Probiotics, Prebiotics, and the Host Microbiome: The Science of Translation

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ABSTRACTS

Economic Assessment of Disease Reduction and Prevention Challenges and Perspectives for Probiotics and Prebiotics, John Hutton, University of York, York, United Kingdom

Over the past 20 years, the use of economic evaluation in decision-making regarding the utilisation and reimbursement of health technologies has become widespread. Health technology assessment (HTA) including the demonstration of the cost-effectiveness of new products has become a requirement for reimbursement of pharmaceuticals in Australia, Canada, Sweden, the UK, and many other European countries. These requirements are now being increasingly applied to medical devices and diagnostics, and in the UK, to public health interventions. The importance of nutrition and nutritional products in achieving improved health outcomes is widely recognized, but at a time when healthcare budgets are under pressure globally, such interventions must be justified in economic as well as clinical terms if they are to be used and financed effectively. The economic evaluation methods used in HTA have been developed with technologies such as pharmaceuticals in mind, which raises the question of whether these methods are equally suited to the assessment of nutritional interventions. The presentation will identify the economic impacts of nutrition-related health interventions and discuss the best approaches to their evaluation.

Impact of Antibiotic Exposures on the Developing Microbiota, Martin J. Blaser, MD, New York University School of Medicine, New York, New York, United States

Although there is enormous variation between individuals, at higher levels of microbiome organization, there is considerable similarity amongst mammals in the encoded functions. Growing evidence suggests a developmental pathway for the microbiome during the critical window in early life when the host also is developing—metabolically and immunologically. However, in recent years, a variety of medical advances have potential impact on the composition of the developing microbiota resulting in selective pressures that substantially differ from the ancient. These include interventions during pregnancy ranging from antibiotics to caesarian sections, and early childhood exposure to antibiotics and other anti-bacterial substances. To study these phenomena, we have developed models in mice of either continuous subtherapeutic antibiotic treatment (STAT) or pulsed antibiotic treatment (PAT), and measured developmental phenotypes. These studies have shown short-term effects on microbiome composition and gene expression in adjacent (terminal ileum and colon) or downstream (liver) tissues, with long-term effects on host morphometry, metabolism, and immune cell populations. In total, these

models indicate that perturbing the microbiota early in life leads to alterations in development with long-term consequences. These studies provide evidence that antibiotic exposures that affect the early life microbiota may have unanticipated biological costs that could affect the risk of developing obesity and metabolic syndromes and the currently epidemic allergic and autoimmune diseases.

MicrobiomeAssembly in Early Childhood, Elizabeth Costello, PhD1, Les Dethlefsen, PhD1, and David A. Relman, MD1,2; 1Stanford University School of Medicine, Stanford, California, United States, 2Veterans Affairs Palo Alto Health Care System, Palo Alto, California, United States

Postnatal microbial colonization prompts the terminal maturation of host intestinal structures, mediates the development of the immune system, and induces resistance to invasion by would-be pathogens. Recent studies using cultivation-independent approaches of the fecal microbiota of premature infants have revealed a low level of diversity, high inter-individual variability, and a capacity for abrupt temporal shifts in species- and strain-level composition. The composition of the human microbiota is body site-specific in healthy adults, yet this is not the case in newborns shortly after delivery. We have been simultaneously tracking the assembly of the gut, oral, and skin microbiota of infants during the first two years of life. Our analysis focuses on the development of habitat specificity, and the factors underpinning compositional variation during this critical timespan, including antibiotic disturbance.

When the Programming Goes Awry: Diabetes, Obesity, and Beyond, Patrice D. Cani, PhD, Université Catholique de Louvain, Louvain, Belgium

