INSIGHT INTO THE ROLES OF GUT MICROBES

Shaifali Gurjar and Suman Kapur

Biological Sciences Group, Birla Institute of Technology and Science, Pilani, 333031, Rajasthan.

Corresponding author:

Dr. Suman Kapur

Dean, Research and Consultancy

Professor, Biological Sciences Group,

Birla Institute of Technology and Science, Pilani, Hyderabad Campus,

Jawahar Nagar, Shameer Pet, Hyderabad-500078, RR District,

Andhra Pradesh, India.

Email: s_kapur@bits-pilani.ac.in, gurjarshail13@gmail.com.

Fax Number: +91-1596-244183

Note: The first and the second authors have contributed equally to this review

Summary

Trillions of microbes that colonize adult intestine function collectively as a metabolic organ that communicates with, and complements the human metabolic apparatus. Manipulation of this gut microbiota can help in identifying novel strategies for treating diseases such as obesity, diabetes, cardiovascular disorder and hypertension. This review is an attempt to summarize various roles attributed to gut-microbiota in the physiology and metabolism of the human body. Emerging information in this area highlights the complex relationship of the endogenous gut microbes with body metabolism, immune surveillance and tolerance, and functions regulated by the central nervous system.

Key Words: Gutmicrobiota, Infant- and adult-gut, Obesity.

Introduction

Mammals, including human beings, are colonized by a vast, complex and dynamic bacterial community. In humans, the number of microbes associated with mucosal surfaces exceeds total number of cells in the body by almost 10 times[1]. Collectively, the microbial genomes, constituted by more than 1000 species, are estimated to contain 100 times more genes than the human genome[2]. In the intestine microflora is in permanent contact and reciprocal interaction with host cells and nutrients and constituting an extremely complex and highly dynamic, but regulated ecosystem. The intestinal microbiota plays an important role in normal gut function and maintenance of host's health. It is established immediately after birth and is now considered essential in priming the immune system[1]. Until recently our understanding of the human intestinal microbiota has been limited due to reliance on conventional microbiological techniques (i.e., selective culturing) as a large fraction of the dominant gut microbes is still reported to be unculturable. As per latest estimates 80% of the phylotypes deriving from intestinal microorganisms have no culturable representatives[1]. However, with the advent of methods like molecular fingerprinting and ecological statistical approaches, which do not require culturing, a much more thorough and reliable assessment of the gut microbiota is now possible. More recently, the metagenomic approaches have also been employed to address the collective genomes of the intestinal microbiota, also known as the microbiome[3, 4]. Indeed, a recent study using large-scale comparative analysis of 16S rDNA sequences of adherent mucosal and fecal microflora has shown 13,355 prokaryotic ribosomal RNA gene sequences[5], greatly facilitating the identification and classification of gut bacteria. Simultaneously, complementary to metagenomics, approaches like meta-transcriptomics, meta-proteomics and meta-metabolomics have been initiated and such studies are expected to provide further insights into the in-situ activity and functions of the intestinal flora.

A comparison of conventionally-raised rodents with germ-free counterparts has revealed a series of anatomical, biochemical and physiological phenotypes collectively termed as Microflora-Associated Characteristics (MACS)[6]. Several factors contribute to the protective functions of gut microbiota such as: maintaining a physical barrier against colonization or invasion by pathogens, facilitating nutrient digestion and assimilation, and providing immunological surveillance signals at the gut mucosa-lumen interface[6]. The presence of a microflora has been reported to increase the turnover of intestinal epithelial cells. Commensal bacteria have been envisaged to directly influence the intestinal epithelium to limit immune activation[6]. Table 1 summarizes the various roles attributed to gut-microbiota in the human body.

Table 1: Various roles played by gut microbiota

Tissue/Stage	Function/ Effect	Reference
Infant	Role of Gut Microbiota in Early Infant Development	R Wall et al., Clinical Medicine: Pediatrics 2009:3 45–54.
Brain	Effects of gut microbiota on the brain: implications for psychiatry	Foster et al., J Psychiatry Neurosci 2009; 34(3):230-1.

Metabolism	Mechanisms underlying the resistance to diet-induced obesity in germ free mice	Backhed et al., PNAS. 2007;104(3): 979-984
Energy Homeostasis	The gut microbiota as an environmental factor that regulates fat storage	Backhed et al., PNAS. 2004;101(44): 15718-15723
Obesity	Gut-microflora and obesity Gut Microbiota and Its Possible Relationship With Obesity Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability Translational research into gut microbiota: new horizons in obesity treatment	Ley et al, Nature. 2006. John K et al., Mayo Clin Proc. 2008;83(4):460-469 Patrice D. Cani et al., Diabètes 2008; P D Cani et al., Gut 2009; 58:1091–1103. Daniela et al., Endocrinol Metab. 2009; 53(2):139-144.

