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Feature Review

Antibiotic-Induced Changes in the Intestinal Microbiota and Disease

Simone Becattini,[1] Ying Taur,[2,3] and Eric G. Pamer[1,2,3,*]

The gut microbiota is a key player in many physiological and pathological processes occurring in humans. Recent investigations suggest that the efficacy of some clinical approaches depends on the action of commensal bacteria. Antibiotics are invaluable weapons to fight infectious diseases. However, by altering the composition and functions of the microbiota, they can also produce long-lasting deleterious effects for the host. The emergence of multidrug-resistant pathogens raises concerns about the common, and at times inappropriate, use of antimicrobial agents. Here we review the most recently discovered connections between host pathophysiology, microbiota, and antibiotics highlighting technological platforms, mechanistic insights, and clinical strategies to enhance resistance to diseases by preserving the beneficial functions of the microbiota.

In the past two decades the gut **microbiota** (see Glossary) has been recognized as a fundamental player orchestrating host physiology and pathology (Box 1). Trillions of bacteria inhabit the gastrointestinal (GI) tract of complex metazoans including humans, greatly expanding the host genetic repertoire [1]. This translates into the possibility for the host to perform functions that are not encoded by its own genome: commensals protect from pathogen invasion, extract additional energy from food, and synthesize key molecules for tissue development in a way that is highly specialized with respect to their location along the GI tract [2–4].

Although the physiology of virtually all organs is influenced by the microbiota [5,6], the intestinal mucosa and its immune components are most affected by this symbiosis [7]. We first review recent findings elucidating the impact of the microbiota on the immune system. Second, we discuss the involvement of gut commensals in the pathogenesis of disease. Third, we examine the role of antibiotics in perturbing or driving these processes. Finally, we discuss the mechanisms of antibiotic resistance development and spread, as well as the proposed approaches to overcome the drawbacks of antibiotic therapy.

Beneficial Roles of the Microbiota

The gut microbiota exerts many beneficial functions for the host, to a level that it can be considered an additional organ [8]. For example, commensal bacteria convert primary bile acids into secondary bile acids and they also produce vitamins of the B and K groups, and ferment otherwise indigestible plant-derived fibers producing **short-chain fatty acids** (SCFAs) that feed **enterocytes** and modulate immune functions [2,3]. Furthermore, the microbiota drives intestinal development by promoting vascularization, villus thickening, mucosal surface widening, mucus production, cellular proliferation, and maintenance of epithelial junctions [9–11]. Notably, the influence of the microbiota is not limited to the intestine, and affects the physiology of most host organs, even the brain [9,12–15].

Trends

The gut microbiota contains trillions of bacteria belonging to hundreds, possibly thousands, of species and is crucial for optimal maintenance of host physiological processes.

The microbiota protects against infections and other pathologies by directly inhibiting invading microbes or by orchestrating appropriate immune responses; conversely, metabolites produced by some gut commensals can promote a variety of diseases such as atherosclerosis or cancer.

Antibiotics alter the microbiota composition, resulting in an increased risk of disease, secondary infections, allergy, and obesity. In addition, they promote the spread of drug-resistant pathogens, making the search for alternative clinical approaches imperative.

Novel strategies are being developed to substitute or complement antibiotic therapies, attempting either to selectively target pathogens without perturbing the microbiota and/or to re-establish commensal communities together with the protective and beneficial effects they confer to the host.

[1]Immunology Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA
[2]Infectious Diseases Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA
[3]Lucille Castori Center for Microbes, Inflammation and Cancer, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA

*Correspondence: pamere@mskcc.org (E.G. Pamer).

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Box 1. The Gut Microbiota: A Structural Overview

The human gut microbiota consists of an estimated 100 trillion bacteria belonging to several hundreds of different species [129]. These fall into four major phyla covering more than 90% of the total bacterial population, namely Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria, and include many additional minor phyla such as Verrucomicrobia and Fusobacteria. The representation of these groups changes along the GI tract, influenced by distinct microenvironments and nutrient availability [2]. The Firmicutes phylum is composed mainly by Gram$^+$ aerobic and anaerobic bacteria. Prominent members are *Clostridia* strains, whose activities range from beneficial and protective (e.g., *C. scindens*, clusters IV–XIVa) to pathogenic (e.g., *C. difficile*, *C. perfrigens*). Potentially pathogenic streptococci, enterococci, and staphylococci are also Firmicutes. Bacteroidetes are Gram$^-$ bacteria that are extremely well adapted to the intestinal environment. Here they ferment otherwise indigestible carbohydrates producing SCFAs, molecules that have been implicated in a plethora of important processes. Actinobacteria are Gram$^+$ bacteria generally considered to be beneficial, such as the *Bifidobacterium* genus, and which are included in many probiotic preparations. The Proteobacteria phylum contains Gram$^-$ bacteria, most notably the family of Enterobacteriaceae, including *E. coli* and *K. pneumoniae*. These are not very abundant under normal conditions, but tend to expand upon dysbiosis.

