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■ ■COMBINATION APPROACHES: MODIFICATION

OF HOST AND TUMOR BY VIROTHERAPY— IMMUNO-ONCOLYTIC VIRUSES These viruses are genetically modified to replicate in malignant but not normal cells. The replicating vectors thus proliferate and spread within the tumor, facilitating eventual tumor clearance. However, physical limitations to viral spread, including fibrosis, intermixed normal cells, basement membranes, and necrotic areas within the tumor, may reduce clinical efficacy, and their activity against metastatic disease has proved limited. Recently, the FDA granted licensing approval to talimo gene laherparepvec, an oncolytic herpes virus containing the human granulocyte-macrophage colony-stimulating factor (GM-CSF) gene, for treatment of melanoma. This success has led to resurgent interest in combining the local tumor destruction and tumor antigen release mediated directly by oncolytic viruses with the recruitment of a systemic immune response mediated by immunostimulatory genes contained within the oncolytic virus. In principle, such immune-oncolytic viruses should produce responses in both local and metastatic disease. Numer ous novel viral agents are now entering early-phase clinical testing.

PART 16 Genes, the Environment, and Disease SUMMARY AND FUTURE DIRECTIONS Cell and gene therapies have progressed from halting beginnings to the current status as the fastest-growing sector of medicine and the health care industry. The speed of technical evolution results in a continuously changing landscape. Key advances likely to assume greater importance in the coming decade include bioengineered AAV capsids selected for tropism and increased expression, leading to lower doses, fewer adverse events, and lower manufacturing burden; extension of therapeutic trials from single-gene disorders to complex acquired disorders, including chronic heart failure, age-related macular degeneration, and Alzheimer’s disease; continued expansion of in vivo genome editing; and application of CAR-T technology to solid tumors and autoimmune disorders

(e.g., systemic lupus erythematosus). The power and versatility of gene therapy approaches are such that there are few serious disease entities for which gene therapies are not under development. Approved products and examples of clinical success are now abundant, and cell and gene therapies are likely to become increas ingly important as therapeutic modalities in the twenty-first century. Realization of the therapeutic benefits of modern molecular medicine will depend on continued progress in cell and gene therapy technology. ■ ■FURTHER READING Al-Zaidy SA et al: AVXS-101 (onasemnogene abeparvovec) for SMA1: Comparative study with a prospective natural history cohort. J Neuromuscular Dis 6:307, 2019. Frangoul H et al: CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. N Engl J Med 384:252, 2021. Fumagalli F et al: Lentiviral haematopoietic stem-cell gene therapy for early-onset metachromatic leukodystrophy: Long-term results from a non-randomised, open-label, phase 1/2 trial and expanded access. Lancet 399:372, 2022. High KA, Roncarolo MG: Gene therapy. N Engl J Med 381:455, 2019. June CH, Sadelain M: Chimeric antigen receptor therapy. N Engl J Med 379:64, 2018. Kanter J et al: Biologic and clinical efficacy of LentiGlobin for sickle cell disease. N Engl J Med 386:617, 2022. Larson RC, Maus MV: Recent advances and discoveries in the mecha nisms and functions of CAR T cells. Nat Rev Cancer 21:145, 2021. Longhurst HJ et al: CRISPR-Cas9 in vivo gene editing of KLKB1 for hereditary angioedema. N Engl J Med 390:432, 2024. Pipe SW et al: Gene therapy with etranacogene dezaparvovec for hemophilia B. N Engl J Med 388:706, 2023. Ruella M et al: Mechanisms of resistance to chimeric antigen receptor-T cells in haematological malignancies. Nat Rev Drug Discov 22:976, 2023. Tabebordbar M et al: Directed evolution of a family of AAV capsid variants enabling potent muscle-directed gene delivery across spe cies. Cell 184:4919, 2021. Verdun N, Marks P: Secondary cancers after chimeric antigen receptor T-cell therapy. N Engl J Med 390:584, 2024.

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The Human Microbiome

in Health and Disease “All disease begins in the gut.” —Hippocrates Nearly two and a half millennia after Hippocrates made this statement, we are just coming to truly appreciate its profundity. Since the begin ning of humankind, scholars have been investigating the underpin nings of disease with an almost singular focus on the human side of the equation. Microbes were not recognized as an important cause of disease until the inception of the “germ theory” in the late nineteenth century. During the first century of medical microbiology, research largely centered on the role of microbes as pathogens. Only recently has there been a resurgence of interest in understanding how commensal organisms—the bacteria, viruses, fungi, and Archaea that make up the microbiota—impact human physiology. The idea that these micro organisms are vital to the well-being of humans has challenged our traditional notions of “self.” Indeed, a human being can most accurately be described as a holobiont: a complex assemblage of human cells and microorganisms interacting in an elaborate pas de deux that drives normal physiologic processes. Aimed at a better understanding of this relationship, myriad stud ies during the past decade have begun to catalogue the microbiota at various body sites and in a multitude of disease conditions. Diseases in virtually every organ system have been associated with changes in the microbiota. Indeed, the microbiota has been linked to intestinal disorders, disturbances in metabolic function, autoimmune diseases, and psychiatric conditions and has been shown to influence sus ceptibility to infection and the efficacy of pharmaceutical therapies. Knowledge of the specific mechanism(s) underlying most of

