The Mammalian Gut Microbiome, Immune Responses and Disease: From Observational to Causal Relationships

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Abstract

The gut is a major organ for the production of immune responses and is colonized by a large variety of microbes. The composition of microbes in the gut influences immune responses qualitatively and quantitatively and is also observationally correlated with enteric and extra-intestinal infectious and non-infectious diseases. Animal models have been extremely useful to unravel the relationships of the gut microbiome with immune responses and various diseases.

The multiple findings of correlations of gut microbiome composition with immune responsiveness and disease entities have motivated the search for causal relationships, and a review of work carried out with this aim is the main objective of this report. It was found that the host's diet is a key variable in shaping the composition of the gut microbiome. In turn, microbiome metabolites may affect various host functions, such as glucose and lipid metabolism and the cardiovascular system.

Fecal microbiome transplantation (FMT) has been instrumental in exploring the pathogenesis of enteric diseases. Probiotic bacteria have the potential to increase immune responses and to ameliorate metabolic diseases.

Keywords: Gut microbiome; Host immune responses; Enteric disease; Extra-intestinal disease; Type 2 diabetes mellitus; Obesity; Diet of host; Fecal microbiome transplantation; Probiotic bacteria; Diet as health promoter

Introduction

The mammalian gut is the largest organ of adaptive immune responses with a total surface area of 300 m² of the gut epithelium. The intestinal mucosa contains at least 80% of the body's activated B cells [1]. The adaptive immune responses mainly consist of secretory immunoglobulin A (IgA) antibodies produced by B cells which have converted to plasma cells in the gut submucosa [2,3]. The bacteria present in the gut are mainly *Proteobacteria, Bacteriodetes, Firmicutis,* and *Actinobacteria* (>90%) [4], mostly located in the colon. The total number of gut bacteria has been estimated to be 10^{14} [4]. They are either commensal or symbiotic, sometimes pathogenic. Besides bacteria, various viruses, fungi, protozoa and helminths can populate the gut, often as pathogens. A disturbance of the gut microbiota accompanied by illness is defined as dysbiosis [5]. The residual and potentially pathogenic gut microbiomes have attracted much attention recently, since their composition was shown to be temporarily correlated with changes in mucosal and systemic immune responses [6]. Animal models have played a major role in elucidating microbiome-host relationships and in improving a mechanistic understanding of gut microbe-host relationship [5]. However, there are still gaps in the knowledge of the molecular mechanisms underlying the interactions of the gut microbiome with host functions such as immune responsiveness, and with enteric and extra-intestinal disease development [6]. Here, new data on the bacterial microbiome will be reviewed, which were obtained with the aim to identify and analyse such causal relationships.

Gut Microbiome and Immune Responses

The gut microbiota of infants, children and adults differ largely in different geographical areas, often depending on the most prevalent socio-economic conditions. In general, the gut microbiomes in children of low-income countries are more diverse and more variable over time, and immune responses to oral and parenteral vaccines in children of low-income countries are low [7,8], possibly also due to intestinal dysbiosis [6,9,10]. In particular, vaccines of high efficacy and effectiveness in high-income countries were found to be much less potent in low- and middle-income countries, due to a number of complex reasons [10,11]. Favourable immune responses in children of low-income countries are associated with the presence of particular gut bacteria, e.g. *Bifidobacterium*, whereas abundance of *Enterobacteria* and *Pseudomonas* were found to be correlated with low immune responses [7,12,13]. High vaccine responders in a low-income country possessed a microbiome the composition of which was similar to that of high vaccine responders in a high-income country [13]. Although numerous studies on germ-free (GF) or specific pathogen-free (SPF) animals colonized with microbiota of different composition have confirmed a correlation of microbiome composition and vaccine responsiveness [5,6,10,13,14] and infectious disease resistance [15], there is a need for molecular/mechanistic understanding of such relationships [5,6,16].

Gut Microbiome and Risk of Disease

Biochemical mechanisms have been recognized by which enteric bacteria can restrict the growth of potentially pathogenic intestinal microbes, such as by the production of antibacterial substances, of secondary bile acids, acetate and other short-chain fatty acids or alpha-galactosyl ceramide; the disease-decreasing functions are exerted by bactericidal activity, inhibition of histone deacetylases, or blocking of inflammation, respectively [17].

Changed microbiome composition in metabolic diseases and transfer of the metabolic phenotype via microbiota transfer fostered the idea that microbial metabolites may affect the metabolic phenotype of the host [18]. Various bacterial products are known to affect mammalian host cell metabolism and may increase the risk of disease [18,19] (Table 1):

- Short chain fatty acids (acetate, propionate, butyrate) have anti-inflammatory activity [20], and microbes generating these compounds are decreased in type 2 diabetes mellitus (T2D) [21]. Furthermore, microbes producing short chain fatty acids have adjuvant activity in oral cholera vaccination [22];

- Bile acids produced in the liver may be metabolized by bacteria in the colon to produce 'secondary bile acids'; those may activate farnesoid X receptor (FXR)-related pathways and stimulate diet-induced obesity [23];

