Metagenomics of the human intestinal tract: from who is there to what is done there

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Metagenomics of the human intestinal tract: from who is there to what is done there

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The human gastro-intestinal tract is colonized by 10^6–10^{14} microorganisms from the three domains, eukaria, archaea and bacteria that are collectively referred as the human gut microbiota. Gut microbiota actively contributes to the digestion of the nutrients, mainly the fibers otherwise undigested by the host, and participate to the host capacity of energy recovery from food. It plays also a key role in gut homeostasis, impacting on its barrier function and regulating the immune and metabolic systems. The target of this review is the diet–microbiota–host immune response triad. Starting from the current knowledge based on intestinal metagenomics, we point out on the role of food in shaping gut microbiota composition and functions, which is therefore mirrored in its healthful or deleterious effect on host immune response.

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Introduction

The digestive system is a complex machinery designed for the conversion of food into simple molecules easily absorbed by the different compartments of the human body. It includes the gastrointestinal tract (GIT), which enclose microbial communities, the digestive organs (liver, pancreas), and the digestive enzymes and hormones. The GIT starts from the mouth where food is introduced, mechanically disrupted by mastication and enzymatically degraded by the action of amylases and lipases, and ends up with the anus by which indigested residues are eliminated. Each section of the GIT is responsible for a precise task of the digestive process and is characterized by specific microbial composition, even though Bacteroidetes and Firmicutes account for 90% of total intestinal microbiota. Only a limited number of bacteria, 10^1–10^3 cfu/ml, mostly *Helicobacter pylori*, is present in the stomach, mainly because of the acidic environment. In the small intestine the number and diversity of bacterial species gradually increase from duodenum to ileum, which is mainly colonized by Enterobacteria, *Bacteroides* and Clostridia. The large intestine counts the highest microbial concentration (10^{12}–10^{14} cfu/ml) of the GIT. Most of the bacteria in this section of the digestive tube are strictly anaerobic.

Non degradable food elements, like resistant starches and vegetal fibers are degraded in the large intestine where they undergo fermentation process by resident microbiota [1]. Short chain fatty acid (SCFA), mainly acetate, propionate and butyrate are one of the end products of fiber fermentation and are used by the host as energy source. Indeed, butyrate account for 85% of the energy needed for colonocytes. The role of SCFA in the prevention of colon cancer or in the promotion of immune tolerance through Treg development has been established [2,3] confirming the key role of diet in shaping gut microbiota composition and promoting either the intestinal homeostasis or the inflammatory associated intestinal dysbiosis.

Metagenomics: a powerful tool to study complex microbial communities

Metagenomics is a recent approach developed to investigate the human microbiota since most of the microorganisms present in our body are anaerobic and cannot be easily cultivated by classic culture-based methods [4]. Metagenomics consists to isolate genomic DNA from an environmental sample (e.g. stool) for both sequencing and functional analysis. In the last decade, the establishment of international consortia including the EU-funded MetaHIT (Metagenomics of the Human Intestinal Tract) and the US HMP (Human Microbiome project) allowed the generation of an extensive reference catalog of microbial genes present in the human intestine [4,5••].

The human host is not open to the whole world of bacterial diversity as among more than 50 phyla existing

in the bacterial world only four phyla represent the dominant human gut microbiota [6,7]. This supposes that strong constraints come into play to condition the gut microbial composition. Evolution, ecology and host environment are such factors.

Comparative studies with hosts phylogenetically distant from human allow understanding how evolution shaped human microbiota. Interestingly, the four main phyla, Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria, are conserved across mammals [8] where they represent more than 95% of the microbial count. Selection mechanisms, like anaerobic condition of the intestine, are responsible for the dominance of certain bacteria during gut colonization. At the species levels, the human gut microbiota appears to be quantitatively specific to each individual, which is paradoxical since the main functions (digestive, immunomodulatory and proliferative functions) are conserved among subjects. It has also been observed that some species (mainly *Bacteroides* and Clostridiales) are shared across subjects constituting a phylogenetic core [9]. The functional core includes genes known to be important to host–bacteria interactions, such as those involved in complex polysaccharides degradation, those necessary to the synthesis of short-chain fatty acids and those necessary to the synthesis of indispensable amino acids and vitamins [4,10]. A core microbiota is also found in animals whatever the feeding sources [11].

