Review Article


Microbes in the gut: A digestable account of host-symbiont interactions

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The human bowel is host to a diverse group of bacteria with over 500 different bacterial species contributing to this diversity. Until recently these bacteria were regarded as residents without any specific functions. The last two decades have seen a radical change in our understanding of the interactions between the gut flora and their eukaryotic hosts and there is a growing appreciation of the spectrum of functions performed by these symbionts. Intestinal bacteria are recognized for their role in nutrient absorption, mucosal barrier function, angiogenesis, morphogenesis and postnatal maturation of intestinal cell lineages, intestinal motility and more importantly maturation of gut associated lymphoid tissue (GALT). Although gut flora are implicated in certain pathological disorders, their remarkable contributions to health and homeostasis of the host need to be recognized and understood.

Key words Barrier - GALT - microbiota - nutrient absorption - symbiotic

Introduction

Microorganisms represent the largest biodiverse component in our world and humans are host to a remarkable variety of these organisms. In fact, the number of microbes associated with the mucosal surfaces of adult humans exceeds the somatic and germ cells by at least an order of magnitude. The largest collection of these organisms reside in the intestine with the microbiota of the gut achieving cell densities much greater than any other ecosystem in the body, serving as a microbial organ within the host organism. A sample of the large bowel contents and feces has been shown to contain $5 \times 10^{10}$ to as many as $10^{11}$ to $10^{12}$ bacterial cells per gram, with bacterial cells contributing to about 50 per cent of the faecal mass. These microbial societies in the gut, collectively referred to as “microbiota”, are believed to be in constant communication with each other and with their eukaryotic hosts. Microbiologists from the days of Louis Pasteur to present day scientists have underscored the importance of understanding this cross-talk, in order to expand our views on “nonpathogenic” (e.g., *Bifidobacterium* spp, *Lactobacillus* spp, etc.) and “pathogenic” relationships (e.g., *Shigella* spp, *Vibrio* spp, *Salmonella* spp). Indeed, it is interesting to speculate on the evolutionary and molecular processes that have helped bacteria to adapt from a free-living lifestyle to a host-dependent form in the human bowel.

The key to understanding these relationships depends largely on establishing the types and numbers of the bacteria in the gut and also determining the molecular cross-talk between microorganisms.
themselves and between microorganisms and their hosts. Efforts in this direction have often been hampered by our inability to cultivate >50 per cent of bacteria residing in the gut. However, the development of nucleic acid based detection methods like 16S rRNA sequence analysis, and the availability of gnotobiotic (germ free) experimental laboratory animals, have served as broad tools to assess these mutualistic partnerships. Better experimental tools now available and the combined use of microarrays and genetically manipulable model organisms like Bacteroides thetaiotaomicron have opened up avenues to more comprehensively investigate the relationships among microbes and their hosts. More information is now emerging, not only on “who is there” in the gut, but also on “what are they doing?” The diversity of microorganisms in the gut, their genomic features, the interactions between bacteria of different niches and their functional stability are being characterized. More significantly, these relationships between the microbiota and their hosts are now believed to be largely symbiotic. Extensive research on the functional aspects of the microbiota clearly highlight that intestinal bacteria modulate expression of genes involved in several different intestinal functions (Table) including nutrient absorption and carbohydrate foraging, mucosal barrier function, xenobiotic metabolism, angiogenesis, morphogenesis and postnatal maturation of intestinal cell lineages, intestinal motility and more importantly maturation of the gut associated lymphoid tissue (GALT). In fact, it is now appreciated that interactions between the microbiota, epithelium and GALT are dynamic, mutual and combinatorial. The relationships between symbiotic residents of the human bowel and their hosts need a wider understanding and this review focuses on emerging aspects of these enduring partnerships.

