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THERAPIES AIMED AT MANIPULATING THE GUT MICROBIOME

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SUMMARY

It is now recognized that disturbances in host microbial populations may be linked to acute infections such as *Clostridium difficile* and to chronic diseases including inflammatory bowel disease, cardiovascular disease, cancer, obesity, diabetes, and metabolic disorders. The gut microbiome is influenced through dietary exposures, drugs, and environmental factors. Features generally associated with health include a high level of diversity, stability and resilience of the gut microbiome over time and a predominance of obligate anaerobic bacteria that greatly outnumber facultative anaerobic species. A dysbiosis or reduction in health promoting metabolites can have a significant impact on host barrier, immune function, and physiology and may, if not cause disease, certainly exacerbate or prolong the disease process. It follows therefore, that strategies such as diet modifications, pre-and probiotics, and faecal microbial transplantation (FMT) may be beneficial in both the prevention and treatment of disease by modifying either microbial composition or function. Probiotics, defined as live microbes that, when ingested, have health-promoting effects have been examined for both preventative and treatment roles in a number of diseases. Despite a multitude of studies demonstrating numerous molecular mechanisms of action, benefits of probiotics in clinical studies remain modest. Recent metagenomic research is identifying shifts in other dominant commensals that are associated with human disease and future probiotic therapy may focus on other possibly more relevant bacterial strains such as *Faecalibacterium prausnitzii* or *Akkermansia muciniphila.* Faecal microbial transplantation delivers a complete microbial ecosystem consisting of a wide array of microbes. FMT has been shown to be highly effective with >90% cure rate in recurrent *Clostridium difficile* infection (RCDI) and is currently being investigated for treatment of numerous diseases. However, in that there is extensive variability between individuals in their microbiota composition, personalized approaches may be required in order to effectively utilize therapies aimed at manipulating the balance of the gut microbiota.

INTRODUCTION

Our gut microbiome changes throughout life and can be influenced by diet, drugs, and environmental exposures. Numerous studies to date suggest that alterations or "dysbiosis" in the gut microbiome are linked with chronic diseases as well as being associated with acute infectious states. This "dysbiosis", defined as a disruption of the normal balance between the gut microbiota and host along with a decrease in overall diversity has been

associated with obesity, type 2 diabetes, irritable bowel syndrome, inflammatory bowel disease, cardiovascular disease, autoimmune arthritis, chronic kidney disease, multiple sclerosis, autism, and cancer (*Backhed* et al., 2004; *Turnbaugh* et al., 2006; *Berer* et al., 2011; *Wang* et al., 2011; *Morgan* et al., 2012; *Qin* et al., 2012; *Kang* et al., 2013; *Ng* et al., 2013; *Wu* et al., 2013; *Brusca* et al., 2014; *Ramezani* and *Raj*, 2014; *Louis* et al., 2014). It is not clear however, whether it is the overall low diversity or the increase or decrease of specific microbial taxa that are most important and whether these changes are causative or associative. However, although many questions remain to be answered, research to date in animal models and human studies supports the concept of developing strategies aimed at targeting the gut microbiota to restore homeostasis through increasing diversity and shifting the balance of gut commensals.

