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PERSPECTIVES

Refocusing Human Microbiota Research in Infectious and Immune-mediated Diseases: Advancing to the Next Stage

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Changes in the microbiota are associated with disease susceptibility, immune system development, and responses to treatment. Refocusing research to elucidate the causal links between the human microbiota and infectious and immune-mediated diseases will be critical to harnessing its power to prevent, diagnose, and treat such diseases.

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Trillions of microbes colonize the human body at or before birth. This microbial community of bacteria, archaea, viruses, fungi, and protozoa, collectively known as the human microbiota, develops in complexity during early childhood and colonizes the host in a remarkable, symbiotic relationship [1]. Over the last decade, advances in emerging technologies and large-scale research programs have catalyzed research on the human microbiota. Significant progress has been made in identifying the composition, diversity, and structure of the microbiota in different human organs and establishing the association of the microbiota with health and many diseases [2].

The influence of the microbiota, the gut microbiota in particular, on infectious and immune-mediated diseases is well established. Changes in its composition and diversity have been shown to be associated with disease susceptibility, development and maintenance of the host immune system, and responses to therapeutic interventions [3]. Although the quantity of microbiota research is increasing, the focus continues on studies associating the microbiota with health or disease. Reductions in sequencing costs, increases in sequencing capacity, especially at large-scale genomic research facilities, advances in next-generation sequencing technologies, the ease of metagenomic analysis of human clinical samples, and improved computational tools all contribute to drive microbiota research toward association studies.

Several microbiota-based therapeutic strategies are emerging from these associations; however, significant research gaps exist. Now is the time to refocus microbiota research beyond association studies and advance research on the mechanisms that underly the causal links between the human microbiota and infectious and immune-mediated diseases. This knowledge will be critical to harnessing the power of the human microbiota to prevent, diagnose, and treat such diseases.

MICROBIAL INTERACTIONS

One area of intense research is the molecular mechanisms of host-microbiota interactions, especially in the gut. These interactions can serve as the basis to develop therapeutic interventions. Commensal organisms influence susceptibility to infection by protecting against invasion, maintaining their own colonization, and resisting subsequent colonization by pathogens. Gut commensals can induce immune responses to pathogens, regulate inflammation, serve as a physical barrier to protect against invading pathogens, compete with pathogens for nutrients, and produce metabolites and other small molecules, such as short-chain fatty acids that can reduce inflammation and restrict growth of enteric bacteria. The importance of a healthy gut microbiota is made evident by the effects of antibiotics, which can wreak havoc by altering the composition and diversity of the gut microbiota and disrupt the ability to prevent colonization by pathogens. Antibiotics can increase susceptibility to bacterial enteric infections, including infections with *Clostridium difficile, Klebsiella pneumoniae, Escherichia coli, and Vibro cholerae* and antibiotic-resistant pathogens, such as vancomycin-resistant enterococci [4]. In addition, reduced bacterial diversity of the human gut microbiota is associated with COVID-19 infection [5].

The specific role of bacterial commensals is starting to be elucidated. Mechanistic studies are starting to shed light on the complex interactions between the gut microbiota and the host and have potential for microbiota-based strategies to restore a healthy microbiota. Short-chain fatty acids and an acidic environment in the gut of mice maintain

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the microbiota and prevent pathogens from causing disease [6]. *Clostridium scindens* has been shown to maintain resistance to colonization of *C. difficile* in the mouse gut microbiota by restoring bile salt inhibition of infection [7]. A defined set of bacterial commensals containing the *Clostridium* cluster XIVa species, *Blautia producta,* and *Clostridium boltcae*, restores resistance to colonization with vancomycin-resistant enterococci [8, 9]. Furthermore, knocking out genes in bacterial metabolic pathways of gut microbiota shows that specific microbiome-based metabolites produced by *Clostridium* species modulate immunoglobulin A–related immune cells [10].

IMPACT OF THE MICROBIOTA ON DEVELOPMENT OF ASTHMA AND ALLERGIC DISEASES

Accumulating evidence links the influence of gut microbiota and the environment to the development of asthma and allergies [11]. Alterations in the developing gut microbiota by antibiotics administered early in life reduce the diversity of gut microbiota which can influence development of the immune system, triggering perturbations to host inflammatory responses, and potentially contributing to the development of asthma. Decreases in the diversity of the human neonatal gut microbiota, with greater abundance of Enterobacteriaceae and lower relative abundance of Bifidobacteriace, have been linked to altering $CD4^+$ T-cell function and susceptibility to and development of asthma [12]. The presence of certain bacterial species in the mouse gut has been shown to protect against milk and other food allergies. For example, transfer of feces from healthy human infants without a milk allergy to germ-free mice protects the mice against allergic responses to cow's milk. Furthermore, a clostridial species in gut microbiota, *Anaerostipes caccae*, may protect mice against cow's milk allergies [13]. Thus, the gut bacteria is a potential target for microbiota-based therapeutics aimed at preventing allergic responses to dietary antigens.