Adult human gut microbiota

The human gastrointestinal tract (GIT) is populated by complex communities of microorganisms, which out-number the eukaryotic host cells by one order of magnitude. Gut microbiota play an important role in extracting nutrients from the diet, regulating fat storage, stimulating intestinal epithelium renewal, and directing the maturation of the immune system. In turn it is itself affected by factors such as food consumption, kinships relationships and environment. The colon is colonized with approximately 10^{12} organisms/g of intestinal content and these distal gut microorganisms comprise of billions of bacteria and archaea[7]. More than 90% of these bacterial populations are obligate anaerobes. Bacteroidetes and Firmicutes, which constitute more than 90% of all phylogenetic types, are the two dominant bacterial divisions in the human and the mouse gut[5]. Other predominant species include Eubacterium, Bifidobacterium, Fusobacterium and Peptostreptococcus[6]. Gut microbiota also contain a few potentially pathogenic bacteria besides the large number of health-promoting nonpathogenic bacteria[7]. The concentration and type of bacteria in the gastrointestinal tract are also influenced by the microhabitat variations such as those in pH, oxygen and nutrient availability throughout the length of tract[8,9].

Establishment of gut microbiota

The neonatal period is crucial for intestinal colonization. The GIT of the fetus is largely sterile but becomes rapidly colonized within the first few days of life. Processes involved in the establishment of microbial population are complex and involve both microbial succession as well as interactions between the mucosa and the microbes in various regions of the infant gut[7]. Changes in the colonization pattern occur up to two years of age, when the microbiota stabilizes and resembles that of adulthood[10,12]. Predominant sources of microbes for the initial colonization of the GIT, following birth, are the maternal microbiota, especially during vaginal delivery and the infant's diet (breast and formula feeding). Other sources include environment during birth, gestational age, hygiene measures and antibiotic treatment[12]. Microbes have also been detected in the amniotic fluid and placenta from mothers and the umbilical cord blood of healthy neonates, which may also play a role in the first colonization of the GIT of the newborn[15]. Mode of delivery has been identified as a key factor that shapes the developing infant's microbiota[12,14]. The microbiota of infants born by caesarean section is characterized by lower numbers of strict anaerobes such as *Bacteroides fragilis* and *Bifidobacteria* compared to vaginally delivered infants[12, 16,18]. When compared with vaginally born infants, the median counts of *B.fragilis* group bacteria and *C.difficile* have been shown to be ~100-fold lower and ~100-fold higher, respectively, for infants born via caesarean section[12]. It is noteworthy that the balance between *Bifidobacterium* and *Clostridium* species is reported to affect immune-physiological development with a heightened risk for diseases associated with fewer *Bifidobacteria* and more C*lostridia*[19,22]. Moreover, use of pre- and/or pro-biotics further impact measures taken for disease prevention and management of specific conditions in infants by increasing the numbers of lactobacilli and Bifidobacteria in the intestine. This also suggests that influencing the composition of gut microbiota in early years may also influence the development of certain diseases in the adult life.

Effect of gut microbiota on metabolism

The distal human intestine has been compared to an anaerobic bioreactor containing trillions of bacteria and archaea, programmed to perform metabolic functions that human body has not been able to evolve on its own, including the ability to harvest the otherwise inaccessible nutrients from diet[23]. Studies showing microbial contribution to nutrient metabolism have been done using the commensal *Bacteroides thetaiotaomicron*[6]. *B. thetaiotaomicron* contains 172 glycosyl-hydrolases that are predicted to cleave most glycosidic linkages encountered in components of most human diets[24]. The capacity of *B. thetaiotaomicron* to degrade a variety of host-derived glycoconjugates, such as chondroitin sulphate, has also been linked to genes, such as csuF, which encodes a potential receptor for these glycoconjugates, and chuR, which controls the expression of the genes involved in the utilization of glycans[25]. Other host molecules, such as mucins and glycosphingolipids, can also be degraded by bacteria-derived enzymes[26]. Using genetically-modified bacteria, many genes have been identified such as those encoding SusC and SusF, outer membrane proteins, which also participate in gut carbohydrate metabolism. These genes are involved in the binding of starch to the bacterial surface allowing its digestion by α-amylases encoded by sus-A and sus-G genes[24]. This symbiotic relationship between host and bacteria also involves microbial fermentation processes. The predominant end-products of bacterial fermentation in the gut are short chain fatty acids (SCFAs), such as acetate, propionate and

butyrate. Acetate is taken up primarily by peripheral tissues and can also be utilized by adipocytes for lipogenesis[27].