MICROBIAL COMPOSITION AND FUNCTION

Studies showing that microbial composition and gene richness were able to distinguish healthy obese individuals from those with metabolic disease (*Le Chatelier*, 2013) suggests that profiling the microbial genome on a functional basis may be useful in predicting disease course in some human diseases (*Fang*, 2013). In addition, animal studies showing that disease phenotypes such as obesity, metabolic syndrome and colitis can be transferred to healthy recipients through faecal transplantation along with studies in humans that faecal transplantation with donor faecal material can cure *C. difficile* colitis (*Turnbaugh* et al., 2006; *Garrett* et al., 2007; *Turnbaugh* et al., 2008; *Aroniadis* and *Brandt*, 2013) argues strongly for a major role of gut microbes in the development and modulation of various human diseases although underlying mechanisms have not as of yet been completely defined. A greater understanding of factors that influence the gut microbiome as well as which components of the microbiota are most important in a particular condition is necessary in order to effectively develop therapeutic interventions based on manipulating the gut microbiome to promote health or treat disease (*Hollister* et al., 2014). If the concept that all healthy humans have a "core" microbiome was true, then achieving this "core" group would represent a clear therapeutic target. However, results from metagenomic studies to date have shown that there are extreme levels of inter-individual variability even among closely related individuals, and there does not appear to be a core microbiome, at least in terms of species (*Qin* et al., 2010; *Shafquat* et al., 2014). Alternatively, the idea exists that a core healthy microbiome may be defined by metabolic and functional aspects, suggesting that identification of these metabolic pathways and specific metabolites may lead to the identification of specific metabolic pathways to target (*Shafquat* et al., 2014). Features generally associated with health include stability and resilience over time and a predominance of obligate anaerobic bacteria that greatly outnumber facultative anaerobic species. Often a reduction in the obligate anaerobes is accompanied by an increase in facultative anaerobes in disease states, including members of the Enterobateriaceae family of the Proteobacteria phylum. This family includes several pathogens including *Salmonella*, *Shigella*,

*Klebsiell*a, *Proteus* and *E. coli*. However, currently it is not known if specific species, a metabolic functional profile, or other factors are most important in the maintenance of health and/or induction of disease, and which should be targeted for therapeutics. How viruses, archaea and eukaryotes interact with bacteria to maintain gut homeostasis also remains to be clearly determined.

BENEFICIAL ACTIVITIES OF GUT MICROBIOTA

One of the key mechanisms by which gut microbes are thought to exert health benefits is through the production of short chain fatty acids (SCFA) by the breakdown and fermentation of polysaccharides. SCFAs include acetate, propionate, and butyrate, with the overall abundance produced dependent upon the diet of the host and microbial composition of the colon. Bifidobacteria and lactobacilli produce mainly lactate and acetate, which can contribute to health benefits through reduction of pH and immune modulation (*Fukuda* et al., 2011), but they do not produce butyrate or propionate, two SCFAs which have been identified to exert highly beneficial local and systemic immunological effects (*Louis* et al., 2014; *Flint* et al., 2015). Butryate and propionate are produced primarily by bacteria belonging to the Clostridium clusters XIVa and IV, and to the Bacteroidetes phylum (*Louis* et al., 2010; *Reichardt* et al., 2014). Complex carbohydrate fermentation by bacteria leads to the production of short-chain fatty acids including acetate, butyrate and proprionate (*Flint* et al., 2008). Acetate maintains gut barrier function and can prevent pathogen translocation (*Fukuda* et al., 2011). Butyrate is the primary energy source for colonocytes and also has numerous anti-inflammatory effects (*Zimmerman* et al., 2012). A lack of butyrate results in colonocyte cell death and autophagy (*Donohoe* et al., 2011). In addition to SCFAs, numerous metabolites and structural components of gut commensals interact with host epithelial and immune cells to influence barrier function and immunoregulatory activity. Gut microbes also provide protection against infection by pathogenic organisms through colonization resistance. This protection may include competition for nutrients or attachment sites on the mucosa, production of antimicrobial compounds, or stimulation of host defences. Colonization resistance may also help keep potentially pathogenic commensals from multiplying and inducing disease. Thus, a dysbiosis or reduction in health promoting metabolites can have a significant impact on host barrier, immune function, and physiology and may, if not cause disease, certainly exacerbate or prolong the disease process. It follows therefore, that strategies such as diet modifications, pre-and probiotics, and faecal microbial transplantation may be beneficial in both the prevention and treatment of disease by modifying either microbial composition or function.