SUSCEPTIBILITY TO INFECTION

The influence of the microbiota on susceptibility to infection is not limited to gut microbiota. Increased susceptibility to human immunodeficiency virus (HIV) acquisition has been linked to decreased dominance of *Lactobacillus* species combined with higher diversity of bacterial species in the vaginal microbiota [14]. Greater diversity of the vaginal bacterial community dominated by anaerobes such as *Prevotella* and *Sneathia* is correlated with increased genital inflammation and an altered immune response, specifically with an increased number of activated mucosal $CD4^+$ T cells compared with that in women with more vaginal *Lactobacillus* species and lower vaginal microbial diversity. The efficacy of topical preexposure prophylaxis has been linked to the composition of the vaginal microbiota and its effectiveness at preventing HIV infection. Women with *Lactobacillus*-dominant vaginal microbiota show significantly reduced risk of HIV acquisition when treated with vaginal tenofovir gel, whereas those with non–*Lactobacillus*-dominant microbiota similarly treated had no difference in HIV acquisition [15]. These effects were not seen with oral preexposure prophylaxis, suggesting that the composition of the vaginal microbiota plays a role in susceptibility to infection, and modulation of this composition could form therapeutic strategies.

THE MICROBIOTA AND THE IMMUNE SYSTEM

The microbiota has a profound and diverse influence on the development and function of the host immune system [16] affecting it in many ways, including priming immune system development and response to invading pathogens, regulating the diversity and function of immune cells, and maintaining epithelial cells that physically deter pathogens. One example is the production of immunomodulatory metabolites of bile acids in the gut. Bile acid metabolites are known to affect the innate and adaptive host immune responses by modulating the balance of T-helper 17 and regulatory T cells [17]. Furthermore, recent studies suggest that the gut microbiota contributes to the production of these immunomodulatory metabolites [18]. Uncovering how the gut microbiome uses bile acids to shape immunity may have future therapeutic applications and provide pathways for therapeutic intervention in regulating T-cell function in autoimmune diseases. The microbiota affects the development of the immune system as well. Development of mucosal-associated invariant T (MAIT) cells rely on exposure to riboflavin-synthesizing commensal bacteria during a specific developmental window, after which MAIT cell development is permanently impaired. Cutaneous MAIT cells promote wound healing, thus suggesting new therapeutic approaches for tissue repair [19].

Recent evidence suggests alteration of the gut microbiota by antibiotics has been linked to vaccine efficacy, highlighting the critical role of the interaction between commensal bacteria and the immune system has on disease prevention. The efficacy of vaccines—including rotavirus, polio, influenza, and others—is associated with changes in the gut microbiota after antibiotic administration. A reduced immune response to the trivalent influenza vaccine was recently demonstrated as a result of less diverse gut microbiota after treatment with antibiotics [20] A reduction secondary bile salts that inhibit inflammatory responses suggest a potential mechanism through which this effect was regulated [20]. Future studies involving diverse populations are needed to elucidate the causal effect of the microbiota on vaccine efficacy.

FECAL MICROBIOTA TREATMENT

As we look ahead, key elements in refocusing microbiota research are within our reach. Current research efforts on the long-term effectiveness and safety of fecal

microbiota treatment (FMT) to restore a healthy microbiota should continue, especially in light of reports of multidrug-resistant bacterial infections associated with FMT [21, 22]. A recent phase 2 clinical trial demonstrated modest therapeutic effects from using a defined set of bacteria for FMT [23]. In addition, the microbiota is more than just bacteria; defining the role of commensal fungi, protozoa, viruses, and bacteriophages play will increase our understanding of the microbiota [24].

LOOKING AHEAD

The wide breadth of microbiota research requires highly collaborative, interdisciplinary teams of scientists that leverage opportunities in data science and emerging technologies to discover the causal links between the human microbiota and infectious and immune-mediated diseases. Recently, data-driven strategies using large-scale computational analysis of publicly available gene sequences were leveraged to jump-start mechanistic studies and therapeutic discoveries by unveiling thousands of novel small proteins associated with the human microbiota [25]. In addition, systems-level analysis to elucidate the complex host-microbiota interactions provided a framework to examine how these molecular interactions may contribute to health and disease.

Despite the success and advances in microbiota research, much remains to be done. A refocused microbiota research program must be prioritized to establish causality of the human microbiota in health and disease and, in turn, inform the development of microbiota-based therapies. This knowledge is critical to harnessing the power of the human microbiota to prevent and treat infectious and immune-mediated diseases.

Notes

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