Obesity and type 2 diabetes are characterized by disturbed inter-organ communications that contribute to the onset of diseases. However, the underlying mechanisms are not fully defined. In this context global changes in the gut microbiota have been observed by a number of culture-dependent and culture-independent methods. We discovered that the gut microbiota contributes to the development of insulin resistance and low grade inflammation characterizing obesity. We described the concept of metabolic endotoxemia (increase in plasma Lipopolysaccharide [LPS] levels) as one of the triggering factors leading to the development of the metabolic inflammation and insulin resistance associated with obesity. We also found that nutritional or genetic-induced obesity and type 2 diabetic rodents display gut barrier dysfunctions leading to the leakage of LPS and possibly other microbiota derived factors. Among the mechanisms, we found that the gut microbiota interacts with the endocannabinoid system as well as with enteroendocrine L-cells (producing GLP-1, PYY and GLP-2). More recently, by using prebiotics or probiotics, we identified novel mechanisms of bacterial interaction with the host that control gut permeability and metabolism during obesity and type 2 diabetes. The key role of Akkermansia muciniphila will be discussed in this context. Altogether, next-generation sequencing methods and targeted approaches have facilitated the identification of bacteria and metabolic functions that might be associated with the development of obesity and type 2 diabetes.

Translating Research into Public Health Policy, Sir Harry Burns, DSc, MPH, Scottish Government, Edinburgh, Scotland

Public Health is concerned with promoting and protecting health and prolonging life through the organized efforts of society. In pursuing this aim, public health wrestles with the problems of complex systems in which outcomes have multiple determinants which interact in obscure ways and attempts to change such systems often have unintended consequences. Not for nothing are such problems often referred to as "wicked problems"! Scotland has been using improvement science to enhance quality and safety in its healthcare system. Such techniques are now being used to transform wellbeing by enhancing the delivery of evidence based public health policy. Examples of current practice will be described.

How Bacteria Can Influence Brain Development, Circuitry, and Behavior, Jane A. Foster, PhD, Sufian Odeh, BSc, and Karen-Anne Neufeld, PhD, McMaster University, Hamilton, Ontario, Canada

The human gut contains an enormous number of microorganisms referred to as commensal bacteria, or gut microbiota. Without this microbiome, we would be unable to digest plant polysaccharides and would have trouble extracting lipids from our diet. Our lab has studied the gut-brain axis in germ free (GF) mice. Our first experiments followed on the observation that GF mice showed enhanced stress-reactivity (Sudo et al., The Journal of Physiology, 2004). Surprisingly, GF mice showed reduced anxiety-like behavior in the elevated plus maze, a well established behavioral test that examines approach and avoidance behavior in mice, in comparison to specific pathogen free (SPF) mice. The low anxiety-like phenotype was accompanied by long-term changes in plasticity-related genes in the hippocampus and amygdala and it persisted after colonization with SPF intestinal microbiota demonstrating that gut-brain interactions influence central nervous system wiring early in life. We next examined the interplay of leptin and central circuits for stress-reactivity and feeding in the presence or absence of gut microbiota and showed that leptin-insufficiency in GF mice leads to long-term changes in the expression levels of the brain's leptin receptors and peptides in feeding centers in the hypothalamus. Hypothalamic neurons provide a biological link between stress and eating that is important to understand. Our ongoing work considers how gut microbiota influences metabolism and mood. Our work aims to understand the developmental factors that influence risk of metabolic disease and mood disorders with a long-term goal to develop prevention strategies for young children and adolescents at risk.

Prebiotic Supplementation Alters Hypothalamic Neuronal Activity and Protects Against the Obesogenic Environment, Gary Frost, PhD and Jimmy D. Bell, PhD, Imperial College London, London, United Kingdom

Obesity is a major health problem worldwide, fuelled by the overconsumption of dietary energy. An important determinant of energy intake is energy density, and dietary carbohydrate is a major determinant of energy density. Increased intake of fermentable carbohydrate leads to decreased body weight and improved insulin sensitivity in animals and humans, although the mechanism associate with this observation remains unclear. The decrease in body weight associated with high intakes of fermentable carbohydrate in mice is associated with increased hypothalamic neuronal activation, which is different from the suppression of neuronal activity observed from anorectic gastrointestinal hormones such as PYY and GLP-1. In humans there is inconsistent evidence that fermentable fibers release PYY and GLP-1. There is an increase in the peripheral plasma of the short chain fatty acid acetate following consumption of fermentable carbohydrate. This has led our team to explore a potential link between acetate and central appetite regulation. We have been able to show that colonially derived acetate does cross the blood brain barrier and has a direct effect on the arcuate nucleus stimulating anorectic signals.