PROBIOTICS

Probiotics, defined as live microbes that, when ingested, have health promoting effects have been examined for both preventative and treatment

roles in a number of diseases (*Ghouri* et al., 2014; *Ferolla* et al., 2015). A multitude of studies have delineated molecular mechanisms of probiotic strains in modulating host physiology and immune function through interactions between the host and various effector molecules, including cell surface proteins, release of bioactive molecules, lipoteichoic acid, peptidoglycan, and exopolysaccharides (*Bron* et al., 2012; *Lee* et al., 2013). Oral intake of probiotics has been shown to significantly alter host gene expression both in a strain-selective manner (*van Baarlen* et al., 2011) and in a host-dependent manner (*Mariman* et al., 2015). There is clinical evidence that probiotics have some efficacy in the prevention of necrotizing enterocolitis in infants, in relieving symptoms of irritable bowel syndrome, and also in the prevention of antibiotic-associated diarrhoea. However, whether probiotics are able to reverse dysbiosis and restore gut homeostasis has not yet been demonstrated. Some studies have demonstrated that intake of probiotics can alter both the composition and metabolic activity of existing microbes in the gut (*McNulty* et al., 2011) while others have shown no effect on composition but significant effects on microbial gene expression (*Lahti* et al., 2013; *Eloe-Fadrosh* et al., 2015). Further, the existing gut microbiota can also have effects on gene expression of the probiotic (*Lahti* et al., 2013) suggesting that the host microbiota may have a significant influence on the individual response to probiotic. Other host factors that change response to probiotics include diet (*Ohland* et al., 2013; *Yadav* et al., 2013; *Degirolamo* et al., 2014; *Tachon* et al., 2014) and the existing microbiome (*Ferrario* et al., 2014). Further, different probiotic strains taken together can have competitive or inhibitory effects on each other (*Ringel-Kulka* et al., 2014) and the host may become adapted to continual ingestion of probiotics and prebiotics or bacterial products (*Dykstra* et al., 2011; *Chambers* et al., 2014; *Komura* et al., 2014). These studies clearly demonstrate that the use of probiotics to mediate human health or treat disease involves a complex reciprocal interaction of the probiotic, host immune function, and commensal microbiota encountered by the probiotic. Further studies are required to truly understand how to properly use these individual strains for beneficial purposes.

FUTURE OF PROBIOTIC THERAPY

While clinical trials have shown modest benefits from probiotic therapy, overall the results have been relatively modest. Most of the strains used as commercial probiotics include lactobacilli and bifidobacteria, even though neither of these are major colonizers of the adult human gut and defects in these have not yet been linked with any human disease in adults. Further, metagenomic research is clearly identifying shifts in other dominant commensals that are associated with human disease (*Backhed* et al., 2004; *Turnbaugh* et al., 2006; *Frank* et al., 2007; *Morgan* et al., 2012; *Qin* et al., 2012; *Kang* et al., 2013; *Ng* et al., 2013; *Wu* et al., 2013). The identification of specific organisms, such as *Faecalibacterium prausnitzii* (*Varela* et al., 2013; *Cao* et al., 2014), which has been shown to be reduced in patients with inflammatory bowel disease, as well as other butyrate producing microbes such as *Roseburia* spp., has led to the suggestion that these organisms should be used as

probiotic preparations in patients with IBD to help manage the disease. In addition, *Akkermansia muciniphila* (*Everard* et al., 2013; *Cani* and *Van Hul*, 2015), which is reduced in patients with metabolic syndrome and diabetes, and specific microbial-produced metabolites in autism spectrum disorders (*Siniscalco* and *Antonucci*, 2013; *Frye* et al., 2015) may be of more relevance for treating specific conditions in adults.

