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Identification of an Intestinal Microbiota Signature Associated With Severity of Irritable Bowel Syndrome

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BACKGROUND & AIMS: We have limited knowledge about the association between the composition of the intestinal microbiota and clinical features of irritable bowel syndrome (IBS). We collected information on the fecal and mucosa-associated microbiota of patients with IBS and evaluated whether these were associated with symptoms. **METHODS:** We collected fecal and mucosal samples from adult patients who met the Rome III criteria for IBS at a secondary/tertiary care outpatient clinics in Sweden, as well as from healthy subjects. The exploratory set comprised 149 subjects (110 with IBS and 39 healthy subjects); 232 fecal samples and 59 mucosal biopsy samples were collected and analyzed by 16S ribosomal RNA targeted pyrosequencing. The validation set comprised 46 subjects (29 with IBS and 17 healthy subjects); 46 fecal samples, but no mucosal samples, were collected and analyzed. For each subject, we measured exhaled H_2 and CH_4, oro-anal transit time, and the severity of psychological and gastrointestinal symptoms. Fecal methanogens were measured by quantitative polymerase chain reaction. Numerical ecology analyses and a machine learning procedure were used to analyze the data. **RESULTS:** Fecal microbiota showed covariation with mucosal adherent microbiota. By using classic approaches, we found no differences in fecal microbiota abundance or composition between patients with IBS vs healthy patients. A machine learning procedure, a computational statistical technique, allowed us to reduce the 16S ribosomal RNA data complexity into a microbial signature for severe IBS, consisting of 90 bacterial operational taxonomic units. We confirmed the robustness of the intestinal microbial signature for severe IBS in the validation set. The signature was able to discriminate between patients with severe symptoms, patients with mild/moderate symptoms, and healthy subjects. By using this intestinal microbiota signature, we found IBS symptom severity to be associated negatively with microbial richness, exhaled CH_4, presence of methanogens, and enterotypes enriched with Clostridiales or *Prevotella* species. This microbiota signature could not be explained by differences in diet or use of medications. **CONCLUSIONS:** In analyzing fecal and mucosal microbiota from patients with IBS and healthy individuals, we identified an intestinal microbiota profile that is associated with the severity of IBS symptoms. Trial registration number: NCT01252550.

Keywords: Functional Bowel Disorder; Bacteria; Microbiome.

Irritable bowel syndrome (IBS) is the most prevalent functional gastrointestinal disorder in Western societies. It affects approximately 11% of the adult population and strongly impairs quality of life, social function, work productivity, and brings substantial costs to health care services.[1] The etiology of IBS remains poorly understood and the search for biomarkers is ongoing.[2]

It is now well accepted that IBS is a disorder involving multiple pathophysiological mechanisms in which composition of gut microbiota has been proposed as one of the potentially important factors.[3,4] Since the first study that investigated the fecal microbiota composition of IBS patients and healthy subjects using a molecular-based approach,[5] many studies have followed using targeted approaches,[6] specifically quantitative polymerase chain reaction (PCR).[6,7] More recently, the use of advanced tools has allowed a better overview of gut microbiota composition,[6,8,9] function,[10] and metabolite production[11,12] in IBS. Even though gut microbiota alterations seem to exist in IBS, no uniform gut microbiota pattern in IBS has been shown. The existing inconsistencies among currently available data in IBS may be attributed to several factors including heterogeneity of gut microbiota profiling methods, inherent individual microbiota variability, and differences in inclusion criteria, as well as sample size. This highlights the difficulty in finding robust microbiota markers associated with IBS clinical parameters,[3]

*Authors share co-first authorship; §Authors share co-senior authorship.

Abbreviations used in this paper: **AUC, area under the curve; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; FODMAP, Fermentable, Oligo-, Di-, Mono-saccharides And Polyols; GI, gastrointestinal; HAD, Hospital Anxiety and Depression; IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; IBS-M, mixed irritable bowel syndrome; IBS-SSS, Irritable Bowel Syndrome Severity Scoring System; JSD, Jensen Shannon divergence; LASSO, least absolute shrinkage and selection operator; OATT, oro-anal transit time; OTU, operational taxonomic unit; PC, principal component; PCR, polymerase chain reaction; rRNA, ribosomal RNA; RV, regression vector.**

Most current article

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CLINICAL AT

Current Biology
Report

Gut Microbiota and Extreme Longevity

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SUMMARY

The study of the extreme limits of human lifespan may allow a better understanding of how human beings can escape, delay, or survive the most frequent age-related causes of morbidity, a peculiarity shown by long-living individuals. Longevity is a complex trait in which genetics, environment, and stochasticity concur to determine the chance to reach 100 or more years of age [1]. Because of its impact on human metabolism and immunology, the gut microbiome has been proposed as a possible determinant of healthy aging [2, 3]. Indeed, the preservation of host-microbes homeostasis can counteract inflammaging [4], intestinal permeability [5], and decline in bone and cognitive health [6, 7]. Aiming at deepening our knowledge on the relationship between the gut microbiota and a long-living host, we provide for the first time the phylogenetic microbiota analysis of semi-supercentenarians, i.e., 105–109 years old, in comparison to adults, elderly, and centenarians, thus reconstructing the longest available human microbiota trajectory along aging. We highlighted the presence of a core microbiota of highly occurring, symbiotic bacterial taxa (mostly belonging to the dominant *Ruminococcaceae*, *Lachnospiraceae*, and *Bacteroidaceae* families), with a cumulative abundance decreasing along with age. Aging is characterized by an increasing abundance of subdominant species, as well as a rearrangement in their co-occurrence network. These features are maintained in longevity and extreme longevity, but peculiarities emerged, especially in semi-supercentenarians, describing changes that, even accommodating opportunistic and allochthonous bacteria, might possibly support health maintenance during aging, such as an enrichment and/or higher prevalence of health-associated groups (e.g., *Akkermansia*, *Bifidobacterium*, and *Christensenellaceae*).

RESULTS AND DISCUSSION

Twenty-four semi-supercentenarians (105+; group S), i.e., 105–109 years old (18 females and 6 males; mean age 106.2), were enrolled for this study in Emilia Romagna and surrounding area, Italy. Fifteen young adults (group Y; eight females and seven males; aged 22–48 years; average age 30.5) were enrolled in the same geographic area. The study protocol was approved by the Ethical Committee of Sant'Orsola-Malpighi University Hospital (Bologna, Italy) as EM/26/2014/U (with reference to 22/2007/U/Tess). Feces were collected, and total bacterial DNA was extracted from all samples (see the Supplemental Experimental Procedures).

