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Gut microbiota correlates with energy gain from dietary fibre and appears to be associated with acute and chronic intestinal diseases

M. Ukhanova[1], T. Culpepper[1], D. Baer[2], D. Gordon[3], S. Kanahori[3], J. Valentine[4], J. Neu[5], Y. Sun[6], X. Wang[1] *and* V. Mai[1]

1) Microbiology and Cell Science, Emerging Pathogens Institute, University of Florida, Gainesville, FL, 2) US Department of Agriculture, Agricultural Research Service, Beltsville Human Nutrition Research Center, Beltsville, MD, USA, 3) Matsutani Chemical Industry Co. Ltd, Itami City, Hyogo, Japan, 4) Division of Gastroenterology, Gainesville, FL, 5) Division of Pediatrics, Gainesville, FL and 6) ICBR University of Florida, Gainesville, FL, USA

Abstract

Improvements in high-throughput sequencing technologies have spurred a large number of studies aimed at obtaining a better understanding of the composition and the dynamics in gut microbiota and its associations with various human diseases, especially those in the intestinal tract. Here we briefly summarize results from three different such studies from our group, all of which used 454 based high-throughput 16S rRNA sequence analysis combined with other microbiota profiling methods to determine faecal microbiota composition. In the first study, a controlled feeding trial, we establish that energy gain from the consumption of up to 50 g/day of a resistant maltodextrin depends on the prevalent microbiota composition. Over time, resistant maltodextrin supplementation increased the proportion of total faecal bacteria as well as potentially beneficial bifidobacteria. Thus, energy gain from resistant maltodextrin in an individual appears to vary over time and depend on the adaptation of gut microbiota. We then illustrate the power of molecular tools for identifying (i) distortions in early microbiota development in pre-term infants and the presence of potentially novel pathogens contributing to necrotizing enterocolitis and (ii) a specific microbiota signature, based on discriminant analysis of the 16S rRNA sequences, that correlates with the prevalence of an early risk marker associated with colorectal carcinogenesis, intestinal adenoma, in elderly adults.

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Corresponding author: Volker Mai, Department of Microbiology and Cell Science and Emerging Pathogens Institute, University of Florida, 2055 Mowry Rd, Gainesville, FL 32611-0700, USA
E-mail: vmai@ufl.edu

Introduction

The role of host-associated microbiota, especially those residing in the intestinal tract, has received renewed interest for potential associations with human health. Molecular 16S rRNA based tools have long helped to overcome earlier limitations of conventional microbiological plating methods in studying gut microbiota composition [1,2]. More recently, the developments of high-throughput parallel sequencing methodologies have increased our ability to analyse microbiota at a previously unimaginable depth. These tools have sparked novel microbiota research including suggestions of associations between the proportion of Bacteroidetes and obesity [3–5]. Although it appears clear from multiple studies that obesity status is associated with prevalent microbiota composition, at least in humans the evidence that gut microbes significantly contribute to the development of obesity needs further investigation. Gut microbes can contribute to obesity by two major means. First, they can generate additional energy in the form of short chain fatty acids from substrates, such as dietary fibre, that reach the large colon. Second, they can affect obesity-associated pathways in the host through signalling, such as the upregulation of Fiaf [6] in response to gut microbes. We present below data suggesting that energy gain from resistant maltodextrin (RM) is dependent on resident gut microbes and conversely that RM shapes microbiota composition.

Due to extensive research funding that recently became available in the USA through the National Institutes of

Health Human Microbiome Project, and similar projects in other regions of the world, there has been an explosion in the number of studies that evaluate associations between microbiota and various disease states. Early results from many of these studies suggest that microbiota is affected by a variety of diseases, but the cross-sectional character of many studies is not well suited for determining associations with disease aetiology. We present below early data from a prospective cohort study in pre-term infants that allowed us to investigate in detail the potential contributions of a distortion in early microbiota development and the presence of novel pathogens to the development of necrotizing enterocolitis (NEC). We then summarize findings from a colonoscopy screening study in adults that shows the utility of advanced 16S rRNA data mining tools [7] to reveal a microbiota signature pattern associated with the prevalence of colorectal polyps. All three studies were approved by the appropriate institutional review boards. Our findings illustrate the need for (i) well controlled feeding studies to link specific dietary components to changes in microbiota composition, and (ii) prospective cohort studies to establish causal links between microbiota and human disease states.