- Trimethylamine N oxide (TMAO), a product of carnitine and choline metabolism of microbiota, is atherogenic and associated with cardiometabolic diseases and T2D [24-27];

- Tryptophan-derived metabolites may have a role in the progression of fatty liver disease [28];

- Para-cresol, a tyrosine-derived metabolite produced by *C. difficile,* provides it with a growth advantage over other gut bacteria and may thus contribute to the survival of *C. difficile* as cause of antibiotic-associated diarrhea [29];

- Histidine-derived imidazole propionate produced by gut microbiota may contribute to the pathogenesis of T2D by impairing glucose tolerance and insulin signalling [30];

- *Bacteroides* spp produce sphingolipids, which may help maintain intestinal homeostasis and are reduced in inflammatory bowel disease (IBD) [31];

- Flavonoids present in diets were found to be enzymatically degraded by bacteria present in the gut of animals on a high-fat diet, and the excessive weight gain could be reduced by oral administration of flavonoids [32]. Another polyphenol-derived, bacterial metabolite is urolithin A which supports the functioning of mitochondria in ageing hosts and has been observed to improve mitochondrial and cellular health in elderly individuals [33].

- Infection of *Caenorhabditis elegans* with *Enterococcus faecium* protected the nematode against *Salmonella* disease by the generation of muramyl-peptides, and this effect could also be reproduced in mice [34]. Similar observations were made with *Bifidobacterium* spp in mice [35].

- Dietary fiber deficiency leads to the gut microbiota utilising host-secreted glycoproteins at nutrient source, thus causing erosion of the mucus barrier of the colon and promoting the development of colitis by *Citrobacter rodentium* [36]. The gut microbiota regulate the maturation of the enteric nervous system (ENS) in mice via serotonin (5-hydroxy tryptamine, 5-HT) signalling as shown by blockage of the 5-HT receptor and depletion of endogenous 5/HT [37].

Microbial metabolite and/ or microbe (where known)	Function and risk increase of metabolic disease	References
Proteobacteria *Bacteriodetes* (sphingolipids) *Firmicutis*	Microbial homeostasis Reduction of inflammation *Bacteriodetes* reduced in IBD	Thursby and Juge [4]; Harris et al., [13]; Brown et al., [31]
Bifidobacterium, Streptococcus *Enterobacteria, Pseudomonas*	Potent immune response Poor immune response	Huda et al., [7]; Harris et al., [12,13] Zhang et al., [48]; Vlasova et al., [5]
Short chain fatty acids *Bifidobacterium spp,* *Bacteroides spp* *Eubacterium rectale*	Anti-inflammatory activity Anti-T2D	Koh et al., [20]; Koh and Baeckhed [18] Wu et al., [21]
Microbiota	Adjuvant of cholera vaccine	Yang et al., [22]; Di Luccia et al., [51]
Microbiota	Enhancement of epithelial barrier Anti-enteric pathogen responses	Rangan and Hang, [17]
Secondary bile acids (Various gut bacteria)	Risk of diet-induced obesity	Wahlstroem et al., [23]
Trimethylamine N oxide	Risk of obesity and T2D, atherosclerosis	Shan et al., [25]; Tang et al., [26]; Li and Tang, [27]
Tryptophan metabolites	Progression of fatty liver disease	Krishnan et al., [28]
Histidine metabolites	Risk of T2D by impaired glucose tolerance	Koh et al., [30]
Bifidobacteria	Use of host glycoprotein for propagation when gut fiber is decreased leading to enteritis	Desai et al., [36]; Schroeder et al., [35]
Para-cresol	Growth advantage in gut for *Clostridium difficile*; survival upon antibiotic treatment diarrhea	Passmore et al., [29]
Flavonoids	Prevent obesity	Thaiss et al., [32]

T2D: Type 2 Diabetes mellitus; IBD: Inflammatory Bowel Disease

For further data on the association of gut bacteria and their metabolites positively or negatively associated with metabolic disease see Koh and Baeckhed [18] (Table 1).

Table 1: Role of gut microbiota and their metabolites in immune responsiveness and various disease conditions

Problems of Proving Causality of Particular Human Gut Microbiome Compositions in Animal Immune Response or Disease Models

The transfer of a human gut microbiome to an experimental animal may encounter the problem that not all of the human bacteria will grow and survive in the new host. This may be due to the fact that the normal diet of the animal differs substantially from that of humans and that diet is an important determinant of gut microbiome composition [38].

In order to move from observation of correlations to causative understanding of the relationships of the gut microbiome with its host, a mouse model for the study of microbe-diet-host interactions has been developed [39]. The procedure consisted of the following steps:

- Construction of a human simplified intestinal microbiota (SIM), containing 10 human bacterial strains with the ability to metabolize dietary fibers;

- Transfer of the SIM into GF mice;

- Keeping mice on three different diets: chow (fibre-rich), high-fat/high-sucrose (low in fibre), zero-fat/high sucrose (low in fibre);

- Investigation of the following relationships:

1) how the dietary fiber, saturated fat, and/or sucrose may affect the abundance and the transcriptome of the SIM bacteria;

2) how SIM-diet interactions may determine the circulation of biochemical metabolites; and

3) how SIM-diet interactions may affect the host metabolism

The following preliminary results were obtained:

1) Dietary changes altered the mode of colonization of mice by the SIM, affecting the bacterial fermentation capacity;

2) SIM-diet interactions affected SIM gene expression and the entry of SIM-produced metabolites (such as formate, acetate, propionate, succinate, butyrate, lactate etc) into the host's system (plasma);

3) The host metabolism depended on the diet taken.