To complete a human aging trajectory, we included extracted fecal DNA, stored at −80°C, from 15 centenarians (group C; 14 females and 1 male; aged 99–104 years; mean age 100.4) and 15 younger elderly (group E; seven females and eight males; aged 65–75 years; mean age 72.5; see also Table S2) enrolled in the same geographic area (Emilia Romagna, Italy), obtained by Biagi et al. [4], in the present study.

For detailed information on physical and cognitive status of the subjects enrolled and a summary of the reported dietary habits, see the Supplemental Experimental Procedures and Tables S1 and S2; in brief, young adults were healthy and medication-free, whereas the physical and cognitive health status of 105+ (as well as that of the centenarians enrolled in the previous study) [4], assessed by ADL (activities of daily living) scale [8] and standardized mini-mental state examination test (SMMSE) [9], mirrored that of the majority of Italian centenarians, as previously characterized by Franceschi et al. [10].

The fecal microbiota of the 69 samples was characterized by Illumina sequencing of the V3–V4 region of the bacterial 16S rRNA gene (see the Supplemental Experimental Procedures; sequences are available at the following MG-Rast link: http://metagenomics.anl.gov/linkin.cgi?project=17761). A total of 1,246,682 high-quality reads were obtained with a mean of 18,068 reads per subject. Reads were clustered in 11,587 operational taxonomic units at 97% of identity.

The four age groups showed a good separation on a principal coordinates analysis (PCoA) based on the unweighted UniFrac distance (Figure 1); indeed, corrected p values obtained by permutation test were <0.05 for all possible comparisons with the exception of groups C versus S. PCo1 separated young subjects

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Omega-3 fatty acids correlate with gut microbiome diversity and production of N-carbamylglutamate in middle aged and elderly women

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Omega-3 fatty acids may influence human physiological parameters in part by affecting the gut microbiome. The aim of this study was to investigate the links between omega-3 fatty acids, gut microbiome diversity and composition and faecal metabolomic profiles in middle aged and elderly women. We analysed data from 876 twins with 16S microbiome data and DHA, total omega-3, and other circulating fatty acids. Estimated food intake of omega-3 fatty acids were obtained from food frequency questionnaires. Both total omega-3and DHA serum levels were significantly correlated with microbiome alpha diversity (Shannon index) after adjusting for confounders (DHA Beta(SE) = 0.13(0.04), P = 0.0006 total omega-3: 0.13(0.04), P = 0.001). These associations remained significant after adjusting for dietary fibre intake. We found even stronger associations between DHA and 38 operational taxonomic units (OTUs), the strongest ones being with OTUs from the *Lachnospiraceae* family (Beta(SE) = 0.13(0.03), $P = 8 \times 10^{-7}$). Some of the associations with gut bacterial OTUs appear to be mediated by the abundance of the faecal metabolite N-carbamylglutamate. Our data indicate a link between omega-3 circulating levels/intake and microbiome composition independent of dietary fibre intake, particularly with bacteria of the *Lachnospiraceae* family. These data suggest the potential use of omega-3 supplementation to improve the microbiome composition.

There is evidence indicating that dietary supplementation with omega-3 polyunsaturated fatty acids (PUFA) may improves some health parameters in humans[1]. Docosahexaenoic acid (DHA) is an omega-3 fatty acid that is a main structural component of the human brain, cerebral cortex, skin, sperm, testicles and retina[2]. Higher circulating levels of DHA are associated with lower risk of future cardiovascular events in three prospective population based cohorts[3]. The other main omega-3 fatty acid is eicosapentaenoic acid or EPA, and omega-3 levels in humans are estimated by the sum of EPA + DHA with docosapentaenoic acid (DPA) being present at much lower concentrations[4]. Positive effects on health from omega-3 fatty acids have been observed for insulin resistance, adult-onset diabetes mellitus[5–7], hypertension[8,9] arthritis[10,11], atherosclerosis[12,13], depression[14,15], thrombosis[16], some cancers[17] and cognitive decline[18,19].

These fatty acids can only be synthesized in mammals from the dietary precursor and essential fatty acid, α-linolenic acid[1]. However, the synthesis pathway requires a number of elongation and desaturation steps, making direct uptake from the diet a more effective route of assimilation. EPA and DHA in the human diet are derived primarily from marine algae (higher plants lack the enzymes for the biosynthesis of these lipids), which is concentrated in the flesh of marine fish where bioavailability is dramatically increased[20]. Some of the mechanisms

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ORIGINAL ARTICLE

High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome

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ABSTRACT

Objectives Habitual diet plays a major role in shaping the composition of the gut microbiota, and also determines the repertoire of microbial metabolites that can influence the host. The typical Western diet corresponds to that of an omnivore; however, the Mediterranean diet (MD), common in the Western Mediterranean culture, is to date a nutritionally recommended dietary pattern that includes high-level consumption of cereals, fruit, vegetables and legumes. To investigate the potential benefits of the MD in this cross-sectional survey, we assessed the gut microbiota and metabolome in a cohort of Italian individuals in relation to their habitual diets.

Design and results We retrieved daily dietary information and assessed gut microbiota and metabolome in 153 individuals habitually following omnivore, vegetarian or vegan diets. The majority of vegan and vegetarian subjects and 30% of omnivore subjects had a high adherence to the MD. We were able to stratify individuals according to both diet type and adherence to the MD on the basis of their dietary patterns and associated microbiota. We detected significant associations between consumption of vegetable-based diets and increased levels of faecal short-chain fatty acids, *Prevotella* and some fibre-degrading Firmicutes, whose role in human gut warrants further research. Conversely, we detected higher urinary trimethylamine oxide levels in individuals with lower adherence to the MD.

Conclusions High-level consumption of plant foodstuffs consistent with an MD is associated with beneficial microbiome-related metabolomic profiles in subjects ostensibly consuming a Western diet.

Trial registration number This study was registered at clinical trials.gov as NCT02118857.

Significance of this study

What is already known on this subject?

- Diet can significantly impact the gut microbiota and metabolome.
- Negligible differences in gut microbiota and faecal short-chain fatty acids were previously reported between habitual omnivores and vegans.
- Mediterranean diet is a recognised healthy dietary pattern that has not previously been related to the composition of the gut microbiota and related metabolome.