Dietary habits are probably the most studied factors influencing gut microbiota composition. For instance, it has been shown that, during the first days of life, fecal microbiota composition between breast-fed and formula-fed infants was different, breast-fed infants showed a higher concentration of lactic acid bacteria. Among adults, herbivores gut microbiota had in average twice more genera than carnivores (omnivores were intermediate). However, in adults, studies differ considering the ability to modulate the microbiota. Wu and colleagues showed that only long term diets were able to shape the microbiota [12], while another study demonstrated that diet rapidly and reproducibly alters the human gut microbiome [13]. Meat-based diet increased the abundance of bile tolerant micro-organisms like *Alistipes* and decreased levels of Clostridiales which are able to metabolize complex plant dietary fibers. The relationship between microbiota, diet and exercise has recently been investigated by Clarke *et al.* [14•]. They observed that professional athletes harbors high microbial diversity (with a higher proportion of *Akkermansia* genus) than control (non-athletes) and that it is correlated to higher protein intake.

According to Arumugam *et al.* [15], microbiota can be stratified into at least three assemblages called enterotypes which correspond to a well-balanced host–microbial symbiotic state. Following the enterotype distribution, Western populations could be split into two populations regarding methane production [16,17••] reflecting the presence of *Methanobrevibacter* species. The dominant presence of *Methanobrevibacter* associated genus *Ruminococcus* characterizes the enterotype described, which comprise on average, half of the individuals. The two other enterotypes reflect the mutual exclusion between *Prevotella* and *Bacteroides* genera. Wu and colleagues showed that *Prevotella* enterotype could correspond to a long term diet enriched in fibers [12]. In parallel to this observation, other studies showed that African countryside population which have a fiber-rich diet are also *Prevotella* enriched [18,19].

Age is another factor that shapes microbiota composition. Before birth, human host may be influenced by microorganisms through umbilical cord [20]. A metagenomic study performed on 320 placentas specimens from healthy mothers demonstrated that placenta harbors a unique microbiome related to the oral microbiome, although the biomass is low [21]. During the first month of life, each host acquires a specific microbiota with an increase of species richness. During this period, the number of Actinobacteria decreases with an enrichment of Bacteroidetes and Firmicutes. Birth delivery mode impacts the early composition of gut microbiota. Newborns are naturally enriched with *Lactobacillus*, the main genera in the vagina microbiota. However, newborns having a C-section delivery display an enrichment in gut microbial species similar to those found on their mother skin microbiota [22••]. Gut microbiota become mature around the age of three [19]. On the opposite, elderly microbiota is sensitive to lifestyle conditions and an increased number of Proteobacteria is associated with low grade inflammation [23].

Immune response is shaped by gut microbiota and conditioned by diet

After birth, mucosal surfaces are exposed to a multitude of microorganisms and dietary antigens that constantly stimulate the host immune system (reviewed in [24]). Indeed, microbiota has an essential role in shaping the gut immune system as demonstrated in germ-free mice models characterized by various immunological defects and where administration of polysaccharide A (PSA) from *Bacteroides fragilis* is sufficient to restore the Th1/Th2 balance, to stimulate Foxp3+ Treg production cells and to protect mice from *Helicobacter hepaticus*-induced severe colitis [25•,26,27]. In a similar way, germ-free mice colonized with conventional mouse fecal microbiota or monocolonized with the segmented filamentous bacteria (SFB), show normal Peyer patches development, normal IgA production and normal capacity of CD4+ T cell differentiation [28].

Under healthy conditions, dietary and microbial antigens are confined in the intestinal lumen. A physical barrier constituted by a monolayer of epithelial cells coated by a thick layer of mucus secreted by goblet cells, avoids direct relationship between bacteria and the

host connective tissue [29•,30]. The essential role played by mucins in maintaining a safe environment has been demonstrated using Muc2-deficient mice that present a marked predisposition to generate colitis [29•]. A recent report has demonstrated the essential relationship between gut microbiota composition and the properties of the mucus layer with potential implications for health and diseases [31].