Effect of Early-Life Pulsed Antibiotic Treatment on T-Lymphocyte Populations, Victoria E. Ruiz, PhD1, Liana Grosinger1, Cornelia Gottwick, BS2, and Martin J. Blaser, MD1; 1New York University School of Medicine, New York, New York, United States, 2Albert Ludwig University of Freiburg, Freiburg, Germany

The human microbiota accounts for about 90% of the cells in the human body. These bacterial communities have co-evolved with humans and have functional roles in metabolism and immunity. The intestinal microbiota shapes mucosal CD4+ T-helper cell differentiation and effector functions. Commensals play a role in immunologic homeostasis, and their perturbation early in life may lead to allergic and autoimmune pathologies. Early-life antibiotic use may be dynamically changing the host microbiota and promoting immunologic dysregulation. Using a model of early-life antibiotic use, our lab has previously demonstrated that high-dose, or pulsed antibiotic treatment (PAT) decreases intestinal pro- and anti-inflammatory cytokine gene expression. We hypothesize that early-life PAT-induced perturbation of the gut microbiota will lead to significant changes in peripheral and systemic T-cell populations. To test this hypothesis, we characterized thymic, splenic, and intestinal T-cell populations in control and PAT-treated C57BL/6 mice. Flow cytometric analysis demonstrated that early-life PAT significantly increased the frequency of thymic and splenic TCRb+ T lymphocytes in male mice, $p<0.05$, $p<0.01$ respectively, and CD4+ Foxp3+ regulatory T-cells, $p<0.05$. These results suggest that early-life antibiotic treatments affect the cellularity of thymic, splenic, and mucosal T-cell populations, phenotypes that may be related to differential selection of the intestinal microbiota.

Beneficial Effects of Prebiotics and Probiotics on the Gut-Brain Axis and Regulation of Body Weight, Helen E. Raybould, PhD, and David A. Mills, PhD, University of California, Davis, Davis, California, United States

Recent attention has turned to putative beneficial effects of prebiotics and probiotics in the treatment of obesity. Animal and human studies using probiotics from the order Lactobacillales show reduced

adiposity and body weight, but the mechanisms of action are poorly understood. The gut epithelium is endowed with numerous sensory mechanisms to detect luminal contents to initiate changes in physiology and behavior to ensure efficient digestion and absorption, and to terminate eating and induce satiety. Intestinal barrier function is compromised in obese, compared to lean, human subjects and in obesity-prone rats fed a high-fat diet, leading to passage of bacterial products across the gut wall. We have evidence to suggest that these bacterial products induce phenotypic changes in neurons innervating the gut that transmit information about lumenal contents to the central nervous system, resulting in an increase in food intake and body weight. Growth of Bifidobacteria infantis on prebiotic bovine milk oligosaccharides (MOs) improves epithelial barrier function in the face of an inflammatory challenge, such as cytokines and ingestion of high fat diets. Investigation of these beneficial effects due to specific prebiotic/probiotic combinations is key in understanding improvement in gut epithelial function as possible treatments for obesity and metabolic disease.

The Effect of Nutrition on the Microbiome in Pregnant Women and the Use of Micronutrient Supplemented Probiotic Yogurt to Improve Outcomes, Megan Enos, BSc1,2, Jordan Bisanz, BMSc1,2, George Praygod, PhD3, Shannon Seney, BSc2, John Changalucha, MSc3, Jeremy Burton, PhD2 and Gregor Reid, PhD, MBA, BSc1,2; 1Western University, London, Ontario, Canada; 2Lawson Health Research Institute, London, Ontario, Canada; 3National Institute of Medical Research, Mwanza, Tanzania