What are the new findings?

- Habitual vegetarian and vegan diets promote enrichment of fibre-degrading bacteria in the gut.
- Subjects who consume a Mediterranean diet rich in fruit, legumes and vegetables have higher levels of faecal short-chain fatty acids, regardless of the diet type.
- Low adherence to the Mediterranean diet corresponds to an increase in urinary trimethylamine oxide levels, a potential risk factor for cardiovascular disease.

How might it impact on clinical practice in the foreseeable future?

- Microbiota modulation through consumption of diets rich in diverse vegetable foods offers the prospect of increasing health and mitigating disease risk.

INTRODUCTION

The role of diet in shaping the gut microbiota is widely recognised, and several recent reviews provide a comprehensive treatment of the subject.[1–6] However, until recently, not many studies have broadly and systematically considered the association between habitual diet and gut microbiota.[2 7]

Some populations eat differently because they have different access to foods, and this can determine significant differences in the taxonomic composition of their gut microbiota, distinguishing agrarian and Western diets.[8–10] Specific compositional patterns of the gut microbiota have also been associated with habitual diet, clearly linking different compositions of the microbiota with animal fat and protein-based diets versus vegetable-based diets.[11] In addition, several studies have shown an association between consumption of fibre, fruit and vegetables and increased microbial richness, at either taxonomic or gene level.[12–14] It is well

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Review

MOLECULAR METABOLISM

A healthy gastrointestinal microbiome is dependent on dietary diversity

Mark L. Heiman [1,*], **Frank L. Greenway** [2]

ABSTRACT

Background: Like all healthy ecosystems, richness of microbiota species characterizes the GI microbiome in healthy individuals. Conversely, a loss in species diversity is a common finding in several disease states. This biome is flooded with energy in the form of undigested and partially digested foods, and in some cases drugs and dietary supplements. Each microbiotic species in the biome transforms that energy into new molecules, which may signal messages to physiological systems of the host.

Scope of review: Dietary choices select substrates for species, providing a competitive advantage over other GI microbiota. The more diverse the diet, the more diverse the microbiome and the more adaptable it will be to perturbations. Unfortunately, dietary diversity has been lost during the past 50 years and dietary choices that exclude food products from animals or plants will narrow the GI microbiome further.

Major conclusion: Additional research into expanding gut microbial richness by dietary diversity is likely to expand concepts in healthy nutrition, stimulate discovery of new diagnostics, and open up novel therapeutic possibilities.

Keywords Microbiome; Microbiota; Gastrointestinal; Dietary diversity; Agrobiodiversity; Microbiota richness

Twenty-five years ago, Epstein et al. described treating a group of 25 children between the ages of 6–12 years of age who were 45% overweight in conjunction with one of their parents who was also overweight. The treatment period lasted for 8 months and consisted of diet instruction, behavioral management training, exercise and contingency contracting in which money was deposited and $5 returned at visits if weight had been lost. The parents and children both lost weight during the treatment period, but ten years after the treatment the children were still maintaining a 7.5% reduction in their percent overweight below baseline. In contrast, the parent that was treated at the same time with the child increased their percent overweight from baseline by 9.1% [1]. The reason for the ability of pre-pubertal subjects to maintain weight loss but not their parents who were treated with them in the same program remains a medical mystery.

That study was performed before there was an appreciation of the interactions between the gastrointestinal (GI) microbiome and physiological systems regulating metabolism. At the end of 2007, the US National Institutes of Health (NIH) launched the Human Microbiome Project (HMP) and, in early 2008, the European Commission and China initiated the Metagenomics project of the Human Intestinal Tract (MetaHIT). These large efforts apply advanced sequencing and bioinformatic tools to characterize the microbes living in and on our bodies. Each community of microbiota is studied as an ecosystem, utilizing the science and language of ecology [2]. Biodiversity is a critical aspect of ecosystem function and has been the intense focus of the HMP and MetaHIT projects [3,4]. Like all healthy ecosystems, some level of species richness characterizes the GI microbiome in healthy individuals [5]. Additions or losses of species with similar roles tend to only have small effects on microbiome function. However, domination by few species or lack of species diversity may impact function significantly.

The pre-adolescent children that were capable of maintaining weight loss may have a much greater level of species richness than their parent. Indeed, biodiversity of the GI microbiome of healthy pre-adolescent children aged 6–12 years of age is much greater than that of healthy adults living in the same city [6]. A greater the biodiversity renders a greater resilience of the ecosystem to recover from or adjust to perturbations. Conversely, a loss in species richness in the GI microbiome is a common finding in several disease states.

During the past 50 years, prevalence of obesity [7], type 2 diabetes [8], and inflammatory bowel diseases [9] sharply increased. A shared discovery for each of these pathologies is a reduction of the GI microbiome biodiversity [10,11,12; respectively]. This biome is flooded with energy in the form of undigested and partially digested foods, and in some cases drugs and dietary supplements. Each microbiotic species in the biome transforms that energy into new molecules, which

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Abbreviations: FXR, farnesoid X receptor; FODMAP, fermentable oligo-, di-, monosaccharides and polyols; FDA, Food and Drug Administration; GI, gastrointestinal; GIMM, GI microbiome modulator; GLP-I, glucagon-like peptide-1; GLUT, glucose transporter; HMP, Human Microbiome Project; MCFA, medium chain fatty acids; MetaHIT, Metagenomics project of the Human Intestinal Tract; NIH, National Institutes of Health; PYY, peptide YY; RYGB, Roux-en-Y gastric bypass; SCFA, short chain fatty acid; SGLTs, sodium–glucose cotransporter; TMA, trimethylamine; TMAO, trimethylamine-N-oxide; VSG, vertical sleeve gastrectomy

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Microbial-Host Co-metabolites Are Prodromal Markers Predicting Phenotypic Heterogeneity in Behavior, Obesity, and Impaired Glucose Tolerance

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SUMMARY

The influence of the gut microbiome on metabolic and behavioral traits is widely accepted, though the microbiome-derived metabolites involved remain unclear. We carried out untargeted urine ^{1}H-NMR spectroscopy-based metabolic phenotyping in an isogenic C57BL/6J mouse population (n = 50) and show that microbial-host co-metabolites are prodromal (i.e., early) markers predicting future divergence in metabolic (obesity and glucose homeostasis) and behavioral (anxiety and activity) outcomes with 94%–100% accuracy. Some of these metabolites also modulate disease phenotypes, best illustrated by trimethylamine-*N*-oxide (TMAO), a product of microbial-host co-metabolism predicting future obesity, impaired glucose tolerance (IGT), and behavior while reducing endoplasmic reticulum stress and lipogenesis in 3T3-L1 adipocytes. Chronic in vivo TMAO treatment limits IGT in HFD-fed mice and isolated pancreatic islets by increasing insulin secretion. We highlight the prodromal potential of microbial metabolites to predict disease outcomes and their potential in shaping mammalian phenotypic heterogeneity.