FAECAL MICROBIAL TRANSPLANTATION

Faecal microbiota transplantation (FMT), a process of transferring stool from a healthy individual to a sick person, has been shown to be highly effective with >90% cure rate in recurrent *Clostridium difficile* infection (RCDI) (*Cammarota* et al., 2014). Unlike probiotic therapy which involves only a few species of microorganisms, faecal microbial transplantation delivers a complete microbial ecosystem consisting of a wide array of microbes. RCDI is one of the most common hospital acquired infections. There has been a large increase in the number of infections along with increased severity and mortality over the past decade, associated with significant health care costs. Following a course of antibiotic therapy, approximately 20-30% of patients will experience a recurrence. Unfortunately, the risk of recurrence continues to increase with each subsequent episode, and no conventional treatment has been proven effective. Recent research suggests that development of RCDI involves alterations in bile acid metabolism. In particular, germination of *C. difficile* spores can be either inhibited or stimulated by a complex mixture of bile salts. Cholate and chenodeoxycholate are metabolized into the secondary bile acids deoxycholate and lithocholate. Deoxycholate stimulates germination while lithocholate inhibits germination (*Sorg* and *Sonenshein*, 2008, 2009). Administration of antibiotics shifts the bile acid pool and allows for spore germination (*Giel* et al., 2010). Antibiotics also reduce the diversity of microbiota and thus decrease competition for available nutrients. In that FMT is so effective at treating RCDI, this procedure is rapidly gaining acceptance throughout the world although questions still remain about the optimal route of administration, quality control, durability of response and long-term outcomes.

Studies have shown that several defined communities of microbes are equally as effective in the treatment of RCDI as is FMT. A combination of 10 facultative aerobes and anaerobes was effective against RCDI (*Tvede* and *Rask-Madsen*, 1989) as was a 33-strain combination (*Petrof* et al., 2013). Recent reports of success of freeze-dried (*Tian* et al., 2015) and encapsulated forms (*Hirsch* et al., 2015; *Stollman* et al., 2015), as well as the use of selected strains of *Clostridium* either in live form or as spores (*Gerding* et al., 2015) (Seres Health Ecobiotic®) suggests that in the very near future a much more targeted approach will be undertaken to cure recurrent *C. difficile* infection. Indeed, it may be possible to use a single strain of *Clostridium* based upon an ability of the particular strain to modulate bile acid metabolism to restore gut homeostasis in patients who are colonized with *C. difficile* (*Buffie* et al., 2015). This is a clear indication of how an approach based on an understanding of the underlying mechanism of disease can lead to effective therapy focusing on manipulating the gut microbiome.

FAECAL MICROBIAL TRANSPLANTATION AND INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease, which includes Crohn's disease and ulcerative colitis, is a chronic, relapsing and remitting set of conditions characterized by an excessive inflammatory response leading to the destruction of the gastrointestinal tract. While the exact aetiology of inflammatory bowel disease remains unclear, increasing evidence suggests that the human gastrointestinal microbiome plays a critical role in disease pathogenesis. Manipulation of the gut microbiome has therefore emerged as an attractive alternative for both prophylactic and therapeutic intervention against inflammation. Probiotics have had limited benefit in ulcerative colitis, and have demonstrated no benefit in the treatment of Crohn's disease (*Wasilewski* et al., 2015). *Borody* and *Campbell* (2012) reported using FMT as an induction and maintenance therapy in patients with IBD, with as many as 69 rectal infusions, to successfully treat 3 patients. However, in most cases, the results are not as consistent or as durable as in the setting of CDI. Most of the published reports consist of small case series, which suffer from significant heterogeneity with regards to disease activity, mode and frequency of delivery, and duration of follow up. A systematic review published in 2012 included 9 case series/reports of FMT to treat IBD (N=26); it found that 19/25 patients experienced symptomatic improvement, 13/17 ceased taking IBD medications within 6 weeks, and 15/24 had no active disease 3-36 months after FMT (*Anderson* et al., 2012). A recent case series also found that 7/9 paediatric patients with mild-to-moderate UC disease activity experienced clinical improvement, and 3/9 achieved clinical remission within 1 week after a 5-day course of daily FMT enema (*Kunde* et al., 2013). On the other hand, another pilot study by *Vermeire* et al. (2012) examined the role of FMT in the management of 4 patients with refractory Crohn's disease who had failed corticosteroids, immunomodulators and anti-TNF therapy. These 4 patients received 3 doses of FMT by naso-jejunal infusion over 2 days but none experienced clinical, biological or endoscopic benefit 8 weeks later. More importantly, the faecal bacterial composition of these 4 patients did not show clustering with their donors after FMT, unlike the cases in recurrent CDI. Intense FMT treatment of 3 paediatric UC patients using a combination of colonoscopy and enemas during a 6-12 week period showed significant clinical benefit and also an expansion of rare taxa and significant changes in colonic mucosal gene expression (*Kellermayer* et al., 2015). Two randomized control trials published recently demonstrated a possible potential for the use of FMT in treating patients with ulcerative colitis, although neither demonstrated a large response (*Moayyedi* et al., 2015; *Rossen* et al., 2015).

