome the best start in life.

Neonatal

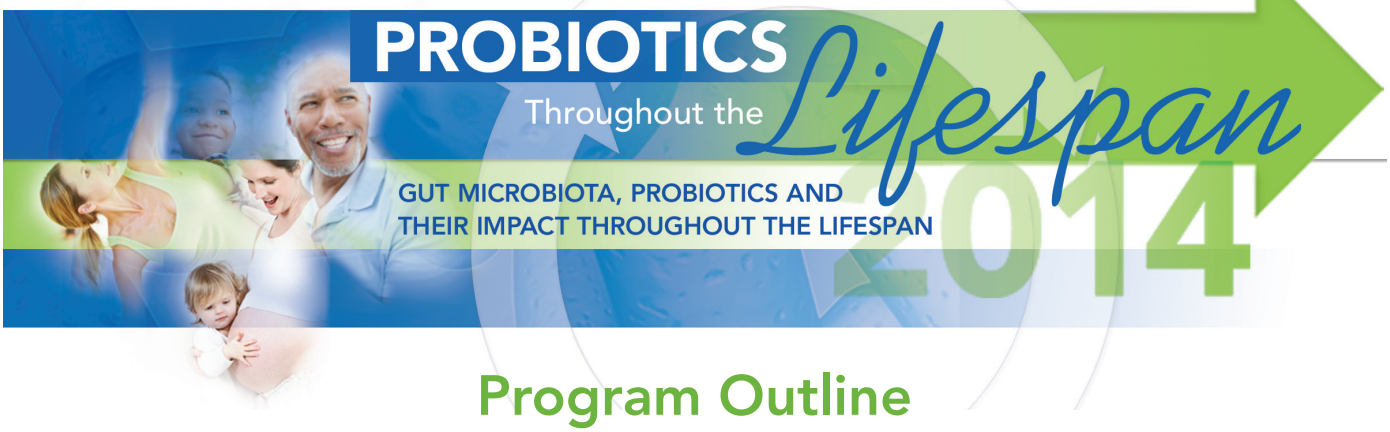
From the moment a baby is born, their microbiome begins to be shaped not only by their mother's microbiome—as it was throughout pregnancy—but by the rest of the world around them. Whether they eat breastmilk or formula, whether they have to stay in intensive care after birth, and whether they take a course of antibiotics in those early days all affect which microbes take up residency in their bodies. The patterns established in their gut now can literally leave microbial signatures that last a lifetime. Studies on the diversity of the microbiome after birth have shown that, for a few weeks, it's an unstable, constantly-changing community of microbes. During this time, the effect of probiotics, food, or environmental changes is particularly profound. Research has already begun on how to influence babies' guts throughout this window to help prevent the development of dangerous conditions such as necrotizing enterocolitis, which affects some tiny premature babies. Other avenues of study are pursuing how to mimic the positive effects of breastmilk on the microbiome in babies that can't breastfeed.

Adulthood

Imbalances in the microbiomes of people's digestive tracts have been linked to diseases from cancer and Alzheimer's disease to Crohn's disease, diabetes, and ulcerative colitis. But distinguishing causation from correlation is hard—are people's microbiomes different because of their disease, or is their disease caused by changes to the microbiome? Researchers are in the early stages of trying to answer this question by studying the effectiveness of probiotics and fecal transplants in treating a variety of conditions. Fecal transplants have already shown effectiveness in curing C. difficile infections. But at the same time, other questions are arising—even the microbiomes of healthy people vary drastically, so what should the desired endpoint be when altering the microbiomes of patients? Studies on both healthy and diseased individuals, as well as animal models, are striving to answer this.

Older Adults

Throughout the lifespan, changes in diet, health, and environment are constantly reshaping the human microbiome. As people reach their elderly years, these factors often shift more than at any other time since infancy. Chronic diseases may appear, long-term residential care may become necessary, and changes in diet may accompany this. The same questions that plague researchers studying the microbiomes of other adults are important to consider in older adults as well. But, additionally, the fast pace of change in both the environment and the microbiome during these later years offer a fertile ground for studying these questions. Clinicians hope that basic research on the balance of microbes in older adults will lead to interventions to help prevent declines in health and memory, chronic infections, and other diseases in the elderly.



Program Outline

Opening Keynote: "Manipulating the Microbiota: Beyond Traditional Probiotics and Fecal Transplant"

R. Balfour Sartor, MD, University of North Carolina

Session IA: Pregnancy

Moderator: Mary Ellen Sanders, PhD, Dairy & Food Culture Technologies

Keynote: "The Microbiota During Pregnancy"

Omry Koren, PhD, Bar Ilan University, Israel

"Probiotic Use During Pregnancy for Protection Against Childhood Diseases"

Erika Isolauri, MD, D. Med. Sc, University of Turku, Finland

"Diet and Microbiotic Exposure During Pregnancy and Immune Protection Against Allergic Manifestations"

Erika von Mutius, MD, MSc, Dr. von Hauner Children's Hospital, Germany

Speakers Discussion

Session IB: Neonatal Period

Moderator: W. Allan Walker, MD, Harvard Medical School

Keynote: "Development of Human Infant Intestinal Microbiota"

David A. Relman, MD, Stanford University

"Antibiotics and Neonatal Colonization'

Martin J. Blaser, MD, New York University Langone Medical Center

"Establishment of a Milk-Oriented Microbiota in Infants: New Insight into Probiotics and Prebiotics"

David A. Mills, PhD, University of California, Davis

"Probiotic Studies in NEC"

W. Allan Walker, MD, Harvard Medical School

"The Perinatal Microbiome: Implications for Health and Disease"

Josef Neu, MD, University of Florida

Speakers Discussion

Session II: Adult Period

Moderators:

Peter R. Holt, MD, The Rockefeller University

Richard L. Guerrant, MD, University of Virginia School of Medicine

"Keynote: Diet and the Gut Microbiota"

Gary D. Wu, MD, University of Pennsylvania

"Microbial Determination of Mucosal Homeostasis vs Chronic Intestinal Inflammation"

R. Balfour Sartor, MD, University of North Carolina

"Microbial Interventions for Obesity and Metabolic Syndrome"

Max Nieuwdorp, MD, PhD, Academic Medical Center, University of Amsterdam, Netherlands

"Microbiota and Brain Gut Axis"

Emeran A. Mayer, MD, David Geffen School of Medicine, University of California, Los Angeles

"Microbiome-gut-brain Interactions in an Autism Mouse Model"

Elaine Y. Hsiao, PhD, California Institute of Technology

Speakers Discussion

Session III: Older Adults

Moderator: Samuel Klein, MD, Washington University School of Medicine in St. Louis

"Keynote: Microbiota in Older Adults–Changes, Associations, and Intervention Prospects"

Paul W. O'Toole, PhD, University College Cork, Ireland

"Probiotics and the Elderly Microbiome – Is it Ever Too Late to Change?"

Patricia L. Hibberd, MD, PhD, Harvard Medical School

"Prevention of Recurrent *Clostridium difficile* Infection"

Ciarán P. Kelly, MD, Harvard Medical School

Diet, Microbiota and Atherosclerosis

Stanley L. Hazen, MD, PhD, Cleveland Clinic

Speakers Discussion

Final Overview Keynote: Future Directions

Jonathan Braun, MD, PhD, David Geffen School of Medicine, University of California, Los Angeles

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