these microbe–disease associations is lacking; it remains unclear whether the disease-associated alterations in the microbiota represent mere biomarkers of disease, a causal relationship, or a combination of the two. Although cause-and-effect relationships are still being eluci dated for many diseases, it is clear that humans coexist in an intricate relationship with commensal organisms. This chapter explores in detail the nature of these host–commensal interactions, focusing on how this information might be translated into clinically meaningful interventions. HISTORICAL PERSPECTIVE Massive undertakings, such as the Human Microbiome Project (HMP) sponsored by the National Institutes of Health and MetaHIT sponsored by the European Commission, have catalogued all the bacteria present at multiple body sites in people with and without disease. Coupled with the confluence of advances in sequencing technologies (Chap. 126), gnotobiotic animal availability, and microbial culture, significant prog ress has been made toward an understanding of the interplay between the microbiota and human health. However, current findings were foreshadowed by work done centuries ago. The human microbiota was first explored in 1683 when Antony van Leeuwenhoek described in a letter to the Royal Society of London the “very little living animalcules, very prettily a-moving” that he had observed in the plaque between his teeth. Leeuwenhoek went on to perform the first comparative “microbiota” studies by assessing how fecal and oral bacteria differ, how oral microbes change in the setting of disease (e.g., alcoholism and tobacco use), and how microbial com position changes across the age spectrum (e.g., in young children vs old men). He attempted—unsuccessfully—to eliminate these bacteria. Although Leeuwenhoek was not taken seriously when he first reported his findings, his studies laid the groundwork for what is now the field of microbiome research, and investigators are still trying to answer

many of the same overarching questions that he raised more than three centuries ago. Although Leeuwenhoek first reported the existence of bacteria and their association with humans at the end of the seventeenth century, the significance of commensal bacteria was not realized until late in the nineteenth century. In 1885, Pasteur suggested that animals could not survive if they were “artificially and completely deprived of the com mon microbes.” Although Pasteur’s preconceived ideas were proven incorrect in 1912 by the advent of germ-free (GF) animals (animals raised without exposure to any microorganisms), the underlying con cept that commensal organisms are critical to health has held up. Élie Metchnikoff made another conceptual advance in this field by suggest ing at the beginning of the twentieth century that clinical outcomes could be altered by the administration of specific beneficial organisms (probiotics). In particular, Metchnikoff believed that aging was caused by toxic bacteria in the gut and that lactic acid–producing bacteria (e.g., Lactobacillus species) present in sour milk and yogurt could miti gate against this process. The data behind this specific claim are still lacking, but contemporary discoveries offer continued hope that the microbiome can be effectively harnessed to protect against and treat a variety of diseases. Thus, although the field of microbiome research is sometimes considered to have emerged over the last two decades, the basic tenets—that the microbiota varies according to body site and clinical characteristics, that microbes are critical for human health, and that specific modulation of the microbiota may lead to improved clini cal outcomes—are far from new. A PRIMER ON TAXONOMY Given that microbiome-based studies have identified and compared microbes at different levels of taxonomic resolution (Fig. 484-1), some understanding of taxonomy is essential for better comprehension of the implications of these studies. Of the ~100 bacterial phyla that exist in nature, only five (Actinobacteria, Bacteroidetes, Firmicutes, Fuso bacteria, and Proteobacteria) are dominant members of the human microbiome. Each of these phyla can be

further categorized into mul tiple classes, orders, families, genera, and species. Early studies on the microbiota focused on changes in the relative abundance at the phylum level between different groups (e.g., obese vs normal-weight patients); however, these comparisons are at such a broad taxonomic level that they often provide little or no biologic insight. As illustrated in Fig. 484-1, drawing comparisons between organisms in two different bacterial phyla is analogous to comparing humans to sea stars: the evolution ary distance between the two is tremendous. Examining microbial profiles at the phylum, family, or even genus level—as is often done at present—ignores the great heterogeneity within different strains of the same bacterial species. The analytical pipelines are beginning to enable strain-level comparisons, and these improvements will likely facilitate our ongoing investigation of host–commensal interactions. Bacteria Firmicutes Bacilli Bacillales Staphylococcaceae Staphylococcus S. aureus G. haemolysans M. equipercicus L. monocytogenes B. anthracis S. pneumoniae E. faecalis C. botulinum C. difficile E. rhusiopathiae E. coli B. fragilis S. epidermidis S. lugdunensis FIGURE 484-1 Juxtaposition of bacterial and human taxonomy highlights the evolutionary distance between different taxonomic levels. The listed species represent exemplars that are members of the taxon to which they are connected but that are not contained within the next-lower-level taxon listed. For example, Clostridium botulinum, Clostridioides difficile, and Erysipelothrix rhusiopathiae are members of the phylum Firmicutes, but are in classes other than Bacilli. Similarly, starfish and humans are both members of the kingdom Animalia, but they are in different phyla.