**We have company**

Humans are born without any microbial communities but colonization begins at birth with all exposed surfaces including the gastrointestinal tract being colonized, effectively making these microbes a postnatally acquired organ. The bacteria pioneering this effort are most often derived from the birth canal. While bifid bacteria predominate in the neonatal bowel, microbial structure becomes more diverse but relatively fixed with time, and the bacteria that predominate in a normal adult gut are called the ‘climax community’. At least 400-500 different species of bacteria have been known to colonize the adult gut although only 30-40 species account for 99 per cent of all flora. Anaerobes far outnumber aerobes and conventional culture techniques used to enumerate microbial flora have shown that Bacteroides, eubacteria, clostridia, ruminococci, peptococci, peptostreptococci, bifidobacteria and fusobacteria represent the predominant flora in adults, while facultative anaerobes such as Escherichia, Klebsiella, Proteus, Lactobacillus and enterococci are some members forming the subdominant genera.

However, conventional cultures techniques have limitations; estimates indicate that 40-80 per cent of bacteria seen in by direct microscopic examination of diluted faecal samples cannot be grown in culture, although there could be variations in the estimates between studies. Molecular biological techniques have helped to overcome some of these problems with 16S rRNA sequence analysis used extensively to characterize the enormously diverse microbial flora of the gut. In a study to characterize the diversity of the intestinal bacterial flora, Eckburg et al. scanned 13,355 prokaryotic RNA sequences and found many sequences corresponding to uncultivated and novel microorganisms. However, it is important to note that not all bacteria that are present/transit through the lumen of the intestine become permanent residents (autochthonous); some only appear as ‘transient’ flora (allochthonous). The autochthonous bacteria establish long term interactions with the host, forming stable populations with a demonstrable ecological function.

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**Table. Beneficial functions of the gut microflora**

<table>
<thead>
<tr>
<th>Function</th>
<th>Beneficial Function</th>
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</thead>
<tbody>
<tr>
<td>Barrier function</td>
<td>Provides a physical barrier; helps to maintain intestinal epithelial integrity</td>
</tr>
<tr>
<td>Nutritive functions</td>
<td>Complex carbohydrate (e.g., plant polysaccharides) utilization; lipid and micronutrient absorption; maintenance of amino acid homeostasis; nitrogen recycling; vitamin synthesis</td>
</tr>
<tr>
<td>Developmental functions</td>
<td>Postnatal intestinal maturation; morphogenesis and epithelial cell lineage differentiation; angiogenesis</td>
</tr>
<tr>
<td>Immune system functions</td>
<td>Influence the development of the gut associated lymphoid tissue (GALT); contribute to both innate and adaptive immune responses</td>
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The spatial differences in the climax community of microflora are also well documented. The number and diversity of bacterial species increases progressively along the GI tract with the stomach having \( <10^7 \) to \( <10^9 \) bacteria/ ml of contents, \( <10^9 \) in the ileum and up to \( 10^{12} \) in the distal colon\(^{10,18,19,23} \) with possible differences in the number of bacteria that are mucosa-adherent and those that are luminal\(^{21,24,24} \). Corresponding differences in bacterial flora that localize in each region are also documented; \textit{Helicobacter} spp. in the stomach, facultative anaerobes and strict anaerobes in the ileum and predominantly anaerobic bacteria in the distal colon\(^{11,25,26} \). Interestingly, microbiota of the climax community can vary within an individual over time and up to 30 per cent between individuals\(^{18,26} \). Despite these differences in the bacterial communities of the bowel, there is enormous functional redundancy, with more than one bacterial species having the ability to carry out the same metabolic process. This key feature helps the host in normal gut functioning, homeostasis and health, even with changes in the number of proportion of individual species.