Relatively little is known about the gut, oral, and vaginal microbiome of pregnant women, especially those who are undernourished. In Africa, maternal and infant morbidity and mortality are major problems with an aberrant vaginal microbiota and poor nutrition being contributing factors. We hypothesize that by using next generation sequencing, we will observe a difference between the microbiotas of undernourished, healthy, and obese women. Thus, in order to target these populations, we chose to perform our study in Mwanza, Tanzania. We also hypothesized that daily supplementation of the diet with probiotic yogurt containing Lactobacillus rhamnosus GR-1 and nutrient-rich ground Moringa leaf extracts, will “normalize” the microbiota and improve pregnancy outcomes. Many challenges arose, from language/cultural barriers, weather extremes, lack of resources, and the need to supervise yogurt production and quality. Nevertheless, we recruited 67 subjects (18 undernourished, 18 nourished, 14 undernourished and 11 nourished randomized to receive probiotic yogurt and 6 obese). Anthropometric measurements and 48 hr dietary recall interviews, 16s rRNA Ion Torrent sequencing and metabolomic data from 700 vaginal, oral, and fecal samples, along with maternal and newborn health status have revealed how complex and interlinked these parameters are. Of interest, the incidence of bacterial vaginosis, a risk factor for preterm labor, was 32% which is similar to Canadian women, and three babies died before term. We hope that this work will lead to novel and practical ways of improving the health of mothers, fetuses, and infants, and stimulate microbiome and probiotic research in Tanzania and other developing countries.

Impact of a Short Chain Galactooligosaccharide on the Human Microbiome and Symptoms of Lactose-Intolerant Individuals, Todd R.Klaenhammer1, Dennis Savaiano2, Andrew Ritter3, and M. Andrea Azcarate-Peril4; 1North Carolina State University, Raleigh, North Carolina, United States, 2Purdue University, W. Lafayette, Indiana, United States,3Ritter Pharmaceuticals, Los Angeles, California, 4University of North Carolina, Chapel Hill, North Carolina, United States

Alteration of the colonic bacteria to effectively metabolize lactose is a novel and potentially useful approach to improve lactose digestion and tolerance. A randomized, double-blinded, multi-site placebo controlled trial was conducted to evaluate the administration of a highly purified, short chain galactooligosaccharide (GOS), named RP-G28, to evaluate clinical impacts on lactose digestion and tolerance. In these individuals, stool samples were collected pre-treatment (Day 0), after GOS treatment (Day 36), and finally 30 days after GOS feeding stopped and consumption of dairy products was encouraged (Day 66). Changes in the fecal microbiome were investigated using both Terminal Restriction Fragment Length polymorphisms (TRFLP) and microbiome analysis of 16S rRNA genes by pyrosequencing. Lactose digestion and overall symptoms of lactose-intolerance trended toward improvement in subjects fed GOS/RP-G28. Subjects on GOS were six times more likely to claim they were lactose-tolerant post-treatment. Principal component analyses showed statistically significant shifts in the microbiome of subjects fed GOS/RP-G28, compared to placebo, at 66 days. Lactobacillus Operational Taxonomic Units (OTUs) were overrepresented. Also within the phylum Firmicutes, the abundance of Faecalibacterium and Roseburia OTUs were significantly elevated in the two clusters that shifted in response to GOS/RP-G28. In the treatment group, a significant reduction in the Clostridia class members of the microbiome, represented among the top 30 OTUs, also occurred during GOS/RP-G28 treatment. The results indicated a definitive change in the fecal microbiome of lactose-intolerant individuals that were clinically responsive to dietary adaptation to GOS/RP-G28.

Challenges to Translating Science to the People with the Greatest Need, Andrew Serazin, DPhil, Matatu LLC, Washington, District of Columbia, United States

Contemporary societal forces and scientific advances forecast a coming revolution in products directed at the microbiome. Most of the world's 7 billion people are currently experiencing deep structural alterations in disease burdens, dietary patterns, and lifestyle. This has been dramatically documented in countries such as India and South Africa, where it is commonplace to find high levels of both stunting and obesity in the same population, indicative of nutritional deficits which manifest in two opposite forms. These changes are not limited to emerging economies, as shifting dietary patterns and consumer preferences in the United States show drastic changes in consumption of major nutrient classes and concurrent rises in chronic disease. In addition to a societal need, there is also a market opportunity. Rising healthcare costs and demands of an aging population have meant growing preference for either prevention or self-treatment of diseases. These products represent an entirely new field at the union of nutrition and medicine and their applications are likely to be profound. Because of such novelty, this surge of scientific inquiry into the structure and function of the microbiome must be matched by a focused and transparent effort to engage industry, health policymakers, and the general public.