Notably, the majority of the studies of the microbiota have been performed in mice, even though the human and mouse microbiota differ in genus representation [130]. Some genera such as *Prevotella*, *Faecalibacterium*, and *Ruminococcus* are abundant in humans, while others, namely *Lactobacillus, Alistipes*, and *Turicibacter*, are highly prevalent in mice [131]. However, a core of common taxa can be identified, and mouse and human intestinal metagenomes appear to be remarkably similar if analyzed from a functional perspective [i.e., representation of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, which depict the overall metabolic potential of a community] [132]. Most importantly, GF animals can be efficiently reconstituted with microbial communities isolated from other species, including humans, reproducing effects observed in a donor in the recipient host [133]. Reconstitution of GF mice with stool samples from obese or malnourished subjects is sufficient to phenocopy patient defects in energy harvest or growth [55,65,134,135], demonstrating that despite interspecies divergences, the mouse model is a valuable tool to study the human microbiota.

One of the most prominent roles of the gut microbiota is to promote the development and education of the immune system, both locally and systemically, as described below.

Education of the Immune System

The close proximity of dense microbial populations to host tissues poses risks of invasion, and the immune system must thoroughly monitor bacteria present in the gut lumen (Box 2). Nonetheless, the microbiota is allowed to prosper on the surface of the intestinal mucosa, orchestrating the overall physiology of the tissue lying underneath. This concept was established with the observation that antibiotic treatment worsens the severity of **dextran sodium sulfate (DSS)-induced colitis** in mice by depleting microbial ligands that normally signal through **Toll-like receptors** (TLRs) and function to ensure expression of tissue homeostasis and repair mediators [16] (Figures 1 and 2).

All branches of the immune system rely on this tonic signaling to properly function (Figure 1). Microbiota-derived lipopolysaccharide (LPS) maintains basal level expression of RegIII-γ (a bactericidal C-type lectin) in intestinal epithelial cells (IECs) and **Paneth cells**. RegIII-γ is not detected in **germ-free (GF) mice** [17], and even short-term antibiotic treatment impairs its expression, rendering mice susceptible to vancomycin-resistant enterococcus (VRE) infection, a defect that can be reverted by oral administration of LPS [18]. Similarly, commensal flagellin sensing by TLR5 on CD103$^+$CD11b$^+$ **dendritic cells** (DCs) in the **lamina propria** (LP) contributes to maintenance of RegIII-γ expression. Upon TLR5 signaling, DCs produce IL-23, promoting IL-22 release by **innate lymphoid cells** (ILCs), and therefore RegIII-γ expression in intestinal epithelial cells (IECs) [19,20].

Granulocytes also receive commensal cues, which they sense while residing in the bone marrow (BM). NOD1-mediated sensing of *meso*-diaminopimelic acid (DAP) promotes neutrophil-mediated killing of pathogens such as *Staphylococcus aureus* and *Streptococcus pneumoniae* [22,23]. In GF mice reconstituted with *Escherichia coli*, DAP was detected in the blood and BM over the course of 3 days, showing that bacterial ligands from the intestinal lumen can have systemic distribution, and therefore a systemic effect [22]. Moreover, perinatal antibiotic

Glossary

Antimicrobial peptides (AMPs): small peptides with bactericidal activity, mainly positively charged, that are produced by microorganisms and host myeloid and epithelial cells.
Bacteriocins: toxins, largely proteins, secreted by bacteria to kill other bacteria.
β-Lactams: antibiotics containing a β-lactam ring in their molecular structure; this class includes penicillins, cephalosporins, and carbapenems.
B1 cells: subset of B cells activated by innate sensor triggering that produce the vast majority of natural IgM against common microbial structures (secreted independently of antigenic exposure).
Cathelicidins: heterogeneous family of AMPs, including LL-37 in humans and cathelicidin-related antimicrobial peptide (CRAMP) in mice, produced mainly by myeloid and epithelial cells.
Colonization resistance: protection against pathogens exerted by commensal bacteria.
Conventionalization: transfer of microbiota into a germ-free mouse; often performed by gavage (intragastric inoculation) of fecal material.
Dendritic cells (DCs): myeloid cells specialized in sampling the outer environment through endocytosis, and which initiate immune responses upon antigen presentation to other immune cells.
Dextran sodium sulfate (DSS)-induced colitis: mouse model of colitis promoted by administration in drinking water of DSS, which damages the intestinal epithelium and promotes inflammation.
Dysbiosis: imbalance (alteration in composition) within a microbiota.
Enterocytes: epithelial cells constituting the intestinal epithelium, characterized by the presence of apical microvilli that enhance their adsorbing functions.
Germ-free (GF) mice: mice born and raised in isolators in the absence of any detectable microorganism inside or outside their body.
Gnotobiotic mice: mice that are born aseptically, bred in laboratories, and that bear known strains of microorganisms.
Inflammasome: oligomeric protein complex that drives caspase 1-mediated maturation of IL-1β and IL-18 upon sensing of