**Cross-talk decoded**

The barrier effect: One of the most significant benefits the host derives from the resident flora is that they serve as a defensive barrier against invading pathogens\(^6 \). Recent reports provide new insights on the barrier effect provided by the intestinal flora by producing antimicrobial substances that are active against several enteropathogens. For example, studies on germ-free mice (C3H) introduced with caecal microflora of hamsters, show that the microflora serve as a barrier against \textit{Clostridium difficile} and this anti-\textit{C. difficile} barrier is attributable to another clostridial species, \textit{C. coeleatrum}\(^{27,28} \). \textit{E. coli} of intestinal origin are also known to produce colicins and microcins that have similar barrier effect against other pathogenic \textit{E. coli}\(^{12} \). Similarly, the E1 strain of \textit{Ruminococcus gnavus}, a strict anaerobic resident in the gut, has been shown to produce ruminococcin A, an antibacterial substance that actively prevents colonization by a range of pathogenic clostridia in experiments involving gnotobiotic rats\(^{29,30} \). Likewise, \textit{Lactobacillus} and \textit{Bifidobacterium} spp. are also known to produce antibacterial substances that are active \textit{in vitro} and \textit{in vivo} against a wide range of pathogens including enteropathogenic \textit{E. coli} (EPEC), enterohaemorrhagic \textit{E. coli} (EHEC), \textit{L. monocytogenes}, \textit{S. enterica} serovar Typhimurium and \textit{S. flexneri}\(^{25} \). This provides some clues on the utility of these organisms (\textit{L. acidophilus} LB, \textit{L. rhamnosus} GG, \textit{L. rhamnosus} DR20, \textit{L. johnsonii} La1) in probiotic formulations. Most molecules with antibacterial properties are low molecular weight, heat stable, protease insensitive and are distinct from the lactic and acetic acids produced by this group of bacteria.

\textit{In vitro} studies involving the activity of \textit{L. acidophilus} LB on \textit{S. enterica} var Typhimurium have shown that the following mechanisms are involved in eliminating pathogenic bacteria: (i) depletion of intracellular ATP, (ii) increase in membrane permeability, (iii) release of lipopolysaccharide (LPS) and sensitization of membrane to lytic action of detergents\(^{13} \). Finally, it is important to remember that ‘barrier effect’ is a sum of the individual contributions of several species of microbial flora, though each may have a unique mechanism to deliver the benefit to the host\(^6 \).

Other mechanisms utilized by the gut bacteria to prevent pathogens from colonizing include impairment of flagellar motility and preclusion of bacterial internalization. \textit{L. johnsonii} La1 and \textit{L. casei} have been shown to inhibit flagellar motility and prevent internalization of \textit{S. enterica} var Typhimurium. \textit{L. acidophilus} LB antagonizes cytoskeleton rearrangements by virulent \textit{E. coli} grown in Caco-2 cells, thus preventing cellular damage. \textit{B. thetaiotaomicron} has been shown to regulate the expression of endogenous protein antibiotic production of \textit{Ang4} by Paneth cells\(^{32,34} \). Although it is not clear whether these mechanisms are used independently or in combination, it would be reasonable to speculate that the intestinal flora may synchronize these mechanisms, imparting maximum benefit to their eukaryotic hosts. Bacteria presumably also benefit as they obtain a degree of control over their microbial neighbourhood.

**Eating together:** The symbiotic relationship between the microbial flora of the gut and the host depends on the metabolic capability of either or both partners to utilize available substrates. This could imply that the microflora and the host actively compete for the same host-ingested nutrients. However, experiments using conventional mice and germ free mice show that the former requires 30 per cent less calorie intake to maintain their body weight as opposed to the latter\(^{35} \), indicating that microbiota help in deriving maximum nutritional value from the available nutrients\(^7 \). In addition, the revolution in genomics has helped to assess the genomic sequences of these intestinal bacteria, and it is evident that much of their genome encodes hydrolytic enzymes that catalyze the degradation of complex...
polysaccharides\textsuperscript{18}, consequently providing a repertoire of enzymes that have not evolved in humans.

