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Review Article

Human Gut Flora

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ARTICLEINFO

Article history:
Received: 1 January, 2020
Accepted: 17 January, 2020
Published: 28 January, 2020

Keywords*:*
Intestinal microflora
immune response
synergy

ABSTRACT

Background: The intestinal microflora is a complex ecosystem. An improved understanding of this hidden organ will reveal secrets that are relevant to human health and to several infectious, inflammatory and neoplastic disease processes. Given the ability of the immune response to rapidly counter infectious agents, it is striking that such a large density of microbes can exist in a state of synergy within the human host.

Objective: The relevance and effect of resident bacteria on a host's physiology and pathology are extremely diverse. An ever-increasing body of evidence implicates the GI microbiota in defining states of health & disease.

Materials and Methods: We review the literature in adult and pediatric GI microbiome studies, the emerging links between microbial community structure, function, infection and disease, and the approaches to manipulate this crucial ecosystem to improve host health.

Conclusions: Manipulation of the flora is becoming a realistic therapeutic and prophylactic strategy for many infectious, inflammatory and even neoplastic diseases within the gut. Based on the influence of prenatal and early postnatal microbial exposures on the developing immune response to gut microbiome, aberrations in adulthood associated with chronic inflammatory diseases, have revealed the complexity of our dynamic relationship with microbes.

Introduction

The human gut is the natural habitat for a large and dynamic bacterial community. However, the relevance and effect of resident bacteria on a host's physiology and pathology has been well documented. Most bacterial species cannot be cultured, but modern molecular methods, such as broad-range sequencing of 16S ribosomal RNA from amplified bacterial nucleic acid extracted from faeces or biopsies, indicate evolutionary divergence that can be used to identify and classify bacteria. The availability of bacterial sequence data has facilitated the development of molecular probes for fluorescence *in situ* hybridization, DNA microarrays and gene chips that can identify and enumerate specific species. These molecular approaches have been used to examine the individuality and stability of the flora over time and to detect shifts in its composition after weaning, exposure to antibiotics or dietary changes. Acid, bile and pancreatic secretions hinder the colonization of the stomach and proximal small intestine by most bacteria. However, bacterial density increases in the distal small intestine, and in the large intestine rises to an estimated 10^{11}–10^{12} bacteria per gram of colonic content, which contributes to 60% of faecal mass. The fetal gut is sterile, but colonization begins immediately after birth and is influenced by the mode of delivery, the infant diet, hygiene levels and medication (Gronlund *et al*, 1999). Probiotics and prebiotics are known to have a role in prevention or treatment of some diseases[2].

Discussion

Host–microbe interactions occur primarily along mucosal surfaces, and one of the largest interfaces is the human intestinal mucosa. The intestine is adapted to bi-directional host flora exchange and harbours a diverse

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http://dx.doi.org/10.31487/j.AJSCR.2020.01.04

bacterial community that is separated from the internal milieu by only a single layer of epithelial cells Enteric bacteria form a natural defence barrier and exert numerous protective, structural and metabolic effects on the epithelium. Collectively, the flora has a metabolic activity equal to a virtual organ within an organ [1].

Table 1: Most Common Anaerobic & Aerobic Genera.

Anaerobic genera	**Aerobic genera**
Bifidobacterium	Escherichia
Clostridium	Enterococcus
Bacteroides	Streptococcus
Eubacterium	Klebsiella

Table 2: Functions of Intestinal Flora.

Protective function	**Structural function**	**Metabolic function**
1.Pathogen displacement. **2.** Nutrient & receptor competition with pathogen **3.** Production of antimicrobial factors e.g. bacteriocins, lactic acids .	**1**. Barrier fortification. **2**. Induction of IgA Antibody **3.** Immune system development	**1**. Control intestinal epithelial cell differentiation & proliferation. **2**. Metabolize dietary carcinogens. **3**. Synthesize vitamins e.g. vit-K, biotin, folate. **4.** Ferments non-digestible dietary residue **5**. Iron absorption **6**.Salvage of energy.

I Patho-Physiology

i Host–Flora Communication at the Mucosal Surface

Host defence requires an accurate interpretation of the microenvironment to distinguish commensal organisms from episodic pathogens and a precise regulation of subsequent responses. The epithelium provides the first sensory line of defence and active sampling of resident bacteria, pathogens and other antigens is mediated by three main types of immunosensory cell .