INTRODUCTION

Phenotypic heterogeneity is generally attributed to gene-environment interactions. However, phenotype variability is also commonly observed in identical twins and in isogenic model systems (Lehner, 2013), which can be exacerbated by high-fat diet (HFD) feeding in mice (Burcelin et al., 2002). This phenomenon is associated with changes in gut microbial communities in isogenic mouse populations (Serino et al., 2012) and in monozygotic twins (Ridaura et al., 2013). With ~10 million genes (Li et al., 2014), there is growing evidence that the gut microbiome contributes to obesity (Cotillard et al., 2013; Le Chatelier et al., 2013; Turnbaugh et al., 2006) and type 2 diabetes (Karlsson et al., 2013; Qin et al., 2012) in the context of Western-style diets rich in saturated fats (David et al., 2014; Muegge et al., 2011). Fecal microbiota transplantations (Smith et al., 2013; Turnbaugh et al., 2006) and metagenomic studies have highlighted the roles of microbiome architecture and richness (Cotillard et al., 2013; Le Chatelier et al., 2013).

However, beyond beneficial bacteria (Dao et al., 2016; Shoaie et al., 2015), the microbiome-derived mediators promoting host health or disease remain elusive: a few microbial metabolite families (e.g., short-chain fatty acids or bile acids) are known to affect human health (Dumas et al., 2014; Russell et al., 2013). To drive a shift in host physiology and potentially affect pathogenesis, microbial metabolite variation should precede changes in host metabolism and physiology and these metabolites should directly modulate traits associated with the disease. In this context, phenotypic heterogeneity observed in discordant twins or in populations of isogenic mice fed HFD offers a unique opportunity to evaluate microbial metabolites as early predictive (i.e., prodromal) markers of disease onset and progression and to assess their impact on disease (Hsiao et al., 2013; Venkatesh et al., 2014; Yoshimoto et al., 2013).

To evaluate microbial metabolites as prodromal markers, we repurposed a pharmaco-metabonomics framework (Clayton et al., 2006), which we developed initially for drug toxicity prediction using pre-dose metabolic phenotypes, to predict complex metabolic and behavior phenotype outcomes following HFD feeding in isogenic mouse populations. We best exemplify the influence of microbial-host co-metabolites through trimethylamine-*N*-oxide (TMAO), a phase 1 oxidation product of gut

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Lien entre les probiotiques et le microbiote : vision du clinicien

Link between probiotics and microbiota: perspective from a clinician

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MOTS-CLÉS
Microbiote ;
Dysbiose ;
Probiotiques ;
Pathologies

Résumé
Un microbiote est l'ensemble des micro-organismes (bactéries, levures, champignons, virus) vivant dans un environnement spécifique. Il existe ainsi de nombreux microbiotes comme un microbiote du sol, un microbiote de l'océan, etc.
Il en existe aussi de nombreux associés au corps humain : microbiote cutané, microbiote vaginal et, le plus étudié actuellement, le microbiote intestinal, appelé auparavant « flore intestinale ». La vision d'un clinicien doit intégrer le fait que la physiologie et la santé résultent des relations symbiotiques existant entre les Hommes et leurs microbiotes. La santé des Hommes, en considérant qu'ils sont des écosystèmes, est l'expression d'une bonne homéostasie entre ces microbiotes et les cellules humaines.
Fort de ce constat, il apparaît intéressant de chercher à influer sur le microbiote. Une approche possible est l'utilisation de produits (aliments ou compléments) probiotiques et/ou prébiotiques. Les premiers sont des micro-organismes qui, en quantités suffisantes, apportent un bénéfice sur la santé de l'hôte qui les ingère. Les prébiotiques sont des substrats qui facilitent le développement de micro-organismes favorables à la santé. L'objectif de cet article est de faire un bilan des connaissances actuelles sur microbiote et probiotique et leur applicabilité en pratique médicale.

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OPEN ACCESS

ORIGINAL ARTICLE

Targeting the gut microbiota with inulin-type fructans: preclinical demonstration of a novel approach in the management of endothelial dysfunction

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ABSTRACT

Objective To investigate the beneficial role of prebiotics on endothelial dysfunction, an early key marker of cardiovascular diseases, in an original mouse model linking steatosis and endothelial dysfunction.

Design We examined the contribution of the gut microbiota to vascular dysfunction observed in apolipoprotein E knockout ($Apoe^{-/-}$) mice fed an n-3 polyunsaturated fatty acid (PUFA)-depleted diet for 12 weeks with or without inulin-type fructans (ITFs) supplementation for the last 15 days. Mesenteric and carotid arteries were isolated to evaluate endothelium-dependent relaxation ex vivo. Caecal microbiota composition (Illumina Sequencing of the 16S rRNA gene) and key pathways/mediators involved in the control of vascular function, including bile acid (BA) profiling, gut and liver key gene expression, nitric oxide and gut hormones production were also assessed.

Results ITF supplementation totally reverses endothelial dysfunction in mesenteric and carotid arteries of n-3 PUFA-depleted $Apoe^{-/-}$ mice via activation of the nitric oxide (NO) synthase/NO pathway. Gut microbiota changes induced by prebiotic treatment consist in increased NO-producing bacteria, replenishment of abundance in *Akkermansia* and decreased abundance in bacterial taxa involved in secondary BA synthesis. Changes in gut and liver gene expression also occur upon ITFs suggesting increased glucagon-like peptide 1 production and BA turnover as drivers of endothelium function preservation.

Conclusions We demonstrate for the first time that ITF improve endothelial dysfunction, implicating a short-term adaptation of both gut microbiota and key gut peptides. If confirmed in humans, prebiotics could be proposed as a novel approach in the prevention of metabolic disorders-related cardiovascular diseases.