Examples of analogous efforts will include issues such as maternal and child health, HIV, polio, and malaria. These lessons will be distilled into a focused set of practical actions that researchers, both individually and collectively, can take to advance the application of important scientific insights.

Probiotic Yogurt for the Developing World, Gregor Reid, PhD1,2, Megan Enos, BSc1,2, Jordan Bisanz, BSc1,2, Grace Ettinger, BSc1,2, Jeremy Burton, PhD1,2, Stephen Rulisa, MD3, George Praygod, MD4, Morris Karmazyn, PhD1,2,and John Changalucha, MSc5; 1Lawson Health Research Institute, London, Ontario, Canada, 2Western University, London, Ontario, Canada, 3National University of Rwanda Teaching Hospital, Kigali, Rwanda, 4Nyamagana District Hospital, Mwanza, Tanzania, and 5National Institute for Medical Research, Mwanza, Tanzania

Of the major causes of morbidity and mortality in the developing world, most are potentially preventable or treatable by use of milk-based probiotic interventions. However, implementation is problematic due to lack of availability of products (and their traditionally high, developed-world costs), lack of cold-chain distribution networks, and poor accessibility of the needy population to healthcare and healthy foods. In an effort to create a grass-roots solution, community kitchens have been established in Tanzania, Kenya, and Rwanda by students, staff, and faculty of Western University in Canada. Run mostly by women, supported by various local authorities and Canadian student interns, the kitchens currently produce yogurt containing probiotic Lactobacillus rhamnosus GR-1 for over 3000 men, women, and children each day. Research studies have shown that this not only provides important societal contributions (self-esteem, social businesses, reduced violence against women, income for farmers and families), but also health benefits ranging from increased energy, reduced infections, better tolerance of anti-retroviral therapy, and improved nutrition markers. The potential exists for probiotic foods to have an effect on ischaemic heart disease, pre-eclampsia and preterm labor, neonatal infection, malaria, and exposure to environmental toxins. The challenges of delivering probiotic food to the bottom-of-the-pyramid must be resolved or we fail humanity and our own self-worth.

Exploiting Probiotics for Childhood Immunization in Resource Limited Settings, Patricia L Hibberd, MD PhD1,2, Abraham Sonenshein, PhD3, Saul Tzipori, DVM, PhD, DSc4, and Elizabeth Hohmann, MD1,5; 1Harvard Medical School, Boston, Massachusetts, United States 2Division of Global Health, Massachusetts General Hospital Boston Massachusetts, United States, 3Department of Molecular Biology and Microbiology, Tufts Medical School, Boston, Massachusetts, United States, 4Department of Microbiology and Infectious Diseases, Tufts Cummings School of Veterinary Medicine, North Grafton, Massachusetts, United States, 5Infectious Diseases Division, Massachusetts General Hospital, Boston, Massachusetts

Vaccines are one of the most successful, cost effective ways of preventing mortality, disease, and long-term disability. When the World Health Organization's (WHO) Expanded Program on Immunizations (EPI) was launched in 1974, less than 5% of the world's children were immunized against polio, diphtheria, pertussis, measles, and tetanus. While childhood immunization has saved an estimated 20

million children's lives over the last 20 years, in 2011, over 23 million children received no immunizations at all. One of the main reasons that these 23 million children could not be reached is because of gaps in the cold-chain that have been estimated to spoil about half of the vaccines produced. The introduction of newer vaccines such as the pneumococcal conjugate and rotavirus vaccines are creating even more pressure on the available space in the cold-chain. Thus there is more urgency than ever before to create vaccines that do not require the cold-chain. Our group has exploited the ability of a probiotic natural product, Bacillus subtilis, to withstand extreme environmental conditions by genetically engineering the bacteria to deliver vaccine antigens – tetanus and rotavirus to date. We have conducted animal safety and immunogenicity studies indicating that the engineered vaccines can be administered intranasally, sublingually, and transdermally; are safe; and can produce protective levels of antibodies, in preparation for testing of the product under a FDA investigational new drug application in humans. The lyophilized vaccines are stable at 45°C without refrigeration for more than 1 year. This approach has the potential of being able to provide childhood vaccines that do not require the cold-chain, needles, or administration by skilled personnel to reach currently unreachable children.