Resistant maltodextrin feeding study

While the amounts of energy derived from consuming fats, proteins and digestible carbohydrates are well established, the metabolizable energy and net energy value associated with the consumption of dietary fibre has been less studied [8]. RM is a soluble non-viscous dietary fibre that, although recalcitrant to human digestive enzymes, is a substrate for fermentation by the commensal microbiota, primarily that of the large intestine. Due to the chemical composition of RM, determination of its fibre content requires a specific analytical method [9]. We performed a human feeding study to determine if energy gain from increased dietary intake of RM was dependent on resident gut microbiota, and if microbiota adapted to increased RM by increasing microbes that can utilize this specific dietary fibre.

In a randomized, controlled dose–response study 14 healthy males were assigned to a random treatment sequence that consisted of three 28-day treatment periods separated by a wash-out period of at least 14 days, with controlled diets: a placebo diet (0 g/day RM + 50 g/day maltodextrin) and two levels of dietary RM (25 g/day RM + 25 g/day maltodextrin and 50 g/day RM + 0 g/day maltodextrin). When the energy value of RM was calculated by bomb calorimetry for each individual, we detected a wide range among individuals, from 0 to 4 kcal/g with a mean of 2.2 kcal/g. We hypothesized that differences in microbial composition and associated activities caused these differences in energy value for RM. Microbiota composition was determined using a comprehensive 16S rRNA based approach that included denaturing gradient gel electrophoresis (DGGE) for initial profiling and quality control, qPCR and fluorescent *in situ* hybridization for targeted quantification and 454 based 16S rRNA sequencing. DGGE indicated that a particular bacterial signature (band) increased upon RM supplementation. Purification and sequencing of the respective band suggested that bacteria grouping closest to Lachnospiraceae increased with RM. No consistent effects of RM were detected when faecal samples from four individuals were submitted to deep 16S rRNA sequencing. Total numbers of bacteria per gram of faeces increased by 22% in subjects consuming 50 g/day RM (p 0.02). While overall microbiota diversity was little affected, the numbers of bifidobacteria increased during RM periods (qPCR, p 0.03) (Fig. 1). Differences in microbiota composition, specifically higher numbers of bifidobacteria in the group gaining more energy, correlated with the variations in energy gain from RM intake. Our findings are consistent with the hypothesis that energy gain from fibre that reaches the large intestine varies between individuals and is dependent on the resident gut microbiota. The intake of RM microbiota changed the microbiota in individuals, probably towards a composition more adapted to utilizing the additional dietary fibre. This observation suggests that as the microbiota adapts energy gain from fibre, it is changing over time even within an individual.

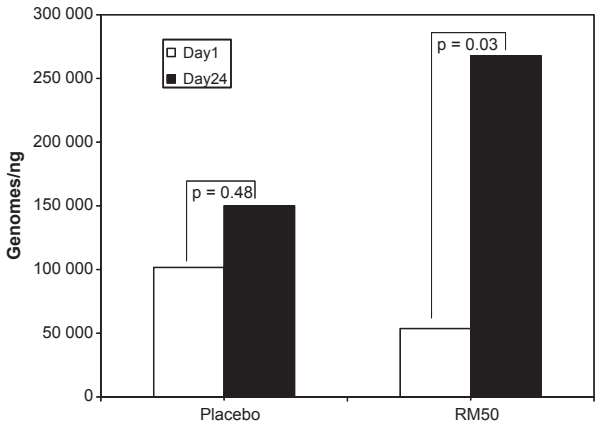

FIG. 1. Change in bifidobacteria by qPCR. The number of genome equivalents for bifidobacteria per nanogram of input DNA are shown for days 1 and 24 during the placebo and the period with 50 g/day of resistant maltodextrin (RM50). p-values are indicated within the intervention periods.