I) First, surface *enterocytes* serve as afferent sensors of danger within the luminal microenvironment by secreting chemokines and cytokines that alert and direct innate and adaptive immune responses to the infected site (Shanahan, 2005).

II) Second**,** *M cells* that overlie lymphoid follicles sample the environment and transport luminal antigens to subadjacent dendritic cells and other antigen-presenting cells.

III) Third, intestinal *dendritic cells* themselves have a pivotal immunosensory role and can directly sample gut contents by either entering or extending dendrites between surface enterocytes without disrupting tight junctions (Rescigno *et al*, 2001). Dendritic cells can ingest and retain live commensal bacteria and travel to the mesenteric lymph node where immune responses to commensal bacteria are induced locally (Macpherson & Uhr, 2004). Thus, acting as a gatekeeper, the mesenteric lymph node prevents access of commensal bacteria to the internal milieu.

The ability of immunosensory cells to discriminate pathogenic from commensal bacteria is mediated, in part, by two major host pattern recognition receptor *(PRR)* systems—the family of Toll-like receptors *(TLRs)* and the Nucleotide-binding Oligomerization Domain/caspase recruitment domain isoforms (*NOD/CARD*; Cario, 2005). These PRRs have a fundamental role in immune-cell activation in response to specific microbial-associated molecular patterns.

ii Effector Mechanisms of the Commensal Flora

Host inflammatory responses to pathogenic bacteria and other stress signals are pivotally controlled by the transcription factor nuclear factor *(NF)-kB.* Several distinct mechanisms by which commensal bacteria limit NF-kB signaling have been elucidated .

I) These include inhibition of epithelial proteasome function.

II) Degradation of the NF-kB counter-regulatory factor or nuclear export of the NF-kB subunit, p65, through a peroxisome proliferator-activated receptor (PPAR) dependent pathway (Kelly *et al*, 2004; Neish *et al*, 2000; Petrof *et al*, 2004).

III) Some commensal bacteria might inhibit specific signalling via TLR4 by elevating PPAR expression and uncoupling NF-kB-dependent target genes in a negative-feedback loop (Dubuquoy *et al*, 2003).

IV) Induction of transforming growth factor-□ and nerve growth factor, and mitogen activated protein kinase and protein kinase B pathways have also been implicated in the anti-inflammatory effects mediated by various commensal bacteria (Ma *et al*, 2004; Yan & Polk, 2002)

V) Molecular mimicry of host molecules: Commensals bacteria display.

Surface molecules resembling those of the surface of the host, could contribute to immune hyporesponsiveness.

II Factors Affecting Gut Flora Development

i Obesity

Compared with lean individuals, obese subjects exhibit a dramatic tenfold shift in the ratio of Firmicutes to Bacteroidetes (from 3:1 to 35:1), two of the major phyla present in the human GI tract [4]. This altered community structure is associated with a shift in function, resulting in increased energy harvest from ingested food; unexpended excess energy is deposited as adipose tissue [5].

ii Diet

Diet can dramatically impact the composition of the gut microbial community [5]. A high-fat diet has been associated with an increase in Firmicutes and Proteobacteria and a concomitant decrease in

Bacteroidetes in both wild-type mice [6]. In humans, a high-fat diet resulted in a similar phylogenetic shift in the GI microbiome associated with obesity; this restructuring is largely due to dietary selective pressure, which promotes organisms optimally poised to metabolize and import readily available carbon sources, particularly simpler sugars, such as glucose, fructose and sucrose [7].

III Administration of Antibiotics

Antibiotic administration dramatically impacts the native microbial community, leading to an unintentional state of dysbiosis [8]. Despite the dramatic impact antibiotic administration has on the gut microbiota, the community in healthy adults appears to be relatively resilient; once administration of antibiotics has ceased, the gut microbiome largely returns to a pretherapy consortium after 4 weeks [9]. However, this dramatically alter the composition of the gut microbiome.

IV Environmental Exposure

Maternal exposure to environmental stimuli, particularly microbes during pregnancy, play an important role in postnatal immune functioning and, in particular, the subsequent development of allergic disease [10-11]. Schaub *et al.* demonstrated that mothers exposed to farms and farm animals during pregnancy were less likely to have children who developed allergies and asthma [12]. These prenatal exposures were associated with increased number and function of cord blood T regulatory (Treg) cells, which are linked to lower Th2 cytokine secretion (increased Th2 cytokine secretion is a characteristic of an allergic response. The exposure to household pets (cats and dogs) has also been shown to protect against allergic disease development and it is hypothesized that this protection is, as with farm animal exposures, mediated via microbes [11]. Pet exposure is associated with lower cord blood IgE levels which is particularly pertinent given the crucial role that IgE plays in fetal immune system functioning and that elevated cord blood IgE levels associated with subsequent development of allergic disorders [13].

