THE HUMAN MICROBIOTA

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How Microbial Communities Affect Health and Disease

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PREFACE

The human body is a marvelously intricate machine, and the parts list includes trillions of microbial cells that colonize epithelial surfaces such as those found in the mouth and gut. There is increasing evidence that these microbes do more than just reside on tissues—they play key roles in human physiology and organ function. Indeed, there are 100 times more genes in our microbiome compared to our human genome, and these microbial genes code for proteins that impact diverse processes such as digestion, immunity, and development. The goal of this book is to provide an overview of the microbial diversity found in humans and to describe efforts linking microbial communities to human health. Attempts to understand human-associated microbial communities were given a boost by Human Microbiome Project (HMP) initiatives in the United States, Canada, Europe, and Asia. The National Institutes of Health in the United States has devoted more than $150 million to support these studies that are now maturing with release of data and a flurry of publications. This is a time of unprecedented discovery, and although still young, the field is sufficiently advanced to warrant a book summarizing progress. Answers to many questions are now emerging. How do microbial communities differ across body sites? What is the variability in microbial composition across healthy and diseased humans at the same body site? How do certain microbial communities foster healthy tissues? What are the microbial community profiles associated with disease states, and are these communities markers of disease or causes of disease? How can microbial communities be manipulated to optimize health and minimize disease risk? How do microbial communities change over the course of human development? What are the internal factors (genetic, anatomic, hormonal, physiologic) and external environmental factors (diet, sexual activity, hygiene) that shape human-associated microbial communities? With partial answers to these questions come many additional questions about the intimate relationships between human and microbial cells in our bodies.

Our excursion into the human microbiome begins with an introduction to the Human Microbiome Project by Lita Proctor and colleagues from the NIH (Chapter 1). This chapter provides an excellent description of the HMP with its many research initiatives and early progress. It also provides some historical context and a vision for future research. We then shift to chapters focused on tools for studying the human microbiome, including a methodological overview chapter (Chapter 2) by Christine Bassis, Vincent Young, and Tom Schmidt that lays the groundwork for later chapters. Bassis and colleagues compare different genomic cultivation-independent methods for characterizing microbial communities, highlighting the advantages and limitations of commonly used techniques. They also consider the role of cultivation methods in the genomic era, and provide advice about designing microbiome studies. In their chapter, Susan Lynch and Eoin Brodie delve more deeply

into the use of phyloarrays for microbial community analysis (Chapter 3), a technique that has some distinct advantages for microbial community profiling. Manuel Lladser and Rob Knight round out our methods section by providing a mathematical perspective on interpreting microbial community structure and diversity. This chapter (Chapter 4) is highly relevant in the era of high-throughput sequencing of phylogenetically informative microbial gene sequences [generated by polymerase chain reaction (PCR) or from metagenomic methods] for describing microbial populations.

We then begin a tour of various microbial niches of the human body, such as the gut, respiratory tract, mouth, and genital tract. These chapters highlight the different microbial populations found in different human tissues, and describe how microbial communities change with conditions such as gingivitis, inflammatory bowel disease, and bacterial vaginosis. Dan Frank and Ellen Li start this tour with a description of how host genetics (immune response) and the gut microbiota may interact to facilitate Crohn's disease (Chapter 5). They also introduce the concept of dysbiosis that will be used in other chapters. Edith Zemanick and J. Kirk Harris then describe the microbiota of the human respiratory tract (Chapter 6), focusing on the normal microbiota and alterations in conditions such as cystic fibrosis and ventilator-associated pneumonia. The oral microbiota is described in a chapter by Angela Nobbs, David Dymock, and Howard Jenkinson (Chapter 7). Here they note some of the physical and metabolic interactions among the 600 different bacteria species that live in the mouth. They also describe the connections between some oral microbial communities and local conditions such as caries, and systemic diseases such as endocarditis. Laura Sycuro and myself review the genital tract microbiota of women and men, with a particular focus on the condition bacterial vaginosis that is associated with numerous adverse health outcomes in women and neonates (Chapter 8). Alexander Swidsinski and Vera Loening-Baucke end this section with a chapter on use of *in situ* hybridization methods combined with fluorescence microscopy for describing the spatial relationships of microbes and human cells in the gut. This chapter (Chapter 9) is notable for moving beyond the description of "who's there" to a description of the structural and functional features of the gut microbiota. Note that not every human body niche is covered in these chapters.