Intestinal microflora is also known to contribute to amino-acid synthesis. As early as 1976, Moreau et al. indicated the key role played by bacteria in nitrogen recycling in the gut as indicated by high concentration of urea in the colons of germ-free rats. Several recent studies using gnotobiotic mouse models, micro-arrays and laser capture micro-dissection have delineated gene expression changes ensuing gut colonization with each component of normal microbiota[6,28,30]. Mechanism based studies have revealed that the microbiota from a conventionally raised mice when colonized in a germ-free mice not only increased caloric harvest, from dietary plant polysaccharide with glycosidic linkages that the host is ill-equipped to cleave with their own complement of glycoside hydrolases, but also modulated host genes that affect energy deposition in adipocytes[31]. Transplantation of microbiota increases glucose uptake in the small intestine as well as fermentation of carbohydrates to SCFAs in the distal gut with subsequent stimulation of *de novo* synthesis of triglycerides in the liver[23]. Microbiota also promotes the storage of triglycerides in adipocytes through suppression of intestinal expression of a circulating lipoprotein lipase (LPL) inhibitor: the fasting-induced adipocyte factor (FIAF)[32]. It also known that microflora deconjugate and dehydroxylate bile acids[33,34], metabolize bilirubin[35], reduce cholesterol[36], and degrade mucus glycoproteins produced by the intestinal epithelium's goblet cell lineage[6].

Gut microbiota and obesity

Metabolic activities of the gut microbiota facilitate extraction of additional calories from ingested dietary substances and also help to store these calories in host adipose tissue for later use[24]. Indeed, it has been suggested that a person's gut microbiota has a specific metabolic efficiency and that certain characteristics of the microbiota composition might predispose an individual to obesity[7]. In a landmark study, Turnbaugh et al showed that the microbiota in the *ob/ob* mice contained genes encoding enzymes that break down otherwise indigestible dietary polysaccharides. They also found more end products of fermentation e.g., acetate and butyrate with fewer calories in the feces of obese mice. Moreover, within two weeks of transfer of gut microbiota, of either ob/ob mice or lean mice to lean gnotobiotic mice, the recipients of the microbiota from *ob/ob* mice extracted more calories from food and showed a significantly greater fat gain as compared to the mice that received the microbiota from lean mice. These results further support the hypothesis of a specific role of microbial component in the pathogenesis of obesity[37]. Ley *et al* have also studied the relative abundance of various types of gut bacteria in obese and lean mice. Based on bacterial 16S rRNA gene sequences from the cecal microbiota of genetically obese (*ob/ob*) mice, their lean *ob/+* and +/+ siblings, and their *ob/+* mothers they reported 50% fewer Bacteroidetes and correspondingly more Firmicutes in *ob/ob* mice than their lean littermates[38].

Put together, these observations suggest that differences exist in the gut microbiota of obese vs lean mice, raising the possibility that the manipulation of gut microbiota could be a useful strategy for regulating energy balance in obese people[39]. Further extrapolating the observations from animal experiments to humans, Ley et al serially monitored the fecal microbiota in 12 obese participants enrolled in a weight loss program for a year[40]. Both Bacteroidetes and

Firmicutes dominated the microbiota and after weight loss the relative proportion of Bacteroidetes increased, while Firmicutes decreased. The underlying mechanism for obese people having more Firmicutes is still unknown. The possible reason can be that the host gut may have uncharacterized properties that may select this bacterial phylum, which contains more than 250 genera with diverse metabolic capabilities and this diversity of Firmicutes may result in more efficient energy extraction from a variety of complex organic matter and lead to obesity.

Studies on obese and diabetic mice also revealed enhanced intestinal permeability and metabolic endotoxaemia often seen as a comorbidity associated with metabolic disorders. Recent data support the idea that a selective increase of Bifidobacterium spp. reduces the impact of high-fat diet-induced metabolic endotoxaemia and inflammatory disorders. Clearly, additional work is needed to better understand the cause-and-effect relationship between obesity and gut microbiota.