Significance of this study

What is already known on this subject?

- Metabolic diseases such as diabetes or non-alcoholic fatty liver disease are associated with macro- and microcirculation and microcirculation damages, initiating an impairment of endothelium-dependent relaxation, a primary driver of cardiovascular diseases.
- Dietary n-3 polyunsaturated fatty acid (PUFA) depletion induces hepatic steatosis and accelerates the development of endothelial dysfunction in mesenteric arteries of apolipoprotein E knockout ($Apoe^{-/-}$) mice.
- Gut microbiota plays a crucial role in the control of host intestinal functions, through the release and/or transformation of metabolites (eg, bile acids and short chain fatty acids) which regulate gut endocrine function, these pathways being mostly studied in the context of obesity.

What are the new findings?

- Inulin-type fructans (ITFs) are able to restore the endothelial dysfunction observed in mesenteric and carotid arteries from n-3 PUFA-depleted $Apoe^{-/-}$ mice without impacting adiposity.
- The improvement of vascular dysfunction by ITF is linked to an activation of the NOS/NO pathway pathway, which could be dependent on events occurring at the microbiota level (increase in NO-producing bacteria) and/or host level (changes in bile acid composition, increase in the L cells density and in glucagon-like peptide 1 production, both acting on the NOS/NO pathway).
- Our data support, for the first time in the context of vascular dysfunction, the concept that changing the gut microbiota has a profound influence on key intestinal functions involved in host cardiometabolic health.

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INTRODUCTION

Nutritional quality of diets underlies or exacerbates several chronic pathologies, including cardiovascular disease (CVD).[1 2] Western diets that are mainly characterised by an imbalance between energy and fat intakes and a lack of key nutrients,

ARTICLE

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Antibiotics in early life alter the murine colonic microbiome and adiposity

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Antibiotics administered in low doses have been widely used as growth promoters in the agricultural industry since the 1950s, yet the mechanisms for this effect are unclear. Because antimicrobial agents of different classes and varying activity are effective across several vertebrate species, we proposed that such subtherapeutic administration alters the population structure of the gut microbiome as well as its metabolic capabilities. We generated a model of adiposity by giving subtherapeutic antibiotic therapy to young mice and evaluated changes in the composition and capabilities of the gut microbiome. Administration of subtherapeutic antibiotic therapy increased adiposity in young mice and increased hormone levels related to metabolism. We observed substantial taxonomic changes in the microbiome, changes in copies of key genes involved in the metabolism of carbohydrates to short-chain fatty acids, increases in colonic short-chain fatty acid levels, and alterations in the regulation of hepatic metabolism of lipids and cholesterol. In this model, we demonstrate the alteration of early-life murine metabolic homeostasis through antibiotic manipulation.

Antibiotics, discovered in the early twentieth century, came into widespread use after the Second World War, with substantial public health benefits. Antibiotic use has increased markedly, now approximating one antibiotic course per year in the average child in the United States[1,2]. However, there is increasing concern that antibiotic exposure may have long-term consequences[3–5].

For more than 50 years we have known that the administration of low doses of antibacterial agents promotes the growth of farm animals, consequently, in the United States, the largest use of antibiotics and related antimicrobial substances is within farms, with low doses fed to large numbers of animals used for food production to increase weight gain by as much as 15%[6,7]. These effects are broad across vertebrate species, including mammals (cattle, swine, sheep) and birds (chickens, turkeys), and follow oral administration of the agents, either in feed or water, indicating that the microbiota of the gastrointestinal (GI) tract is a major target. That the effects are observed with many different classes of antibacterial agents (including macrolides, tetracyclines, penicillins and ionophores) indicates that the activity is not an agent-specific side effect, nor have the effects been observed with antifungals or antivirals.

The vertebrate GI tract contains an exceptionally complex and dense microbial environment, with bacterial constituents that affect the immune responses of populations of reactive host cells[8] and stimulate a rich matrix of effecter mechanisms involved in innate and adaptive immune responses[9]. The GI tract also is a locus of hormone production, including those involved in energy homeostasis (such as insulin, glucagon, leptin and ghrelin) and growth (for example, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1))[10]. Alterations in the populations of the GI microbiota may change the intra-community metabolic interactions[11], modify caloric intake by using carbohydrates such as cellulose that are otherwise indigestible by the host[12], and globally affect host metabolic, hormonal and immune homeostasis[13]. Full (therapeutic) dose antibiotic treatments alter both the composition of the gastrointestinal microbiota[14] and host responses to specific microbial signals[15]. In combination with dietary changes, antibiotic administration has been associated with changes in the population structure of the microbiome. However, the effects of exposure to subtherapeutic antibiotic dosages have not been described.

Early studies of the effects of gut microbiota on metabolism were limited by the use of culture-based technologies that interrogated <5% of the extant GI tract microbes[16]. Culture-independent investigation of small-subunit ribosomal RNA sequences allows the microbial population structure[17] of the gut microbiota to be characterized with greater resolution. Despite inter-individual differences, substantial similarities exist[18] among mammalian species in the GI microbiota at higher taxonomic levels and functional pathways, indicating a basis for the conserved responses to early-life subtherapeutic antibiotic treatment (STAT) within farms. Previous work has shown that obesity leads to variation in the GI microbiome[12,19]; we use the insights provided from modern agricultural practices to suggest an alternative approach, using a murine model of STAT to explore how antibiotic exposure modulates host metabolic phenotypes.