V Serious Illness & Long-Term Intravenous Feeding

Although a well-functioning human intestine teems with a variety of microbial life, serious illness, long-term intravenous feeding, wipe out much of this diversity.When a patient spends a long time in ICU, “the gut undergoes near-complete ecologic collapse [2].

VI Vaginal Delivery

Leads to exposure to maternal vaginal microbiome, typically composed of commensal organisms commonly found in the lower GI tract [14]. These infants typically possess higher abundances of certain *Bifidobacterium* and *Bacteroides* species, which have been associated with health-promoting effects**.**

VII Breast Feed Vs Formula – Based Diet

Breast milk provides nutritional support,it facilitates transfer of bioactive agents, for example, maternal secretory IgA, which provides passive immunoprotection & sequester commensal species in the neonatal intestine and promote biofilm formation an aspect that has been argued to facilitate colonization by protective native gut bacteria (immune inclusion) and prevent colonization by pathogenic species (immune exclusion) [15, 16]. Other components of breast milk modulate the developing mucosal immune response, while the presence of indigestible oligosaccharides promotes the growth of specific bacterial families such as the Bifidobacteriaceae and may act as decoy ligands for pathogens, preventing their mucosal attachment. Thus, the components of breast milk serve to both directly and indirectly enhance mucosal barrier function and shape immune development. In addition to protection against allergic disease development, breastfeeding has also been associated with defense against neonatal diarrhea, necrotizing colitis, obesity (meta-analysis is provided in [17]) and Type II diabetes.

VIII Disease & Disorders due to Alteration of Gut Flora

Aberrations in gut microbiota associated with a number of diseases and disorders including allergic disease development colon cancer and even progression and severity of HIV [18- 21]. Disruption of the gut microbiome, termed dysbiosis, is frequently accompanied by overgrowth of pathogenic bacteria or fungi, in conjunction with significant loss of microbial diversity or key functional groups and an inflammatory response by the host which contributes to disease development [21-23]. Dysbiosis has been associated with an imbalance between populations of inflammation-mediating T-helper cells (Th1, Th2 and Th17) and anti-inflammatory Treg cells. Prolonged overproduction of Th1- and Th17-associated cytokines has been linked with Inflammatory Bowel Disease (IBD) (overproduction of Th1 for Crohn's disease and Th17 for both CD and ulcerative colitis [24-25]. IBD patient appears unable to reprogram towards a noninflammatory state suggesting that low levels of certain microbial species maintain a proinflammatory state [26].

At a functional level, the butyrate-producers, which belong to the *Clostridium leptum* group of the Firmicutes, are less abundant in the GI microbiome of IBD patients. Butyrate is an essential regulator of gene expression, inflammation, differentiation and apoptosis, and is a major energy source for the mucosa-associated microbial community. *Faecalibacterium prausnitzii*, a butyrate-producing bacterium with anti-inflammatory properties is significantly reduced in abundance in the ileum of CD patients in parallel with increased numbers of *E. coli* [23, 27]. This emphasizes the key role a number of distinct bacterial species play in maintaining inflammatory homeostasis and that loss of key functional organisms leads to pathogen overgrowth associated with chronic inflammatory illness.

Role of Probiotics: Owing to the increased prevalence of diseases and disorders associated with gut microbiota imbalances (Table 3) and the fact that traditional treatments such as antibiotic administration appear to have the potential for long-term disruption, microbial manipulation of the host microbiome to treat chronic diseases has become the focus of recent renewed interest. Manipulation may be elicited through pro-, pre- or synbiotics.

Table 3: Diseases & Disorders Due to Aberrations in the Human Gut Microbiome.

