

Targeting the microbiota in the management of gastrointestinal and liver disease

Apuntando a la microbiota en el manejo de las enfermedades gastrointestinales y hepáticas

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ABSTRACT

Thanks to rapid advances in technology the details of the human microbiome and its functions in health and disease are being progressively revealed. Though many reports have linked various disease states with an altered microbiome and while some associations between the microbiome and disease states are well established, many of these studies are largely descriptive and the changes reported in the microbiome have yet to be shown to be causative. A number of strategies are available to modify the microbiota; some such as the use of antibiotics for specific indications, are well established, others such as the use of probiotics and prebiotics in a variety of disease states are supported by more limited data. Fecal transplantation has emerged as an exciting, albeit rather drastic, intervention for intestinal and, perhaps, other disorders. Other approaches, such as the isolation, purification and formulation of small molecules with specific biological actions, derived from the microbiota look very promising.

Key words: *Gastrointestinal diseases; Intestinal diseases; Liver diseases (source: MeSH NLM).*

RESUMEN

Gracias al rápido avance de la tecnología los detalles del microbioma humano y sus funciones en salud y enfermedad están siendo conocidos progresivamente. A pesar que muchos reportes han relacionado varios estados de enfermedad con un microbioma alterado y mientras algunas asociaciones entre el microbioma y estados de enfermedad están bien establecidas, muchos de estos estudios son solo descriptivos y los cambios reportados en el microbioma todavía tienen que demostrarse que son la causa. Existen muchas estrategias para cambiar la microbiota; algunas como el uso de antibióticos para indicaciones específicas, están muy bien determinadas, otras, como el uso de probióticos y prebióticos en una gran variedad de enfermedades, están sustentadas en data más limitada. El trasplante fecal ha surgido como una alternativa muy emocionante, aunque algo drástica, para las enfermedades intestinales y quizás también para otras patologías. Otros abordajes como el aislamiento, purificación y formulación de pequeñas moléculas con acciones biológicas específicas, derivados de la microbiota aparecen como muy prometedoras.

Palabras clave: *Enfermedades gastrointestinales; Enfermedades intestinales; Enfermedades del hígado (fuente: DeCS BIREME).*

BACKGROUND

Due largely to rapidly evolving advances in analytical techniques in microbiology, molecular biology and bioinformatics⁽¹⁻³⁾ the true diversity of the population of micro-organisms that inhabits the gastrointestinal tract of man (collectively referred to as the human gut microbiota) is being revealed and its contributions to homeostasis in health and to the pathogenesis of disease appreciated. Formerly, bacteria in the gastrointestinal tract had to be individually identified by tedious and cumbersome culture-based techniques and, while it is theoretically possible to characterize the entire population of bacteria in the gut employing this approach, the resources and time involved would be absolutely prohibitive. The advent of high-throughput techniques based on molecular methodologies has revolutionized the field and now permits the rapid identification of all species and strains from a given sample⁽¹⁻³⁾; these methodological

advances (coupled with dedicated bioinformatics expertise) are largely responsible for the study of the microbiota being currently one of the most active and exciting in all of science. One has only to glance through the pages of such major scientific journals as *Nature* and *Science* to appreciate the prominence of this field. Major collaborative projects have been initiated to assess the variability of the microbiota across various populations in health⁽⁴⁻⁷⁾ and relationships to phenotype in diseases as varied as diabetes^(8,9) and cystic fibrosis⁽¹⁰⁾.