Early-life STAT increases adiposity

We exposed C57BL/6J mice at weaning to penicillin, vancomycin, penicillin plus vancomycin, chlortetracycline, or no antibiotic in their drinking water at levels in the mid-range of US Food and Drug Administration (FDA)-approved levels for subtherapeutic antibiotic use in agriculture[6,7]. After a 7 week exposure, the observed weights were within the expected range of growth for female C57BL/6J mice, and there was no significant difference in overall growth between the STAT and control mice (Fig. 1a). However, by dual energy X-ray absorptiometry (DEXA) scanning, (Fig. 1b) total fat mass was significantly higher in all four groups of STAT mice than in the control group (Fig. 1c). Per cent body fat also was increased in most STAT groups compared to controls (Fig. 1d). Lean weight was not significantly ($P = 0.24$) different in the STAT mice (15.0 ± 0.1 g (mean $\pm$ standard error)) compared to controls (15.4 ± 0.3 g)

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ARTICLE

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Human gut microbes impact host serum metabolome and insulin sensitivity

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Insulin resistance is a forerunner state of ischaemic cardiovascular disease and type 2 diabetes. Here we show how the human gut microbiome impacts the serum metabolome and associates with insulin resistance in 277 non-diabetic Danish individuals. The serum metabolome of insulin-resistant individuals is characterized by increased levels of branched-chain amino acids (BCAAs), which correlate with a gut microbiome that has an enriched biosynthetic potential for BCAAs and is deprived of genes encoding bacterial inward transporters for these amino acids. *Prevotella copri* and *Bacteroides vulgatus* are identified as the main species driving the association between biosynthesis of BCAAs and insulin resistance, and in mice we demonstrate that *P. copri* can induce insulin resistance, aggravate glucose intolerance and augment circulating levels of BCAAs. Our findings suggest that microbial targets may have the potential to diminish insulin resistance and reduce the incidence of common metabolic and cardiovascular disorders.

Insulin resistance (IR) and metabolic syndrome are risk factors for both type 2 diabetes and ischaemic cardiovascular diseases, pathologies that are in epidemic growth worldwide. Mounting evidence suggests a link between the gut microbiome and human metabolic health[1–4], with transferability of insulin resistance phenotypes through faecal microbiome transplants[5,6]. These effects may partly be mediated through the metabolome[7]. Serum levels of amino acids, most consistently the BCAAs[8,9], triacylglycerols with low carbon number and double bonds[10,11], as well as specific membrane phospholipids[12], have previously been associated with IR and future risk of metabolic and cardiovascular morbidities. However, the origin of the abnormal IR-associated serum metabolome is largely unknown[13].

To explore the relationships between the fasting serum metabolome and the gut microbiome in the states of IR and metabolic syndrome, we examined 291 non-diabetic Danish adults, of whom 277 had available gut microbial data from the MetaHIT[1] study population. IR was estimated by homeostatic model assessment (HOMA-IR), which in epidemiological studies is a widely applied measure of IR, primarily as an estimate of hepatic IR[14]. Since IR is largely influenced by body mass index (BMI), we also estimated the IR index adjusted for BMI ($HOMA\text{-}IR_{BMIadj}$). Metabolic syndrome was defined according to recommendations by the International Diabetes Federation[15] (see Methods). For comparative analyses we further included 75 Danish type 2 diabetes patients[4] with quantitative gut metagenomics data generated by the same experimental protocol. Characteristics of the study samples are given in Supplementary Table 1 and Extended Data Fig. 1.

Results

In the present study, we assessed the role of the gut microbiome as a source for key features of the serum metabolome profile, predicting metabolic and cardiovascular disorders in non-diabetic lean and obese people. Untargeted metabolome profiles were generated on fasting serum samples, applying two mass spectrometry-based analytical platforms and providing information about 325 polar metabolites (94 known, 231 unknown) and 876 molecular lipids (289 known, 587 unknown), respectively (here collectively termed serum metabolites). These were binned into 74 co-abundance clusters across all individuals (Fig. 1a, Supplementary Tables 2 and 3). We found 19 of the 74 metabolite clusters (26%), comprising 26 polar metabolites and 367 lipids, to be significantly associated with both IR and metabolic syndrome, with consistent directionality across the 291 non-diabetic

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Increased Abundance of *Clostridium* and *Fusobacterium* in Gastric Microbiota of Patients with Gastric Cancer in Taiwan

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Helicobacter pylori **is recognised as a main risk factor for gastric cancer. However, approximately half of the patients with gastritis are negative for *H. pylori* infection, and the abundance of *H. pylori* decreases in patients with cancer. In the current study, we profiled gastric epithelium-associated bacterial species in patients with gastritis, intestinal metaplasia, and gastric cancer to identify additional potential pathogenic bacteria. The overall composition of the microbiota was similar between the patients with gastritis and those with intestinal metaplasia. *H. pylori* was present in half of the non-cancer group, and the dominant bacterial species in the *H. pylori*-negative patients were *Burkholderia*, *Enterobacter*, and *Leclercia*. The abundance of those bacteria was similar between the cancer and non-cancer groups, whereas the frequency and abundance of *H. pylori* were significantly lower in the cancer group. Instead, *Clostridium*, *Fusobacterium*, and *Lactobacillus* species were frequently abundant in patients with gastric cancer, demonstrating a gastric cancer-specific bacterial signature. A receiver operating characteristic curve analysis showed that *Clostridium colicanis* and *Fusobacterium nucleatum* exhibited a diagnostic ability for gastric cancer. Our findings indicate that the gastric microenvironment is frequently colonised by *Clostridium* and *Fusobacterium* in patients with gastric cancer.**

The microbiota is an essential component of the human epidermal and mucosal environments. It is well recognised that specific microbes are associated with specific pathological conditions, especially in the alimentary tract where microbes are particularly abundant. These disease-associated microbes become more abundant in the microbiota under pathogenic conditions and are likely contribute to disease progression[1–3]. Such disease-promoting bacterial infections are best exemplified by the role of *Helicobacter pylori* in gastritis and gastric cancer[4,5]. Recent studies have also demonstrated that *Fusobacterium nucleatum* is enriched in colorectal cancer lesions and plays a role in promoting cancer invasiveness[6–8]. Hence, the colonisation of the alimentary tract by specific pathogenic microbes drives the development of gastrointestinal cancers.