Microbiota and necrotizing enterocolitis in pre-term infants

Pre-term infants are at an increased risk for developing a variety of perinatal diseases. NEC is a severe disease mostly observed in pre-term infants; almost 5000 NEC cases are identified each year in the USA alone with a mortality rate of 15–30%. The disease can leave surviving infants with long-term health problems and is a large burden on the health-care system. NEC is an inflammatory disease of the bowel with a diffuse disease presentation [10]. Our earlier studies evaluated microbiota composition in pre-term infants [11]. We are currently performing a prospective cohort study in infants delivered at a gestational age of 32 weeks or younger with a birth weight of 1250 g or less at three hospitals (Gainesville and Jacksonville, FL, USA). In a preliminary analysis we matched nine NEC cases identified by a neonatologist, using modified Bell's criteria phase II and III, with nine controls by gestational age, birth weight, date and centre. We analysed microbiota in faecal samples collected from cases 1–2 weeks before and during the week of diagnosis and in gestational age matched samples from controls. Initial DGGE profiling did not indicate the presence of distinct molecular signatures in cases either before or during the week of NEC diagnosis. However, an extensive 454 based 16S rRNA analysis suggested that at the time point before NEC diagnosis the proportion of Proteobacteria, Actinobacteria and Bacteriodetes was lower in cases than in controls [12???]. Based on Unifrac analysis there appeared to be more heterogeneity in microbiota composition before NEC diagnosis among cases compared with controls. Overall diversity did not differ between cases and controls [12]. At the pre-diagnosis time point the proportion of Firmicutes was higher in cases (61%) than in controls (31.5%). During the week of diagnosis we observed a bloom in Proteobacteria (from 36% to 71%) in cases only; their Proteobacteria proportion was now higher than that in controls (71% vs 56%) while Firmicutes decreased (61–29%). Actinobacteria and Bacteriodetes remained low in cases (<1%). When we analysed specific operational taxonomic units (OTUs) we detected 11 OTUs exclusively in cases during the week of NEC diagnosis but not 2 weeks before and not in controls. These OTUs grouped to various known Proteobacteria and other known pathogens, but some represented 'unknowns' grouping to γ-Proteobacteria, a group of bacteria that contains many established enteric pathogens. Our findings suggest an association between a lack of early colonization and a later bloom of Proteobacteria and NEC. Furthermore, molecular signatures suggest that currently unknown pathogens grouping closest to γ-Proteobacteria might contribute to NEC in a subset of pre-term infants. These still speculative findings need to be corroborated in more cases from our and other ongoing studies in pre-term infants.

Gut microbiota and colorectal carcinogenesis

We have previously shown evidence from a study in APCMin mice that caloric restriction, diet composition and exercise affect both intestinal microbiota composition and polyp numbers [13]. Some bacterial signatures appeared to be associated with reduced intestinal carcinogenesis in that mouse model, indicating that the physiological conditions in the gut of mice with multiple polyps might vary from those in 'healthy' mice, probably affecting microbiota composition. We have further shown in four individuals undergoing a screening colonoscopy that microbiota composition after a colonoscopy procedure differs from that before for up to 4 weeks [14]. In another study we established that microbiota composition correlates with dietary habits and differs between African Americans (AA) and Caucasian Americans (CA) [15]. AA suffer from an increased risk of colorectal cancer (CRC). Thus, correlating dietary habits in AA with microbiota composition and markers of colorectal carcinogenesis might help to identify targets for a microbiota-directed prevention regimen to reduce their disease burden. Colorectal carcinogenesis is a long process that involves multiple mutations that probably affect gut physiology [16]. At the time that CRC is symptomatic the physiological conditions in the gut have changed and the microbiota has probably adapted to those conditions. Thus, analysis of microbiota of subjects that already suffer from CRC is unlikely to identify many microbes that contributed to the onset of the disease. In contrast, an analysis of microbiota in subjects with early lesions, small to medium sized adenomas that are unlikely to have a large effect on gut physiology, might be better suited for identifying contributions of microbiota to the development of CRC. Ideally, a prospective study design should be performed to follow changes in microbiota in a large number of subjects and identify what changes in microbiota occur at the earlier stages of colorectal carcinogenesis.