The application of molecular techniques to the study of microorganisms has generated a whole new terminology; the major terms are listed and defined in Table 1. It should be stressed that the term microbiome is now to be preferred to the older term "flora" as, unlike the latter, the microbiome includes viruses, fungi and other microorganisms which are clearly normal residents of the human gut. It is estimated that the intestinal

Table 1. Terminology

Term	Definition
Microbiome	The totality of microbes, their genetic elements (genomes), and environmental interactions in a defined environment, e.g the human gut. The microbiome includes microbiota and their complete genetic elements.
Metagenome	The human metagenome is a composite of <i>Homo sapiens</i> genes and genes present in the genomes of the microbiome.
Metabolome	The complete set of small-molecule metabolites (such as metabolic intermediates, hormones and other signaling molecules, and secondary metabolites) to be found within a biological sample, such as a single organism.
Proteome	The entire set of proteins expressed by a genome, cell, tissue or organism.

microbiota composed of 10^{13} to 10^{14} microorganisms; whose microbiome contains more 100 times as many genes as the human genome. Given this discrepancy one could reasonably ask: who is the host? While the emphasis in this review will be on the composition of the gut microbiota and how it may be modified, it should be noted that the study of the metabolome (metabolomics) is a rapidly advancing field which is revealing what the microbiome actually does.

The microbiota in health

The contributions of the microbiota (“the forgotten organ”) to homeostasis in health are being more completely understood and appreciated. Some of these functions, such as the synthesis of vitamin K and folate, deconjugation of bile acids and production of short chain fatty acids are well known⁽¹¹⁾; others are more recent revelations⁽¹²⁾. Some of the main functions of the microbiota are listed on Table 2.

Table 2. Important homeostatic functions of the gut microbiota.

a. Metabolic Role
i. Caloric salvage
ii. Produces short chain fatty acids (SCFA's)
iii. Produces arginine and glutamine
iv. Synthesizes vitamin K and folic acid
v. Participates in drug metabolism, e.g. activates 5-amino salicylic acid (5-ASA) from sulfasalazine
b. Deconjugates bile acids
c. Prevents colonization by pathogens
d. Immunological effects:
i. Stimulates IgA production
ii. Promotes anti-inflammatory and down-regulates pro-inflammatory cytokines
iii. Induces regulatory T cells

Central to the beneficial interaction between the microbiota and man is the manner in which bacteria and, most likely, other microorganisms, contained within the gut “talk” to the host’s immune system and participate in a variety of metabolic processes of mutual benefit to the host and the microbe. Indeed, through the development of tolerance, the microbiota and the host learn to live together in a symbiotic relationship^(13,14). These immunological effects are mediated, at least in part, via dendritic (antigen-presenting cells, APCs) cells⁽¹⁵⁾. Interactions with the mucus layer and the epithelial barrier contribute to the maintenance of intestinal barrier integrity.

More recently the metabolic functions of the microbiota have received considerable attention in large part related to observations linking alterations in the microbiota to obesity and such complications as diabetes and the metabolic syndrome. The essential observations here were, firstly, that the gut microbiota of the genetically obese *ob/ob* mouse contained 50% fewer *Bacteroidetes* and more *Firmicutes* than a lean mouse⁽¹⁶⁾, secondly, that an obesity-inducing diet decreased the diversity of the microbiota by decreasing the proportion of *Bacteroidetes* and increasing that of *Mollicutes*⁽¹⁷⁾ and, thirdly and most critically, that an obese microbiome was highly efficient at extracting energy^(18,19). While the contribution of the microbiome to obesity in animal models and in man continues to be explored^(20,21), others have focussed on the ability of components of the microbiome to induce subtle and highly specific alterations in the composition of various organs and tissue compartments⁽²²⁻²⁶⁾. The demonstration that the oral administration of a single bacterial strain with commensal properties can induce compositional changes in organs as remote as the brain is a fascinating illustration of the potent effects of the microbiota^(25,26). The idea that the microbiome could interact with distal organs has been taken a step further in the concept of the microbiome-gut-brain axis. This is based on a number of observations in a number of animal models which have shown how manipulation of the microbiota can alter behaviour, brain function and morphology^(27,28). Furthermore, various commensal bacteria have been shown to produce small molecules with anti-bacterial⁽²⁹⁾, analgesic and neuromodulatory properties⁽³⁰⁻³³⁾. Observations such as these may well herald a new era in therapeutics: pharmabiotics⁽³⁴⁻³⁶⁾.