The extreme acidity and thick protective mucosa of the gastric environment limit the growth and colonisation of bacteria. Therefore, the complexity of the gastric microbiota is generally much lower than that of the intestinal and oral microbiotas, and most of the gastric bacteria remain in the gastric juice[9]. Previous studies that have profiled the gastric microbiota have shown that *Streptococcus*, *Prevotella*, *Rothia*, *Porphyromonas*, and *Veillonella* are the most common bacterial genera. *Neisseria*, *Fusobacterium*, *Klebsiella*, and other potential pathogens have also been detected[10]. In addition, the composition of the microbiota is subject to rapid changes caused by food consumption. Indeed, most of the bacteria found in the gastric microbiota are undergoing passage from the oral cavity to the intestines[9]. However, *H. pylori* can penetrate the mucus layer to colonise the gastric mucosa and establish a long-term infection[5,11]. Once *H. pylori* establishes a growing colony under the mucosal layer, it produces urease and elevates the pH of its microenvironment[12]. *H. pylori* not only elicits a strong inflammatory response but also actively alters the cellular functions of the gastric mucosa[11,13]. This is accomplished by a type IV

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Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice

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There is emerging evidence that the commensal microbiota has a role in the pathogenesis of multiple sclerosis (MS), a putative autoimmune disease of the CNS. Here, we compared the gut microbial composition of 34 monozygotic twin pairs discordant for MS. While there were no major differences in the overall microbial profiles, we found a significant increase in some taxa such as *Akkermansia* in untreated MS twins. Furthermore, most notably, when transplanted to a transgenic mouse model of spontaneous brain autoimmunity, MS twin-derived microbiota induced a significantly higher incidence of autoimmunity than the healthy twin-derived microbiota. The microbial profiles of the colonized mice showed a high intraindividual and remarkable temporal stability with several differences, including *Sutterella*, an organism shown to induce a protective immunoregulatory profile in vitro. Immune cells from mouse recipients of MS-twin samples produced less IL-10 than immune cells from mice colonized with healthy-twin samples. IL-10 may have a regulatory role in spontaneous CNS autoimmunity, as neutralization of the cytokine in mice colonized with healthy-twin fecal samples increased disease incidence. These findings provide evidence that MS-derived microbiota contain factors that precipitate an MS-like autoimmune disease in a transgenic mouse model. They hence encourage the detailed search for protective and pathogenic microbial components in human MS.

gut microbiome | multiple sclerosis | experimental autoimmune encephalomyelitis | twin study | germ-free mice

The risk of developing multiple sclerosis (MS) is driven by both genetic factors and environmental exposures (1). Risk genes have been determined by large-scale genome-wide association studies (GWAS), which identified more than 200 different DNA variants associated with disease susceptibility (2). Environmental risk factors include smoking, reduced exposure to sunlight, and infection with Epstein–Barr virus (3). Very recently, the intestinal microbiota emerged as an additional potential triggering factor (4, 5).

The notion that commensal gut bacteria are causally related to brain autoimmunity is supported by a transgenic mouse model of spontaneous experimental autoimmune encephalomyelitis (EAE). In this model, nearly all animals raised in specific pathogen-free (SPF) conditions develop a relapsing–remitting (RR) variant of the disease within months of age (6). Importantly, when kept in a germ-free environment, animals from the same strain remain disease-free. However, spontaneous disease promptly follows exposure of germ-free mice to SPF-derived fecal material (7). Taken together, these observations indicate that the encephalitogenic immune response observed in these mice is mediated by the intestinal microbiota.

Translation of these experimental observations into human MS poses considerable practical challenges. Complicating factors are, in particular, genetic diversity (8) and lifestyle, such as diet (9), both of which profoundly impact the individual gut microbiota. In addition, age, therapy, and neurological condition (10) might also affect the gut microbial composition. In an attempt to eliminate genetic variance and reduce environmental variance to a minimum, we identified and recruited 34 monozygotic (MZ) twin pairs, discordant for MS, for a microbiome study. All the probands were of Caucasian origin and had grown up together with their healthy twins to adulthood in Germany. We studied their gut microbiota in two tiers: first, intestinal microbial profiles of MS twins and healthy twins were compared by 16S ribosomal RNA (rRNA) amplicon and metagenomic shotgun sequencing. Second, we transplanted fecal samples from selected twin pairs to germ-free mice to assess functional differences in the human intestinal microbiota of MS and healthy twins.

Results

MZ Twin Cohorts Discordant for MS. We assembled a cohort of 34 MZ twin pairs clinically discordant for MS. In each pair, one twin has clinically definite MS according to the current diagnostic criteria (11), whereas the co-twin is unaffected. Our MS twin cohort resembles the general MS population with respect to female

Significance

Studies using experimental models have indicated that multiple sclerosis (MS)-like disease can be triggered in the gut following interactions of brain autoimmune T lymphocytes with local microbiota. Here we studied the gut microbiota from monozygotic human twin pairs discordant for multiple sclerosis. When we transferred human-derived microbiota into transgenic mice expressing a myelin autoantigen-specific T cell receptor, we found that gut microbiota from multiple sclerosis-affected twins induced CNS-specific autoimmunity at a higher incidence than microbiota from healthy co-twins. Our results offer functional evidence that human microbiome components contribute to CNS-specific autoimmunity.

Author contributions: K.B., L.A.G., R.H., G.K., and H.W. designed research; K.B., L.A.G., L.K., U.S., T.K., and G.K. performed research; K.B., L.A.G., E.C., X.J., L.X., Z.X., C.L., L.K., S.E.B., and G.K. analyzed data; and K.B., R.H., G.K., and H.W. wrote the paper.

The authors declare no conflict of interest.

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Data deposition: Normalized datasets related to this paper are available from the UCSF Data Sharing Service (Dash) at https://doi.org/10.7272/Q6N58JH2 and raw data have been deposited in the European Bioinformatics Institute (EMBL-EBI) database, https://www.ebi.ac.uk (accession no. ERP101460).

See Commentary on page 10528.

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Microbiome

RESEARCH Open Access

The gut microbiota in conventional and serrated precursors of colorectal cancer

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Abstract

Background: Colorectal cancer is a heterogeneous disease arising from at least two precursors—the conventional adenoma (CA) and the serrated polyp. We and others have previously shown a relationship between the human gut microbiota and colorectal cancer; however, its relationship to the different early precursors of colorectal cancer is understudied. We tested, for the first time, the relationship of the gut microbiota to specific colorectal polyp types.

Results: Gut microbiota were assessed in 540 colonoscopy-screened adults by 16S rRNA gene sequencing of stool samples. Participants were categorized as CA cases ($n = 144$), serrated polyp cases ($n = 73$), or polyp-free controls ($n = 323$). CA cases were further classified as proximal ($n = 87$) or distal ($n = 55$) and as non-advanced ($n = 121$) or advanced ($n = 22$). Serrated polyp cases were further classified as hyperplastic polyp (HP; $n = 40$) or sessile serrated adenoma (SSA; $n = 33$). We compared gut microbiota diversity, overall composition, and normalized taxon abundance among these groups. CA cases had lower species richness in stool than controls ($p = 0.03$); in particular, this association was strongest for advanced CA cases ($p = 0.004$). In relation to overall microbiota composition, only distal or advanced CA cases differed significantly from controls ($p = 0.02$ and $p = 0.002$). In taxon-based analysis, stool of CA cases was depleted in a network of *Clostridia* operational taxonomic units from families *Ruminococcaceae*, *Clostridiaceae*, and *Lachnospiraceae*, and enriched in the classes *Bacilli* and *Gammaproteobacteria*, order *Enterobacteriales*, and genera *Actinomyces* and *Streptococcus* (all $q < 0.10$). SSA and HP cases did not differ in diversity or composition from controls, though sample size for these groups was small. Few taxa were differentially abundant between HP cases or SSA cases and controls; among them, class *Erysipelotrichi* was depleted in SSA cases.