We have recently collected pre-colonoscopy stool samples as well as biopsy samples taken during the procedure in more than 100 individuals to determine if a microbial signature profile is associated with an individual's risk of harbouring at least one adenoma and if such signature is stronger in subjects with high risk adenomas. We selected 30 cases with

at least one adenoma and compared their microbiota to that in 30 matched controls (age, gender, body mass index category). In our studies we defined subjects to be at high risk if they had multiple polyps or at least one polyp larger than 5 mm. Using 454 based 16S rRNA analysis we initially determined that microbiota in biopsy samples differs from that in stool samples (Fig. 2). Thus, microbiota samples from biopsies need to be analysed separately from faecal samples. When evaluating overall microbiota composition, using Unifrac analysis of >250 000 sequence reads (average length 214 nucleotides), we did not detect any pattern that allowed us to differentiate subjects by case status (polyp/no polyp) or race (AA/CA) in either stool or biopsy sample. We did, however, detect many operational taxonomic levels (98% similarity) in which prevalence differed by case or race. The fold differences were larger when we compared microbiota in controls with high risk cases rather than all cases. This observation is encouraging as it suggests that there are more dramatic differences in subjects with high risk lesions, which more strongly correlate with CRC. Because we performed these analyses on a few thousand OTUs most findings are probably false positives. We are currently working on developing the appropriate statistics for such analyses that appropriately correct for the multiple analyses performed. We then used a novel discriminant analysis [7] to develop a microbial pattern that can distinguish microbiota in the stools of cases from that in matched controls. At a level of 0.08 sequence dissimilarity we detected a microbiota pattern based on 27 OTUs that allowed for a distinction between cases and controls with a high correlation (area under the curve 0.81).

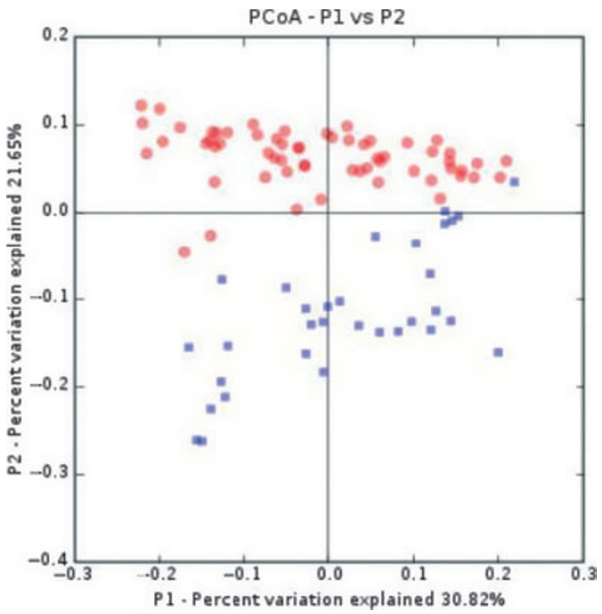

FIG. 2. Unifrac based analysis of microbiota composition in biopsy and stool samples. Principal components analysis plot based on weighted Unifrac analysis based on 454 16S rRNA sequences from biopsies (squares) and stool samples (circles) in the colonoscopy study.

Conclusions

Our studies are consistent with the hypothesis that diet, particularly dietary fibres that reach the colon, can affect microbiota composition. Energy gain from dietary fibre varies between individuals as well as within an individual over time. Microbiota pattern as well as signature sequences appear to be associated with NEC and colorectal polyp prevalence. A prospective study design appears necessary to determine if microbiota contributes to the aetiology of various diseases. Such studies should now be developed to allow us to determine within the next decade(s) if and how microbiota affects human health. Once we have a thorough understanding of causative associations, microbiota-based diagnostics and a preventive regimen can be developed that might extend healthy life years and reduce the burden of various diseases.

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Transparency Declaration

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