Interactions between bacteria and its host are mediated through the recognition of bacterial motifs and antigens by the pattern recognition receptors (PRRs) localized on both the apical and the basolateral side as well as in the endosomes (Toll-like receptors, TLRs), or in the cytoplasme (NOD-like receptors) of epithelial cells (reviewed in [32,33]). Bacteria and their antigens can cross the epithelial barrier via the M (microfold) cells situated in the Peyer's patches to be delivered by transcytosis to the antigen presenting cells situated in the subepithelial dome, or sampled by $CX3CR1^{+}$ phagocytes directly in the lumen through the tight junctions [34,35•]. Once antigen is recognized by DCs, these cells migrate to the MLN to prime naïve CD4+ T cells. Epithelial cells are able to regulate DCs functions via the secretion of immunomodulatory molecules. Transforming growth factor-beta (TGF-β) and retinoic acid (RA) are such of these mediators whose secretion is associated to homeostatic conditions through the induction of $CD4^{+}Foxp3^{+}$ Treg [24]. The effect of TGF-β has long been associated to those of vitamin A metabolite RA secreted by DCs [36] or by lamina propria macrophages and which is recognized by RA receptors (RARs) and retinoid X receptors on CD4+ T cells. Insufficient amounts of dietary vitamin A have been linked to impaired immune response to pathogens probably due to the RA property to stimulate the CD4+ T cells homing capacity [37].

During the last ten years, several studies have focused on the Aryl hydrocarbon receptor (AhR) for its property to regulate immune as well as non-immune cells and to stimulate IL-22 secretion by innate lymphoid cells (ILCs) [38] which, in turn, are involved in the production of antimicrobial peptides as well as in mucus secretion [39•]. AhR recognize natural chemicals, food antigens and bacterial metabolites. Dietary tryptophan from cabbage, broccoli and cauliflower is converted in L-kinurenine, an AhR ligand, by the action of Indoleamine 2,3-dioxygenase 1 (IDO1). Gut microbiota, especially some *Lactobacillus* species like *L. reuteri* and *L. johnsonii* use dietary tryptophan as energy source and release indole-3-aldeyde which in turn activates AhR, stimulate IL-22 transcription and protects mice against *Candida albicans* colonization (reviewed in [40]). Recently, a screening of a chemical library derived from *Propionibacterium freudenreichii*, a bacterium isolated from Swiss-type cheese, allowed to identify the 1,4-dihydroxy-2naphthoic acid (DHNA) as an AhR activator *in vitro* and *in vivo* models [41]. Since AhR is down regulated in the intestinal tissues of IBD patients as well as in mouse models of colitis [42,43], its activation by dietary ligands could represent a valuable way to attenuate the symptomatology associated to the disease.

With the aim to decipher the link existing between diet, microbiota and cardiovascular diseases (CVD) and to identify metabolic biomarkers for the early detection of the disease, Wang and colleagues set up a metabolomic study on plasma from healthy and disease individuals [44]. They found that derivatives of dietary phosphatidylcholine and carnitine (from red meat, eggs, among others), notably the trimethylamine oxide (TMAO), are associated to the risk of CVD. TMAO results from choline conversion into trimethylamine (TMA) through enzymes produced by gut microbiota and subsequent TMA oxidation into TMAO by liver flavin monoxygenase. Indeed, Kymberleigh *et al.*, starting from a screening of intestinal bacteria identified eight strains able to generate TMA from choline [45]. Interestingly, no TMAO production has been observed in germ-free mice underlying the important role of gut microbiota.

It is noteworthy that western diet contributes to the development of gastrointestinal diseases. Fiber-rich diet promotes SCFA production by the intestinal microbiota, which, in turn, contributes to maintain intestinal homeostasis. In addition to their contribution to colonocytes energy supply, SCFA action is often associated to the inhibition of lysine or histone deacetylase (HDACs) [2], but they also act as signaling molecules via the interaction with G-protein coupled receptors (GPR) especially free fatty acid receptor 2 (FFAR2/GPR43) and FFAR3/GPR41, although GPR109A has also been proposed to be involved (reviewed in [46]). The activation of these receptors on epithelial cells, entero-endocrine cells as well as immune cells may be involved in SCFA-dependent effects [3]. Commensal bacteria through their fermentation end products can therefore contribute to protective function. It is noteworthy that *Faecalibacterium prausnitzii*, a butyrate producing bacteria from *Clostridium leptum* group, is deficient in intestinal bowel disease (IBD) patients characterized by chronic intestinal inflammation [47], although this intriguing commensal is producing a lot of other metabolites with potential protective activities [48•].

Conclusions

Large amount of data shows how diet contributes to the composition of intestinal microbiota and, therefore, either to healthy conditions or to the exacerbation of pathologies like IBD, metabolic and cardiovascular diseases. Fate knowledge of nutrients metabolism by intestinal microbiota has allowed the identification of new antigens and the respective receptors on the human host. However, more efforts must be done to better understand how food

interacts with microbiota and how the latter dialog with the host. To this goal, a multi approach based on the 'Omics' technologies together with *in vivo* studies and finally human trials are necessary.

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