REVIEWS

Gut Microbiota: Mining for Therapeutic Potential

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The resident microbiota of the human intestine exerts a conditioning effect on intestinal homeostasis, delivering regulatory signals to the epithelium and instructing mucosal immune responses. Pattern recognition receptors are key mediators of innate host defense, and in healthy individuals, the mucosal immune system exhibits an exquisitely regulated restrained response to the resident microbiota. However, in genetically susceptible hosts, unrestrained mucosal immune activation in response to local bacterial signals can contribute to the pathogenesis of inflammatory bowel disease. Manipulation of the microbiota to enhance its beneficial components thus represents a potential therapeutic strategy for inflammatory bowel disease. Moreover, the microbiota might be a rich repository of metabolites that can be exploited for therapeutic benefit. Modern molecular techniques are facilitating improved understanding of host-microbe dialogue in health and in several disease processes, including inflammatory bowel disease. It follows that elucidating the molecular mechanisms of host-microbial interactions is now a prerequisite for a "bugs to drugs" program of discovery.

The chronic inflammatory bowel diseases (IBDs), Crohn's disease and ulcerative colitis, are characterized by bouts of uncontrolled, chronic mucosal inflammation, followed by remodeling processes that occur during periods of remission. Together, these result in tissue-damaging inflammatory responses in the intestine that might result in much personal suffering and impaired quality of life. Crohn's disease and ulcerative colitis have a combined prevalence of 250/100,000 in the Western world, and they represent a substantial economic burden on health care resources. Although the precise etiology of IBD remains to be elucidated, a complex interaction of environmental, genetic, and immunoregulatory factors contributes to the initiation and perpetuation of the disease.[1]

Under normal circumstances, the mucosal immune system is exquisitely regulated and exhibits a restrained response to the resident microbiota while retaining an ability to mount appropriate immune responses to pathogenic bacteria. However, several lines of evidence indicate that genetically influenced dysregulation of the mucosal immune response to antigens of the indigenous microbiota can contribute to the pathogenesis of IBD.[2,3] In susceptible individuals, environmental triggers can impact on the initiation or reactivation of disease, and tissue damage might result from immune cell misperception of danger within the indigenous microbiota or from failure of normal tolerance to enteric bacteria.[2,4] Within the gastrointestinal tract, the inflammatory capacity of commensal bacteria is varied. Some resident bacteria are proinflammatory, whereas others attenuate inflammatory responses.[5–11]

The 2005 Nobel prize awarded to Barry Marshall and Robin Warren is a sobering reminder that the solution to some chronic disorders cannot be unraveled by exclusive investigation of the host response. The host response must be considered in terms of bacterial residents and prokaryotic-eukaryotic interactions. Modern molecular techniques are facilitating our understanding of microbe-microbe and host-microbe communications at mucosal interfaces and are providing glimpses of tangible therapeutic strategies. Therefore, the intent here is to present an overview of the commensal microbiota, their interactions with the intestinal mucosa, and clinical relevance to IBD.

Indigenous Gut Microbiota

Composition of the Normal Gut Microbiota

Dialogue between commensal bacteria and the host occurs primarily along mucosal surfaces, and the largest interface is the human gastrointestinal mucosa. The intestine is habitat to a dynamic and diverse bacterial community that is separated from the internal milieu by only a single layer of epithelial cells. Intestinal bacteria outnumber human somatic and germ cells 10-fold[12] and represent a combined microbiome well in excess of the human genome. Co-evolution of the host and indigenous microbes has fostered mutually beneficial and cooperative interactions mediated by bidirectional host-microbiota exchange.

Traditionally, studies of indigenous bacteria focused on the characterization of fecal diversity. Detailed analysis was hindered by conventional microbiology techniques, and most bacterial species still cannot be cultured. Modern molecular techniques such as broad-range sequencing of 16S ribosomal RNA (rRNA) from amplified bacterial nucleic acid extracted from feces or biopsies indicate evolutionary divergence and can be used to identify and classify bacteria. The availability of bacterial sequence data has facilitated the development of molecular probes for DNA microarrays, fluorescent in situ hybridization, and gene chips that identify and enumerate specific microbial species. These molecular approaches are being used to examine the individuality and temporal stability of the microbiota and

Abbreviations used in this paper: AIEC, adherent-invasive *Escherichia coli*; CARD, caspase recruitment domain; DC, dendritic cell; IBD, inflammatory bowel disease; IL, interleukin; IFN, interferon; MAP, *Mycobacterium avium* subspecies *paratuberculosis*; MDP, muramyl dipeptide; NF, nuclear factor; NOD, nucleotide-binding oligomerization domain; PPAR, peroxisome proliferator activated receptor; PRR, pattern recognition receptor; rRNA, ribosomal RNA; Th1/Th2, T-helper cell type 1/2; TLR, toll-like receptor; TNF, tumor necrosis factor.