Disease /disorder	Role of microbiome	Recent finding
Atopy & asthma[19]	Pre- and postnatal microbial exposures appear key to appropriate immune development. Mode of delivery and nutrient uptake are important factors for GI community development and protection against subsequent atopic disease development .	• Mothers exposed to farm animals during pregnancy are less likely to have children who develop asthma and allergies • Early exposure to cat(s) and/or dog(s) is protective against atopic diseases, presumed to be microbially mediated . • Infants born through the birth canal are exposed to and colonized by specific Bifidobacteria and *Bacteroides* species • Breast-fed infants possess functionally distinct GI microbiomes compared with formula-fed children.
Colon cancer (CC) [20]	High abundance *of Clostridium leptum* and *Clostridium coccoides* subgroups in CC patient GI bacterial communities .	• Overall bacterial diversity increased for CC patients compared with healthy controls • CC patients exhibited GI bacterial community instability compared with healthy controls • Microbial butyrate production is thought to reduce the chances of CC development [23]
HIV [21]	Gut microbiome dysbiosis may be critical for pathogenesis	• Dysbiosis and intestinal inflammation may be critical to impairment of the GI microbial structure and function in early stages of HIV infection. •Low abundance of Bifidobacteria and lactobacilli detected in gut microbiomes of early-stage HIV infection [23]
IBD [24, 28]	• Immune response to gut microbial Community. • Composition of GI microbiota contributes to inflammation. • Treg-promoting organisms depleted; overgrowth of bacteria that induce proinflammatory Th17 cell populations .	**Crohn's disease (IBDC)** • Characteristics of GI dysbiosis include lower counts of *Clostridium leptum* , *Bacteroides uniformis* , Firmicutes, *Bacteroides* and higher abundances of *E. coli* , Proteobacteria and *Bacteroides ovatus* • Lower number of *Faecalibacterium prausnitzii*, butyrate producing bacterium, found in parallel with increased *E. coli* . **Ulcerative colitis (IBDU)** • Lower levels of Bifidobacteria and *Clostridium coccoides* reported in comparison to healthy individuals • Differential *Clostridium leptum* and lactobacilli profiles identified by DGGE analysis of IBDU and healthy subjects [28]

CC: Colon cancer; DGGE: Density gradient gel electrophoresis; GI: Gastrointestinal; IBD: Inflammatory bowel disease; IBDC: Irritable bowel disease–Crohn's disease; IBDU: Irritable bowel disease–ulcerative colitis; IBS: Irritable bowel syndrome; NEC: Necrotizing enterocolitis.

IX Probiotics

First suggested by Metchnikoff in 1907, probiotic therapy represents alteration of the gut microbiota by supplementation with live microorganisms that function to inhibit pathogen adherence to the mucosa

1. improve the intestinal epithelial and mucosal barrier function.
2. produce bacteriocins.
3. increase IgA production.
4. downregulate proinflammatory cytokine secretion.

Prebiotics, originally defined as nondigestible food ingredients that improve host health by stimulating the growth or activity of colonic bacteria, have now been reclassified to include components that are resistant to gastric acidity, hydrolysis by host enzymes and absorption by the upper gastrointestine, are fermented by the gut microbiota, and stimulate growth of microbial species beneficial to the host's health. Synbiotics are supplements composed of a combination of both probiotics and prebiotics.

Prebiotics also represent a promising approach for the management of inflammatory diseases. They have the advantage of promoting subsets of existing native GI bacterial community members (e.g., Bifidobacteria)

capable of degrading them and can increase production of important anti-inflammatory compounds, such as butyrate, without the conventional caveats of probiotic competitiveness or colonization efficiency.

Conclusion

Manipulation of the flora is becoming a realistic therapeutic and prophylactic strategy for many infectious, inflammatory and even neoplastic diseases within the gut. However, the promise of pharmabiotics is unlikely to be completely fulfilled without greater attention to the secrets held within the forgotten inner organ represented by the enteric microflora. Based on the influence of prenatal and early postnatal microbial exposures on the developing immune response to gut microbiome ,aberrations in adulthood associated with chronic inflammatory diseases, have revealed the complexity of our dynamic relationship with microbes. Relatively recent changes in lifestyle, diet and the use of antimicrobials are just some of the factors implicated in increased prevalence of a range of inflammatory disorders that have a demonstrated basis in altered GI microbial community structure and function.

With respect to pre-, pro-, synbiotics initial results are promising, even though randomized, large-scale studies with well-characterized patient populations who are supplemented with controlled dosages of specific well-characterized supplements, and whose samples are examined using high-resolution microbiome composition and functional analyses, are necessary to fully appreciate the therapeutic effects.

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