The human diet primarily contains carbohydrates, and humans are well equipped to breakdown disaccharides to monosaccharides and subsequently absorb them. However, humans have limited capacity to hydrolyse and utilize other complex polysaccharides particularly components of plant origin (cellulose, xylan, pectin) and these undigested dietary carbohydrates pass to the distal part of the gastrointestinal tract\textsuperscript{17}. Thus, the overwhelming presence of an anaerobic population in the distal colon, equipped with specific enzymes for degradation of these complex polysaccharides is not entirely unexpected\textsuperscript{16}. Interestingly, a comparison of the glycosciome of \textit{B. thetaiotaomicron} with that of humans shows the bacterium has 226 predicted glycoside hydrolases as against the 98 known or putative glycoside hydrolases in the 2.85 Gb human genome\textsuperscript{4}. Consequently, \textit{B. thetaiotaomicron}, an abundant colonizer of the gut, has become the model symbiont and the starch utilization system (involving Sus, a group of eight genes involved in starch utilization) in this bacterium is one of the best known examples of complex polysaccharide utilization\textsuperscript{1}. The starch utilization system of the bacterium helps to utilize starch in the distal colon of the host by absorbing and breaking it down to short chain fatty acids (SCFA; acetate, butyrate and propionate)\textsuperscript{9}. These SCFAs are utilized by different organs of the host (acetate by the peripheral tissues, butyrate by the colonic epithelium and propionate by the liver) where these meet different metabolic fates. Other than starch, SCFAs are derived from complex polysaccharides like pectin and xylan and together account for >10 per cent of daily absorbed calories\textsuperscript{17}. Thus “eating together” helps the bacterium to derive energy and establish a stable niche while the host benefits in utilizing otherwise indigestable carbohydrates.

\textit{Bacteroides} spp. is also known to possess the ability to degrade a variety of host-derived glycoconjugates (glycans) including chondroitin sulphate, mucin, hyaluronate and heparin\textsuperscript{14,37,38}. Interestingly, these bacteria have been found to be very versatile in their utilization of glycans and ‘carbohydrate foraging’ is now a well recognized phenomenon where they have the ability to utilize host polysaccharides and glycans when the dietary sources are limiting\textsuperscript{4}.

Experiments in NMRI (Naval Medical Research Institute) mice have shown that bacteria like \textit{B. thetaiotaomicron} have the ability to induce the host to produce specific glycoconjugates, thereby aiding the colonization of the gut with only the select species which can utilize these complex carbohydrates. This is beneficial to the host as it facilitates the creation of a suitable ecosystem. In addition, intestinal microbiota are also known to play a role in maintaining the amino acid homeostasis, nitrogen recycling in the intestine and in vitamin synthesis\textsuperscript{15,35,37}. Thus these “master physiologic chemists” appear to have devised strategies and endured the harshest conditions of evolutionary selection to provide the host with maximum nutritional benefit.

**Architectural contributions:** The microflora of the gut has a direct impact on the architecture and morphology of the gut\textsuperscript{8}. For example, the villi of the small intestine are longer; crypts are shorter and contain fewer cells in germfree mice than in age-matched conventional animals\textsuperscript{39}. In fact, the largest difference is in areas with the highest bacterial density. Since the microbiota are chiefly responsible for the degradation of mucus glycoproteins, the absence of the flora in germfree mice results in the accumulation of these components in the distal small bowel and proximal colon with corresponding enlargement. However, this enlargement can be easily reversed with monoaacociation with \textit{Peptostreptococcus micros} or conventionalization with flora from normal mice\textsuperscript{16}. These important observations on the contribution of microflora to the architecture of gut have been possible primarily because of the use of animal models such as conventionalized or germ free mice. However, it is essential to remember that the pattern of colonization of the microflora (mucosal adherent/non-adherent), epithelial cell renewal, cells/crypt and crypt/villus ratio in the gut of mice could be different from humans\textsuperscript{5}. Nevertheless, these animal models have been instrumental in providing us insights into the contributions of the microflora to gut morphology. Also, these preliminary findings highlight the importance of normal colonization of the gut during postnatal development, though precise morphometric methods will be required to determine the proportional microbial contributions.