Thus, while manipulating the gut microbiome still remains a potential therapeutic target in patients with IBD, the question remains as to why some individuals have dramatic improvements while others do not and also why continual therapy appears to be necessary. In IBD patients with active inflammation, the luminal environment that the transplanted microbiota enters contains factors (e.g. nitrate, reactive oxygen species, viruses, phages) that may prevent successful colonization of some species and allow for the bloom of microbes received from the donor that are adapted to living in

inflammatory environments (*Winter* et al., 2013). In the colon, bacterial species are primarily anaerobes which lack the ability to respire oxygen and rely on fermentation of complex polysaccharides for growth. Dysbiosis in patients with intestinal inflammation is characterized by a marked decrease in obligate anaerobes and an increased relative abundance of facultative anaerobes such as Gammaproteobacteria and Bacilli (*Frank* et al., 2007). Members of the Enterobacteriaceae family are adapted to survival in the presence of inflammatory mediators such as reactive oxygen and nitrogen species (*Winter* et al., 2013). In the inflamed gut, increased growth of these pathogenic organisms can act to reduce gut barrier function and stimulate the immune system, thus propagating inflammatory responses. At the same time, a reduction in anaerobic bacteria that produce either butyrate or other anti-inflammatory molecules would also contribute to an increased inflammatory state. Thus, under these conditions, the newly transplanted microbes may initially exert an anti-inflammatory effect due both to the production of immunoregulatory molecules (e.g. short-chain fatty acids) and generation of signalling molecules (e.g. secondary bile acids), but over time a susceptible individual that has a genetically-determined defect in handling bacteria or in barrier function may develop an inflammatory response directed towards the newly transplanted microbes. Thus, under these conditions, while targeting the microbiota may initially be effective in reducing gut inflammation at induction FMT, due to patient genetic susceptibility, continual maintenance FMT would be necessary.

FUTURE OF FAECAL MICROBIAL TRANSPLANTS

Many questions remain to be answered before FMT becomes a feasible treatment option for diseases other than *C. difficile* infection. Optimal dosage, frequency of treatment, preparation of donor material, and route of administration needs to be determined. In that each donor is different, this represents a clear challenge to implementation of this type of therapy. In addition, potential long-term effects and the risk of transferring either infectious material or susceptibility to disease needs to be evaluated.

CONCLUSION

In order to develop new therapies aimed at returning our microbiome to a healthy state, future research should seek to understand why and how our gut microbiome changes and to understand the functional consequences of those changes. It is clear that interactions between the gut microbiome and the host have a major role in health and disease; therefore, manipulation of gut microbiota represents a therapeutic target. However, in that there is extensive variability between individuals in their microbiota composition, personalized approaches may be required in order to effectively utilize therapies aimed at manipulating the balance of the gut microbiota. In the future, a detailed analysis of an individual's gut microbes may indeed be part of their health care and biomarkers identified that can be followed for changes that may herald imminent onset of disease.

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