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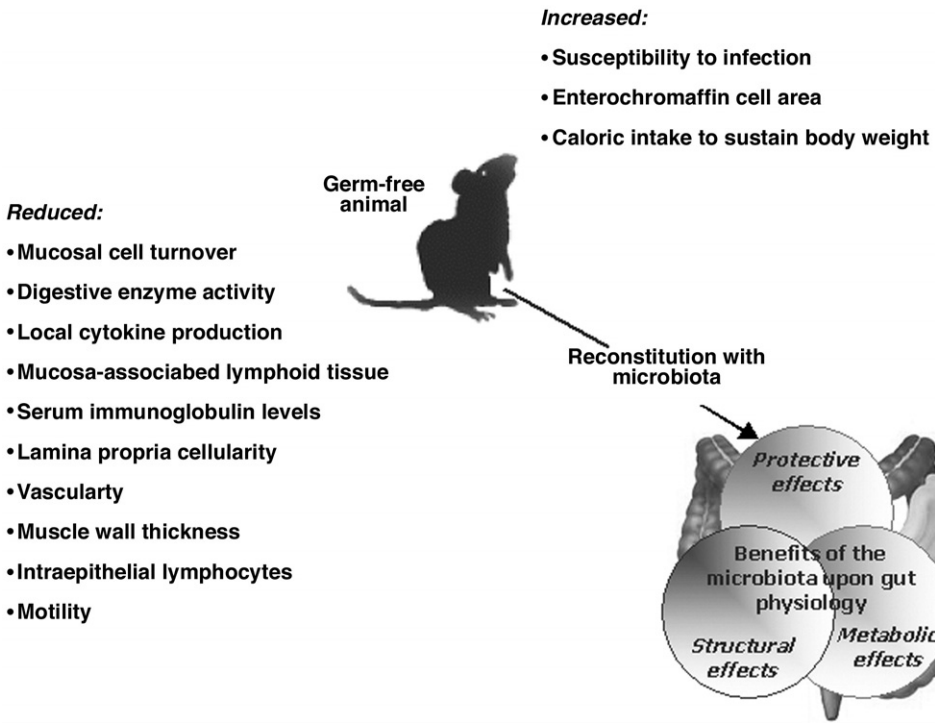

Figure 1. Changes in intestinal structure and function in germ-free animals compared with colonized animals raised under conventional conditions. Reconstitution of germ-free mice with a microbiota restores the mucosal immune system, and commensal bacteria exert numerous protective, structural, and metabolic effects on the intestinal epithelium.

the impact of weaning, antibiotics, or dietary changes on its composition. It is now realized that the mucosa-associated community is significantly different from the luminal and fecal communities.[13] Although every adult intestine harbors a particular combination of predominant species, the composition of the intestinal microbiota might alter with lifestyle, diet, and age.[14,15] Nonetheless, a comparative study of the microbiota of human adults with varying degrees of genetic relatedness, including monozygotic twins, emphasized the prevailing influence of host genotype over diet in determining the microbial composition of the gut.[16]

Acid, bile, and pancreatic secretions hinder the colonization of the stomach and proximal small intestine by most bacteria. However, bacterial density dramatically increases in the distal small intestine and in the large intestine increases to approximately 10^{11}–10^{12} bacteria per gram of colonic content, which contribute to 60% of fecal mass.[17,18] Although archaea and eukarya are also represented,[19] bacteria predominate, and colon-residing bacteria achieve the highest cell densities recorded for any ecosystem.[20] The most common anaerobic genera include *Bifidobacterium*, *Clostridium*, *Bacteroides*, and *Eubacterium*, and aerobic *Escherichia*, *Enterococcus*, *Streptococcus*, and *Klebsiella* are also found. However, sequencing of 16S rRNA gene clone libraries has indicated that a significant fraction of the bacteria represent uncultivated species and novel microorganisms.[13]

Mammalian fetuses are born germ-free, but immediately after birth, establishment of the resident microbiota is driven by environmental factors such as mode of delivery, type of infant diet, hygiene levels, and medication.[21,22] Enterobacteria and bifidobacteria species represent early colonizers, but differences in gut microbial composition and incidence of infection occur between breast-fed and formula-fed infants.[21] These pioneer bacteria can modulate gene expression in the host to create a suitable environment for themselves and can prevent growth of other bacteria introduced later in the ecosystem.[23,24] Increased credence is being given to the hypothesis that the modern sanitized environment of developed societies has altered the normal pattern of gut colonization during infancy. This might result in a lack of tolerance to otherwise harmless food proteins and other antigens, including those of the intestinal microbiota.[25]

Functions of the Normal Gut Microbiota

Enteric bacteria confer many benefits to intestinal physiology including protective, structural, and metabolic effects. Their influence on intestinal structure and function has been demonstrated in comparative studies of germ-free and colonized animals. Some of the differences between animals raised under germ-free and conventional conditions are listed in Figure 1. Such comparisons, together with studies indicating that reconstitution of gnotobiotic mice with a microbiota is sufficient to restore the mucosal immune system,[26] illustrate that the microbiota provide regulatory signals that instruct intestinal development and function. Indeed, colonization of germ-free mice with a single species, *Bacteroides thetaiotaomicron*, affects the expression of a variety of host genes influencing nutrient uptake, metabolism, angiogenesis, mucosal barrier function, and the development of the enteric nervous system.[23] Furthermore, ligands from resident bacteria and commensal-derived symbiosis factors influence the normal development and function of mucosal immunity.[27,28] Indigenous bacteria educate the mucosal immune system and modulate the fine