**Effects on intestinal motility:** Intestinal microflora contributes to the development and maintenance of gut sensory and motor functions, including intestinal propulsive activity\textsuperscript{40}. Experimental evidence shows that spatial and temporal spread of migrating motor complexes (MMC) is slower and more restricted in germfree mice as compared to conventional mice\textsuperscript{8}. Similarly, gastric emptying and intestinal transit are more delayed in
germfree animals. In vivo functional recordings in animal models demonstrate that the microbiota of the gut promote the propagation of interdigestive motor migratory complexes, as well as intestinal transit during fasting and feeding. These bacteria release certain chemotactic peptides that affect the enteric nervous system. In addition, the SCFA released as metabolic end products also affect motility, although different experimental systems have yielded contradictory results. However, the direct impact of SCFA on the colonic and ileal smooth muscle contractility has been documented. The SCFAs also affect the serotonin- and motilin-containing enteroendocrine cells in the colon and the ileum. It is essential to point out that this interaction is bidirectional, with intestinal motility serving as a major control in sweeping the excessive bacteria from the lumen, thus bacteria can be retained but are more contained.

**Effects on epithelial cell lineages, morphogenesis and angiogenesis:** The components of the microflora possess the ability to modify the differentiation of epithelial lineages. An example is the Paneth cell lineage, which emerges as the crypts form. Normal mice have Paneth cells with apical secretory granules by postnatal day seven (P7) and lysozyme by P10, but a delay in maturation is seen in germfree mice in whom lysozyme becomes detectable only by P14. The flora also appears to modify the intestinal epithelial cells to produce glycoconjugates that can be utilized by the microbes. Experiments using germfree mice provide evidence that glycoconjugate expression regulated by the microflora is sustained by altering the epithelial cell lineages during differentiation. However, not all bacteria that colonize the intestine have comparable abilities to modulate epithelial cells. *P. micros* and *B. infantis* have little or no effect on glycoconjugate expression of the host cells, but *B. thetaiotaomicron* can induce production of glycoconjugates and enhance co-colonization of the gut by other bacteria like *B. infantis*. Although there is growing experimental evidence to demonstrate the effect of microflora on epithelial cell morphogenesis, the interactions directed by the host will have to be investigated to achieve a more complete picture. The epithelial cell response to the colonizing microflora will have to be defined at different time points during postnatal development and DNA arrays may be a useful tool to understand interactions directed by host responses.

Gut microflora are also known to play a key role in the construction of the villus mesenchymal microvascular network and Paneth cells are central components in this microbiologically regulated angiogenesis. The time of appearance of Paneth cells during development and their strategic position in the crypts make them ideal candidates to perform this function. Knockout mice that lack Paneth cells do not show any increase in microvasculature despite induction with gut microflora. On the other hand, germfree animals upon conventionalization show an increase in vasculature, as angiogenesis is restarted. While improved microvascular network is beneficial to the host in improving the intestinal absorptive capacity, microbial regulation of angiogenesis also helps bacteria to achieve greater cell densities and more species complexity.

**Tripartite talks: Effect on diffuse gut-associated lymphoid tissue (GALT):** The gastrointestinal tract performs the important function of nutrient absorption and the large surface area of the epithelium, approximately 400 m², is a necessary requirement. This thin epithelial lining with a large surface area could make the host vulnerable, not only to the antigenic load that comes through the diet, but also to the abundant microbiota residing in the gut. This may partially explain the high proportion of lymphoid cells in the gut mucosa, with an estimated 80 per cent of all B cells in the body being gut-associated B cells. Interestingly the gut immune system in healthy individuals appears to be tightly regulated to prevent responses against gut flora and food components. In fact, the gut epithelium can sense the commensals from the pathogens and integral to this function are the mammalian pathogen recognition receptors (PRRs). Both the Toll-like receptors (TLRs) and the Nod receptors expressed by the epithelial cells recognize differences in muropeptide motifs and activate pro-inflammatory pathways alerting the host to infection, while remaining non-responsive to gut microbiota.