Conclusions: Our results indicate that gut microbes may play a role in the early stages of colorectal carcinogenesis through the development of CAs. Findings may have implications for developing colorectal cancer prevention therapies targeting early microbial drivers of colorectal carcinogenesis.

Keywords: Microbiome, Microbiota, Adenoma, Polyp, Colorectal, Cancer, Serrated

Background

Colorectal cancer (CRC) is the third most common cancer and fourth most common cause of cancer death worldwide [1]. CRC represents a heterogeneous group of cancers arising through different combinations of genetic and epigenetic events [2]: the "conventional" pathway to CRC is characterized by adenomatous polyposis coli (APC) mutation, chromosomal instability, and paucity of CpG island hypermethylation, while the "serrated" pathway is characterized by B-Raf proto-oncogene, serine/threonine kinase (BRAF) mutation, chromosomal stability, and high CpG island hypermethylation [3]. The majority of CRC cases (~60%) arise via the "conventional" pathway, with ~20% arising from the "serrated" pathway and ~20% arising from an alternate pathway [4]. These distinct molecular pathways originate with different precursor lesions: the "conventional" pathway with conventional adenomas (CAs) and the "serrated" pathway with sessile serrated adenomas

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Systematic Review: Gut Microbiota in Fecal Samples and Detection of Colorectal Neoplasms.

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Author information

Abstract

Colorectal cancer (CRC) is a leading cause of cancer morbidity and mortality. Dysbiosis in the gut microbiota may be associated with CRC. This systematic review focuses on differences in gut microbial community between people diagnosed with CRC or adenoma and healthy individuals using fecal samples, emphasizing non-invasive fecal microbiome models for CRC early diagnosis. Nineteen studies were identified in a systematic literature search of Pubmed, Web of Science and ScienceDirect. Several bacteria were reported to differ in abundance between CRC and adenoma cases and healthy controls, with Fusobacterium the most common. Fecal multi-bacterial predictive models used to distinguish CRC patients from healthy controls had reported areas under the receiver operating curve (AUCs) in external validation populations of 0.68-0.77. Though advanced sequencing techniques could in the future complement current non-invasive methods for CRC early detection, more studies with high statistical power, comparable and reproducible methods and external validation of predictive models are needed.

KEYWORDS:

bacteria; cancer; colorectal; fecal; fusobacterium; microbiome; microbiota

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The role of the intestinal microbiome in ocular inflammatory disease.

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Abstract

PURPOSE OF REVIEW:

The intestinal commensal microbiota are important in shaping immune cell repertoire and are influenced by host genetics. Because of this intricate interaction, an intestinal dysbiosis has been associated with multiple immune-mediated polygenic diseases. This review summarizes the literature on how alterations in the intestinal microbiota contribute to immune-mediated ocular disease, and how to potentially target the gut microbiome for therapeutic benefit.

RECENT FINDINGS:

Several groups have demonstrated the importance of the intestinal microbiome in uveitis pathogenesis. Two groups showed that altering the microbiota with oral antibiotics results in reduced uveitis severity, and another group demonstrated that a commensal bacterial antigen activates retina-specific autoreactive T cells, potentially indicating a commensal trigger for uveitis. We have found that commensal bacterial metabolites, short chain fatty acids, can suppress autoimmune uveitis. Age-related macular degeneration is associated with an intestinal dysbiosis, which can be influenced by genetic risk alleles and age-related eye disease study (AREDS) supplementation. Strategies that might be effective for targeting the intestinal microbiota might involve several approaches, including the use of antibiotics, drugs that supplement beneficial bacterial components or target inflammatory bacterial strains, dietary strategies or microbial transplantation.

SUMMARY:

The intestinal microbiota are potentially crucial in propagating inflammatory diseases of the eye, and can be targeted for therapeutic benefit.

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The gut microbiome and elevated cardiovascular risk in obesity and autoimmunity.

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Abstract

Cardiovascular disease associated with obesity and autoimmunity is the leading cause of death in these populations and significant residual risk remains despite current treatment approaches. Obesity, type 1 diabetes mellitus (T1DM), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) are linked to chronic inflammation, and subjects with these disorders have characteristic shifts in their gut microbiome composition. Recent data suggest that alterations in gut microbial and metabolic composition may be responsible, in part, for induction of chronic inflammation, thus promoting cardiovascular disease. Common microbiome changes observed in obesity, T1DM, RA, and SLE include a decrease in the ratio of bacteria, such as Gram-positive Firmicutes to Gram-negative Bacteroidetes, as well as an overabundance or depletion of certain species, including Prevotella copri. The consequent effects of these shifts include alterations in the metabolic composition of the gut, hyper-activation of toll-like receptor 4 (TLR-4), upregulation of inflammatory pathways, e.g. c-Jun N-terminal kinase and nuclear factor-kappa B (NFκB), increased intestinal permeability, increased C-reactive protein, and increased levels of trimethylamine N-oxide (TMAO). Differential microbiome compositions may also explain sex differences observed in autoimmunity, where a male gut microbiome promotes anti-inflammatory processes as compared to a female pro-inflammatory gut microbiome. Intervention at the level of the microbiota appears to attenuate symptoms in these inflammatory syndromes with probiotic treatment, such as Lactobacilli, playing a uniquely beneficial role in restoring intestinal health, decreasing inflammation, and reducing cardiovascular disease. This review will discuss obesity, T1DM, RA, and SLE in the context of how each unique microbiome profile contributes to elevated cardiovascular risk.

KEYWORDS:

Atherosclerosis; Inflammation; Microbiota; Obesity; Rheumatoid arthritis; Short-chain fatty acids; Systemic lupus erythematosus; Type 1 diabetes mellitus