Fecal Transplantation for Obesity and Type 2 Diabetes Mellitus, Shanti Undayappan1, Willem M de Vos, PhD2, and Max Nieuwdorp, MD1; 1University of Amsterdam, Amsterdam, The Netherlands, 2Wageningen University, Wageningen, The Netherlands

Recently, alterations in intestinal microbiota are associated with obesity and insulin resistance in both animals and humans. We recently showed that fecal transplantation (infusing intestinal microbiota from lean donors) in male recipients with metabolic syndrome has beneficial effects on the recipients' microbiota composition and insulin sensitivity up till 6 weeks after infusion (Vrieze et al., Gastroenterology, 2012). Moreover, we found that small intestinal levels of Eubacterium hallii (anaerobic gram positive belonging to Firmicute phylum) were increased upon lean donor fecal transplantation. We have now preliminary data suggesting that four weeks of daily gavage with cultured Eubacterium hallii is safe and has beneficial effects on glucose metabolism in male db/db mice, most likely via altered fecal short chain fatty acid production. Combined our data suggest that intestinal microbiota might be developed as therapeutic targets to increase insulin sensitivity in humans.

Faecalibacterium prausnitzii and other Bioactive Commensals for Immune-Mediated Diseases, Joël Doré, PhD, Philippe Langella, PhD, and Herve M. Blottiere, PhD, Institut National de la Recherche Agronomique (INRA), Jouy-en-Josas, France

The crosstalk between bacteria and human cells is central to the protective role of intestinal commensals for health and wellbeing. Yet, while much is known on the mode of action of pathogens, our understanding of beneficial mutualistic interactions is still very poor. Clinical trials and, most recent, comparisons of microbiomes between patients and healthy controls have highlighted potential anti-inflammatory commensals such as Faecalibacterium prausnitzii. This leads to targeted mechanistic exploration of crosstalk mechanisms. While Faecalibacterium prausnitzii is consistently

underrepresented in patients' microbiota, it also appeared as a robust biomarker of high gene-count microbiota, which is associated with the most beneficial outcomes in immune-mediated chronic disorders (low relapse rate in inflammatory bowel disease, best response to probiotic-driven microbiota stabilization in ulcerative colitis or to dietary restriction in obesity). Bacteria-cell crosstalk has further been investigated using a functional metagenomics approach. Human cell lines are engineered with stably transfected reporter genes allowing assessing modulation of transcription regulators such as NFkB, AP-1, or PPAR-gamma, or production of proteins such as TSLP, TGF-beta, or Fiaf. High-throughput screening of interactions between over 20,000 metagenomic clones bearing large genomic inserts of culturable and non-cultured bacteria and human cells have allowed the identification of several bioactive clones that modulate cellular activities with relevance to immune response, proliferation, or metabolism. Genes involved are identified and bioactive signal molecules are sought for, using biochemical or genetic approaches. We are uncovering mechanisms by which commensal bacteria modulate cellular functions with potential extension to the exploration of probiotic-host interactions.

Overcoming the Regulatory Roadblocks to Non-Drug Applications of Microbiome-Based Health Interventions, Fred H. Degnan, JD, King & Spalding LLP, Washington, District of Columbia, United States

The US Food and Drug Administration ("FDA") maintains an understandable and justifiable interest in the conduct of clinical studies. FDA's authority, however, with respect to such studies varies depending upon the regulatory classification ("drug," "new drug," "food," "food additive," "dietary supplement," etc.) that applies to the substance under investigation. The investigation of a probiotic product can fall along a continuum of regulatory classifications, each having implications on the attending nature and degree of regulatory requirements and, ultimately, for claim substantiation and market access. It follows that researchers need to understand applicable federal restraints on the contours of clinical research well before deciding on a given course of investigation.