The microbiota in the pathogenesis of gastrointestinal and liver disease

While the role of the gut microbiota in a number of disease states, ranging from enteric infections to *Helicobacter pylori* infection, *Clostridium difficile*-associated disease, small intestinal bacterial overgrowth, portal-systemic encephalopathy, spontaneous bacterial peritonitis and biliary and pancreatic sepsis, is well established, the advent of high-throughput methodologies has resulted in explorations of changes in the microbiota in a host of intestinal and liver diseases and disorders^(11,12,37-41). While

some of the reported associations have resulted in plausible hypotheses to support a role for the microbiota or its interactions with the host in the pathogenesis of these disorders a number of notes of caution need to be mentioned. The first of these relates to source of the microbial samples; for reasons of convenience this has usually been fecal and not mucosal and colonic rather than small intestinal or gastric. These discrepancies are not unimportant as different bacterial populations may inhabit the lumen and the juxta-mucosal surface, in the biofilm^(42,43). Furthermore, while metabolic interactions between the microbiota and food or products of digestion are most likely to occur in the lumen, juxta-mucosal species and strains may be more relevant to immunological interactions^(44,45). Similarly, immunological interactions are more likely to be the province of the microbiota of the small intestine, a population that, for obvious reasons, has been scarcely studied, given that this organ contains a greater mass of immune tissue than the colon. Furthermore, most studies have been based on single point in time "snapshots", have failed to account for potential instability in the microbiota over time⁽⁴⁶⁾ and the likely impact of diet and other environmental factors⁽⁴⁷⁻⁴⁹⁾. For these and other reasons, such studies are usually incapable of correlating microbial signatures with disease activity.

However, well characterized examples of the consequences of perturbing the microbiota do exist. A vivid example is provided by antibiotic-associated diarrhea and its deadliest manifestation, *Clostridium difficile* colitis⁽⁵⁰⁾. Similar perturbations in the flora are thought to be involved in a devastating form of intestinal inflammation that may occur in new-born and, especially, premature, infants: necrotizing enterocolitis⁽⁵¹⁾. In other situations, bacteria may simply be where they should not be: impaired motility and/or acid secretion from the stomach, promote an environment conducive to the proliferation, in the small intestine, of organisms normally confined to the colon and small bowel bacterial overgrowth (SIBO) ensues⁽⁵²⁾. While SIBO has traditionally been regarded as a cause of the malabsorption syndrome there has been some enthusiasm, of late, to extend the clinical manifestations of SIBO into unexplained diarrhea and, most controversially, irritable bowel syndrome (IBS)⁽⁵³⁾. In other situations, such as inflammatory bowel disease, the host-microbiota immune interaction goes awry with the host coming to recognize commensals, not as friend, but as foe and mounting an inappropriate inflammatory response⁽⁵⁴⁻⁵⁷⁾. If damage to the intestinal epithelium renders the gut wall leaky and permits enteric bacteria (in whole or in part) to gain direct access to the submucosal compartments or translocate into the systemic circulation the stage is set for the development of potentially catastrophic sepsis syndromes; a scenario all too familiar to those who work in the intensive care unit⁽⁵⁸⁻⁶⁰⁾. A similar scenario has been described in the aftermath of inflammatory disorders⁽⁶¹⁾.

Most recently, qualitative changes in the microbiota have been invoked in the pathogenesis of a global epidemic, obesity (and its attendant consequences, the metabolic syndrome and non-alcoholic fatty liver disease (NAFLD))^(17,62). It has been postulated that a shift in the composition of the flora towards a population where bacteria that are more avid extractors of absorbable nutrients delivers more calories to the host and thus contributes to obesity.

Further layers of complexity continue to be added to the host-microbiome interface. Firstly, it must be remembered that the microbiota-host interaction is a two-way conversation; it is evident that the host can also affect the composition and function of the microbiome⁽⁶³⁾.

Interventions to modify the microbiota

A variety of strategies may be employed either singly or in combination to modify the microbiota.