Two chapters focus on the use of animal models to manipulate the microbiota and understand how changes impact health. June Round tackles the use of a variety of animal models to study host immunity, including the fruit fly, zebrafish, and mouse (Chapter 10). She highlights key lessons that can be learned from these models regarding human immune responses to our indigenous microbiota. The team of Bethany Rader and Karen Guillemin describe new insights that have been produced by animal models, including fish (Chapter 11). Several important questions are more easily answered with animal models, including how microbial communities assemble in space and time, and identifying the relative contributions of host genetics, environmental factors (such as diet), and stochastic sampling. Microbes and animals can be studied in the laboratory in ways that are not possible in human studies.

The use of cultivation-independent methods suggests that many human-associated microbes still resist cultivation in the laboratory. Karsten Zengler tackles this issue in his chapter on cultivation procedures in the genomic age (Chapter 12), reinforcing the challenges and rewards of cultivating microbes from the human

body. So, how can we cultivate fastidious members of the human microbiota? The chapter by Slava Epstein, Maria Sizova, and Amanda Hazen (Chapter 13) provides many novel approaches for cultivating human microbes using cutting-edge techniques in order to resolve the "great plate count anomaly."

Finally, George and Sandra Macfarlane (Chapter 14) address a key issue in the microbiome field: how one can manipulate the human microbiota. These investigators provide a thoughtful and balanced review on the use of prebiotics, probiotics, and synbiotics to alter the human indigenous microbiota for the purpose of enhancing health.

What does it mean to be human? The authors of these chapters provide a compelling argument that we are far from alone, and that our microbiota helps mold the human form. Please enjoy the insights from this outstanding collection of investigators who are unraveling the mysteries of the human microbiome.

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THE NIH HUMAN MICROBIOME PROJECT

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1.1. INTRODUCTION

The human microbiome is the full complement of microbial species and their genes and genomes that inhabit the human body. The National Institutes of Health (NIH) Human Microbiome Project (HMP) is a community resource project designed to promote the study of complex microbial communities involved in human health and

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disease. The HMP has increased the appreciation for the features of the human microbiome that all people share as well as the features that are highly personalized. Host genetics, the environment, diet, the immune system, and many other factors all interact with the human microbiota to regulate the composition and function of the microbiome. As a scientific resource, the HMP has publically deposited to date or made available over 800 reference microbial genome sequences, hundreds of microbial isolates from the human microbiome, over 3 terabases (Tbp) of metagenomic microbial sequence, over 70 million 16S rRNA reads, close to 700 microbiome metagenome assemblies, over 5 million unique predicted genes, and a comprehensive bodywide survey of the human microbiome in hundreds of individuals from a healthy adult cohort. A number of demonstration projects are contributing a wealth of knowledge about the association of the microbiome with specific gut, skin, and urogenital diseases. Other key resources include the development of new computational tools, technologies, and scientific approaches to investigate the microbiome, and studies of the ethical, legal, and social implications of human microbiome research. This chapter captures the historical context of the HMP and other international research endeavors in the human microbiome, highlights the multiple initiatives of the HMP program and the products from this activity, and closes with some suggestions for future research needs in this emerging field.

1.2. GENESIS OF HUMAN MICROBIOME RESEARCH AND THE HUMAN MICROBIOME PROJECT (HMP)

It sometimes seems that research on the human microbiome blossomed overnight. However, the conceptual and technological foundations for the study of the human microbiome began to emerge before the 1990s and can be found within many disciplines. Microbial ecologists who studied microorganisms and microbial communities in the environment recognized early on that most microorganisms in nature were not culturable and so developed alternate approaches to the study of microbial communities. An early and broadly adopted approach for investigating microorganisms in the environment, based on the three-domain system for biological classification [1], was the use of the 16S ribosomal RNA gene as a taxonomic marker for interrogating microbial diversity in nature [2]. With the growth of non-culture-based, molecular techniques in the 1980s and 1990s for study of environmental microorganisms and communities, some medical microbiologists turned these tools to the human body and found far greater microbial diversity than expected, even in well-studied sites such as the oral cavity [3–5].

In the infectious disease field, recognition was growing that many diseases could not satisfy Koch's postulates as the pathogenesis of many of these diseases appeared to involve multiple microorganisms. The term *polymicrobial diseases* was coined to describe those diseases with multiple infectious agents [6]. We now recognize that many of these formerly classified polymicrobial diseases, such as abscesses, AIDS-related opportunistic infections, conjunctivitis, gastroenteritis, hepatitis, multiple sclerosis, otitis media, periodontal diseases, respiratory diseases, and genital infections, are associated with multiple microbial factors, that is, with the entire microbiome. In an essay on the history of microbiology and infectious disease, Lederberg [7], who coined the term *microbiome*, called for "a more