Though epithelial proinflammatory responses to commensal bacteria do exist, most individuals are able to maintain the abundant colonizers without acquiring disease. In fact, certain non-pathogenic bacteria are known to regulate inflammation negatively, e.g., the avirulent strains of *Salmonella* in the gut are known to inhibit the activation of NF-κB in the epithelial cells by blocking the polyubiquitination of phosphorylated IκB. Normal bacterial flora is now known to help in maintaining epithelial integrity and preventing toxic insult and Rakoff-Nahoum et al. in their elegantly designed experiments, have helped to demonstrate that TLR ligands which are continuously derived from the luminal bacteria play an important role in deriving...
TLR-mediated protection and contribute to a new, non-immune TLR role— that of epithelial homeostasis. The gut flora has an impact on the composition and spatial complexity of the GALT. For example, the numbers of αβ T-cell receptors on intraepithelial lymphocytes (IELs) increase on conventionalization of germ-free mice with normal gut flora. Further, germ-free mice are known to possess small, underdeveloped Peyer patches lacking germinal centers, a few IgA plasma cells and CD4 cells in the lamina propria and fewer IELs. All these features are altered when germ-free mice are conventionalized with normal gut flora. However, it is not clear whether the spatial difference among the bacteria of the bowel is related to the spatial organization of the components of GALT and whether differences in the components of GALT affect the establishment of microbial niches.

At war or peace?

The relationship between the gut and its microflora appears mutualistic and symbiotic, but emerging research indicates that the microflora can cross the continuum from symbiosis to pathogenesis. As a large polymicrobial community, gut microbiota benefit the host in more than one way. But as single entities, many have opportunistic traits with the ability to expand their habitat when the opportunity presents; minor components of this flora may expand in large numbers when their ecosystem is disrupted, e.g., with use of antibiotics; newly acquired metabolic activities may result in mutagenic end products; acquisition of gene cassettes by horizontal transfer may confer new virulent traits. But these opportunistic/pathogenic bacteria are usually restricted in number and disruption of their ecosystem appears to be a central event in pathogenesis. For example, C. difficile associated pseudomembranous colitis appears only in conjunction with usage of antibiotic or immunosuppressive drugs. However, reconstitution of the ecological balance is directly curative emphasizing that these bacteria are ‘innocent’ until they are forced to be guilty. This also focuses attention on the potential for use of probiotics rather than antibiotics in the treatment of certain infections.

The relationship between the gut flora and the immune system is delicately balanced with the immune system perpetually prepared for war in order to ensure a lasting peace. In this situation, a disturbance to this equilibrium can result in massive gut inflammation. The microflora of the gut are now thought to play an important role in inflammatory bowel disease (IBD) and that tolerance to autologous bacteria is lost in intestinal inflammation. Moreover, T cells of patients with IBD have been found to be hyperactive against bacterial antigens and increased intestinal mucosal secretion of IgG antibodies against commensal bacteria and increased attachment of bacteria to epithelial cell surfaces, are pointers to bacterial involvement in the pathogenesis of IBD. Although no aetiological agent has been clearly identified, the potential for inflammatory responses has to be balanced against the benefits of mutualistic relationships. More recently, gut flora has also been linked to obesity and an increased expression of key enzymes (acetyl CoA synthetase and fatty acid synthetase) involved in fat metabolism in conventional mice.

While the list of beneficial aspects linked with our microbial associates seem to grow, a number of new and unanswered questions on these interactions are also appearing. Important areas for investigation include the factors that provide ecological stability in the gut, the role of the less investigated bacterial species in the gut, enumeration of all bacterial species residing in the gut, factors that promote the creation of an ideal habitat for colonization and factors that favour infection with pathogenic bacteria. These thrust areas for research stress that facilitating our understanding of the normal is as important as the study of pathogenic interactions.

References


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