The application of this procedure will permit further studies on the causative role of gut bacteria present in humans, which had in the past been found to be positively or negatively correlated with metabolic diseases such as T2D, type 1 diabetes mellitus (T1D), and obesity (Table 1 in: [18]). From those data it can be tentatively concluded that bacteria negatively associated with disease are of potential benefit and bacteria positively associated potentially harmful [18]. In detail, observational studies with partial explanations of molecular mechanisms have shown beneficial effects of particular bacteria in reducing obesity, glucose intolerance and gut permeability [40,41], T2D [20], and liver steatosis [42]. Observational data should be followed up by studies permitting causality-based conclusions on particular microbiome-host relationships.

Fecal Microbiome Transplantation

Fecal microbiome transplantation (FMT) from healthy human donors to patients suffering from chronic diarrhea caused by antibiotic-resistant *Clostridium difficile* was found to lead to cures [43]. However, since there are still major gaps of knowledge of the functionality of particular gut microbiota [44], since uncertainty exists on whether particular gut microbiota are cause of, consequence of, or incidental to specific enteric or extra-intestinal diseases [45], and since the gut microbiome of healthy humans

may contain potentially pathogenic bacteria [46], FMT as a therapy is at present restricted for the treatment of *C. difficile* recurrent infection and disease [45]. However, microbes are beginning to be considered as potential 'drugs' for the prevention and amelioration or treatment of disease [47].

Use of Bacteria as Probiotics

Some gut bacteria, e.g. *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium lactis Bb12* (Bb12), have been shown to enhance adaptive responses to vaccination, acting as probiotics [48,49]. More recently, the combination of rice bran and probiotics, which protected gnotobiotic piglets against rotavirus challenge, was demonstrated to alter the concentration of various metabolites in the piglets' large intestine and in serum, which indicated enhancement of anti-diarrheal functions and immune responses [50]. Malnourishment of children often leads to dysbiosis and poor vaccine responsiveness. Gnotobiotic mice colonised with the perturbed microbiota of malnourished Bangladeshi children improved their IgA responses to oral vaccination against cholera, when their food was augmented by a 'nutraceutical' supplement (consisting of prebiotic spirulina, amaranth, flaxseed and micronutrients and of 5 probiotic bacterial strains of the *Bacteroides*, *Clostridioides* and *Fusobacteria* spp) which corrected the dysbiosis. Interestingly, mice initially colonized with microbiota from poor vaccine responders improved their immune response when they were in contact with and infected by cage mates colonized with microbiota determining a stronger immune response to vaccine [51]. The data are considered proof-of-concept that diet and microbiota can jointly favour immune responsiveness; further testing of 'unfavourable' and 'favourable' microbiota in GF or SPF animals will be required before this knowledge can be translated into human studies.

Diet as a Health Promoter

In order to decide whether the composition of the human gut microbiome was a determinant or precondition for the development of obesity, mice that were depleted of their native residual microbiome by treatment with broad-spectrum antibiotics, received fecal microbiome transplants (FMTs) from lean or obese human donors. In addition, they were fed for 22 weeks on three different diets: 1) a control AIN93G diet (a mouse diet), 2) a mimic of the average American diet (called Total Western Diet, TWD), and 3) a 45% high-fat diet-induced obesity (DIO) diet. Mice in receipt of FMTs from obese donors had significantly different microbiomes compared to mice that had received FMTs from lean donors. It was expected that mice in receipt of FMTs from obese donors would develop

obesity and aberrant glucose metabolism. However, after 22 weeks it was found that the diet influenced the microbiome composition irrespective of the donor body type, strongly suggesting that the diet of the host is a key determinant in structuring the gut microbiome after FMT [38].

It has been shown that malnutrition reduces the concentration of amino acids in plasma and is accompanied by or correlated with dysbiosis of the gut microbiome [52]. Food is a primordial need for well-being. However, diet is not only essential to maintain human growth, reproduction and health, but also modulates and supports the symbiotic microbial communities that colonize the digestive tract, the gut microbiota. Type, quality and origin of food shape the composition of the human gut microbiomes and affect their composition and function [53,54]. Dietary components which favour the growth of 'beneficial' gut bacteria have been termed 'prebiotics' [55]. Dietary fiber impacts gut microbial ecology, host physiology, and health. Thus, it should be considered how diet can be used for modulation of gut microbial ecology to promote health [54].

Conclusion

The move from studies observing correlations to causality-based studies of the gut microbiome and its effect on host metabolism [18] is considered to be a major step forward toward rationally-based optimization of vaccination strategies and better informed treatment procedures for amelioration of metabolic and cardiovascular diseases.

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