1. Diet

Not only is it evident that changes in diet can dramatically alter the composition of the microbiome^(21,22,47-49), but it has also been postulated that consequent changes in bacterial metabolism resulting in alterations in the production of short-chain fatty acids, polysaccharide A and peptidoglycan, molecules with known immunomodulatory and immuno-regulatory functions⁽⁶⁴⁾, may play a role in the genesis of inflammatory disorders. Metabolic products resulting from the impact of the microbiota on dietary constituents (in this case phosphatidylcholine) have even been implicated in the pathogenesis of cardiovascular disease⁽⁶⁵⁾. Indeed, diet, either deliberately or inadvertently, may well represent the most common strategy given the presence of substances with prebiotic properties in a variety of food-stuffs and additives.

2. Antibiotics

Antibiotics are the most time honored approach and their impact may be complicated by unwanted changes in the commensal flora; changes that may be more extensive and long-lasting than formerly thought. One major consequence of antibiotic use is the suppression of the commensal population; while many species and strains may rebound promptly on cessation of therapy, not all do⁽⁶⁶⁻⁶⁹⁾.

The use of antibiotics in inflammatory bowel disease (IBD) has witnessed somewhat of resurgence with the publication of studies illustrating benefits for antibiotic therapy in Crohn's disease⁽⁷⁰⁻⁷²⁾ and pouchitis⁽⁷³⁾ and of strategies directed against specific organisms in ulcerative colitis⁽⁷⁴⁾. In irritable bowel syndrome, the poorly absorbed antibiotic rifaximin has been associated with modest, though consistent, symptomatic benefit; its precise method of action remains unclear⁽⁷⁵⁾. Another twist to the

antibiotic use in IBD has been provided by a number of studies associating risk for IBD in later life with antibiotic exposure in early childhood⁽⁷⁶⁻⁷⁸⁾; further evidence of the potential clinical impact of perturbing the microbiota at a time of its most rapid evolution⁽⁷⁹⁾.

The roles of antibiotic therapy in hepatic encephalopathy and spontaneous bacterial peritonitis have well documented though relationships between these effects and changes in the microbiota remain unclear. Antibiotic therapy has also been shown to reduce mortality and re-bleeding after variceal hemorrhage and its impact on liver disease, per se, has also been explored, especially, in relation to jejunio-ileal bypass-related liver disease and other liver diseases that have been linked to SIBO, translocation, bacterial antigens such as intestinal failure-related liver disease and primary sclerosing cholangitis⁽⁴¹⁾. Given the evidence accumulating for a causative role for the microbiota in the various manifestations of non-alcoholic fatty liver disease (NAFLD)^(40,80,81), studies of antibiotic therapy or other manipulations of the microbiota in this disease will be of especial interest.

3. Prebiotics and probiotics

Though probiotics and prebiotics have been extensively used to address a host of digestive symptoms and disorders, firm clinical data is somewhat thin on the ground^(82,83). For probiotics, the best evidence for efficacy lies in diarrheal disease⁽⁸⁴⁻⁸⁶⁾ and necrotizing enterocolitis⁽⁸⁷⁾. Studies in inflammatory bowel disease are supportive of their use in pouchitis and, possibly, in the maintenance of mild ulcerative colitis but results in Crohn's disease have been disappointing⁽⁸⁸⁾. Though used empirically by sufferers for decades, more recently, a rationale for the use of probiotics in irritable bowel syndrome has emerged⁽⁸⁹⁾. While meta-analyses suggest overall efficacy for probiotics and bifidobacteria, in particular, in irritable bowel syndrome⁽⁹⁰⁾, it is likely that these effects, as elsewhere, are strain specific⁽⁹¹⁾. There is, as yet, no convincing evidence for a role for probiotics in liver disease or its complications.

Fecal transplantation is now a widely accepted approach for the treatment of resistant, recurrent *C difficile* infection⁽⁹²⁻⁹⁴⁾ and its application to other indications is being explored⁽⁹⁵⁾.

The future

What is the future for this area? Avenues currently under active exploration include the use of "dead" bacteria, bacterial components and small molecules produced by bacteria. The possibility that bacteria could be genetically modified to transport biologically active compounds or vaccines represents another exciting application of this technology⁽⁹⁶⁾.

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