Gut microbiota and immune response

Several hundred grams of bacteria living within the colonic lumen are known to affect the host homeostasis in more than one way. Some of these bacteria are potential pathogens and can be a source of infection and inflammation, while majority co-exist with the host and several health benefits are attributed to these. The enteric microbiota is a metabolically active partner in host defenses that influence the normal structural and functional development of the mucosal immune system. Establishment of a normal microbiota provides the host with a substantial antigen challenge, with a strong stimulatory effect for maturation of the gut associated lymphoid tissue (GALT) and mucosal immunity[41,42]. The fact that approximately 80% of all immunologically active cells of the body are located in the GALT is an affirmation of the importance of microbe-gut immune system interactions[43]. Studies have indeed shown that germ-free mice have an under-developed sparse mucosal immune system, with small germinal centers and small T cell zones. Furthermore, their lamina propria contains essentially no immunoglobulin A (IgA), plasma cells or CD4 cells, and intraepithelial lymphocytes[44, 45]. However, reconstitution of germ-free mice with intestinal microbiota leads to a rapid expansion of the immune system[46]. Intestinal bacteria are not uniform in their ability to drive mucosal inflammatory responses. Some species such as *Bacteroides vulgatus* are proinflammatory[47] while other species such as *bifidobacteria* and *lactobacilli* lack inflammatory capacity[48, 49]. Thus, commensal bacteria such as bifidobacteria and lactobacilli exert protective effects by attenuating proinflammatory responses induced by different pathogens[49-51]. Other studies have demonstrated that recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis, explaining why disequilibrium in this signalling pathway can lead to the initiation of inflammatory bowel diseases[52]. Kelly et al. proposed a mechanism by which commensal flora may regulate host inflammatory responses and maintain immune homeostasis, by promoting nuclear export of NFkB subunit relA, through a PPAR-γ-dependent pathway[51]. Recent evidence also indicates that certain enteric bacterial components can ameliorate radiation induced mucosal injury[52, 53].

Gut microbiota and Central Nervous System

Emerging evidence support that gut microbiota influence central nervous system (CNS) functions and behavior[54]. Energy balance and food intake are centrally mediated processes even though the direct link between gut microbiota and central feeding circuits has not yet been

deciphered[55-57]. Studies involving intestinal microbiota indicate that events occurring in the gut also have an impact on the development and function of the CNS. It has been demonstrated that early life stress in a rodent leads to altered stress reactivity in later life, with a concomitant alteration in gut microbiota profile[58].

Almost 50 years ago, Gustafsson[59-61] developed germ-free animal models as a scientific tool[59-62]. These models have no commensal intestinal microflora and, exhibit an undeveloped immune system[63-66]. Sudo et al have reported that when compared with specific pathogen-free mice, adult germ-free mice show an exaggerated stress response with increased plasma corticosterone and adreno-corticotrophic hormone levels in response to restraint stress[54]. An additional finding of this report was that colonization of the gut microbiota and the resultant constitution of the immune system at six weeks of age (adolescence) leads to normalization of the stress axis. However, when mice were colonized in early adulthood (8 weeks of age or later), the altered stress response persisted throughout adulthood[54]. All these observations support a direct influence of microbiota on stress reactivity and anxiety-like behavior impacting overall CNS development. Among psychiatric illness, mood disorders are the most common disorders and studies have shown that nearly 50% of patients with irritable bowel syndrome suffer from mood disorders. Whereas most clinical and preclinical studies have focused on top-down treatment options for intestinal disorders, emerging work involving germ-free mice suggest novel treatment options for psychiatric disorders potentially being targeted to systems outside of the CNS, including the GIT.

Conclusion

The human intestine is more densely populated with microorganisms than any other organ and manipulation of the gut microbiota may represent an alternate novel approach for treating human diseases right from the infancy to adulthood. Mounting evidence that gut microbiota composition differs between obese and lean humans has led to the proposition that gut microbiota manipulation can ameliorate obesity. Different mechanisms have been proposed to explain the link between gut flora and obesity. The first assumption is based on the fact that gut microbiota increases energy extraction from indigestible dietary polysaccharides. The second assumption suggests a role of gut flora in modulating plasma LPS levels leading to chronic low-grade inflammation seen in patients of obesity and diabetes. The third mechanism proposes that gut microbiota may induce regulation of host genes that modulate how energy is expended and stored. It remains to be seen whether these small changes in energy extraction and food assimilation actually contribute to clinically meaningful differences in weight? How do genetic changes in the host, such as genetic mutation in leptin in the ob/ob mouse, result in differences in the composition of the gut microbiota? Do these differences persist overtime? Future studies are distinctly warranted to answer these queries and as yet un-understood mechanisms. Considering the importance of microbiota in combination with clinical work, examining the impact of antibiotics and probiotics on CNS development and function, will enlighten us about the importance of bottom-up control of brain function. The results from work undertaken in this emerging area may provide novel targets for intervention in psychiatric and metabolic illnesses. Thus, in conclusion, gut microbiota is truly emerging as a metabolic organ within the human body that holds an important regulatory place in the entire physiology of human body and a healthy microbiota is seemingly essential for the entire homeostasis of the organism.

Conflict of interest: None

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