THE MICROBIOTA AND HUMAN HEALTH

■ ■OVERVIEW OF THE HUMAN MICROBIOTA

The overwhelming majority of microbiota studies have focused on stool, given that this sample type represents the most ecologically rich anatomic site, is easy to obtain, and can readily be followed longitudi nally in the same individual. A landmark study by the HMP sought to define the “normal” microbiota throughout the entire body in healthy Western adults. To this end, the microbial populations at 15–18 body sites were characterized in 242 people. One striking finding was that all samples from a given body region (e.g., skin) were more similar to each other than they were to samples from a different body region (e.g., stool), even in the same individual (Fig. 484-2A). In essence, the effect of the anatomic site on microbial composition is far greater than the effect of heterogeneity between individuals. That said, there was a remarkable amount of interindividual variation at any given body site (Fig. 484-2B). In stool, for example, the abundance of the phylum Bac teroidetes ranged from ~10% in some individuals to >90% in others. Remarkably, even with person-to-person variability and differences among body sites, the functional capacity of the microbiota—assessed using metagenomic data to identify gene pathways—was quite similar across different people and different body sites (Fig. 484-2C). This dis crepancy between the substantial differences in microbial composition and the little or no resulting change in the functional properties of the microbiota reflects an important ecologic property of the microbiota: the microbial communities at different body sites and in different people assemble in such a way that all the core metabolic functions are maintained. This finding also hints at the likely possibility of sig nificant functional redundancy within the microbiota, with different species executing the same biologic functions in different people and/ or at different anatomic sites.

While the HMP provided the first large-scale catalogue of the microbiome in multiple people and at many different body sites, the amount of data generated by what, at the time, was by far the largest microbiome study has been dwarfed by subsequent studies. These more recent studies have confirmed the HMP’s major

tenets: the com position of the microbiota differs by body site, there is tremendous interindividual variation, and the microbial gene content is relatively conserved irrespective of the body site or individual. No microbial species are ubiquitous in all individuals and at all body sites, but some species are highly prevalent at a given body site: in the HMP study, Staphylococcus epidermidis was present in 93% of nares samples and Escherichia coli in 61% of stool samples. These findings highlight the remarkable personalization of the human microbiome. While the human genome is typically >99.5% identical in different people, the microbiotas of two individuals may not overlap at all. Although the “precision medicine” approach currently focuses on teasing out how differences in the human genome relate to different clinical end Eukarya Domain Animalia Kingdom Chordate Phylum Mammalia Class Primate Order Hominidae Family Homo Genus Species

Gastrointestinal Urogenital PC2 (4.4%) PART 16 Genes, the Environment, and Disease Oral Skin Nasal PC1 (13%) A Phyla B Metabolic pathways C Anterior nares RC Buccal mucosa Supragingival plaque Tongue dorsum Stool Posterior fornix FIGURE 484-2 The human microbiome exhibits significant taxonomic variability among body sites and between individuals while maintaining core metabolic pathways. A. Principal coordinates (PC) plot showing variation among samples demonstrates that primary clustering is by body area, with the oral, gastrointestinal, skin, and urogenital habitats separate; the nares habitat bridges oral and skin habitats. Each circle represents an individual sample. B, C. Vertical bars represent microbiome samples by body habitat, with each bar within a given body site representing a different individual. Bars indicate relative abundances colored by microbial phyla (B) and metabolic pathways (C). The legend on the right indicates the most abundant phyla/pathways. RC, retroauricular crease. (Reproduced with permission from Human Microbiome Project Consortium: Structure, function and diversity of the healthy human microbiome. Nature 486:207, 2012.) points, the human microbiome clearly represents a critical component for consideration. ■ ■THE MICROBIOTA BY THE NUMBERS It has long been known that the human-associated microbiota is numerically dense. Leeuwenhoek estimated that there were more “animals living in the scrum on the teeth in man’s mouth than there are men in a kingdom.” Specific enumeration of the components of the microbiota has been challenging, in part because of its variability across time, space (body region), and clinical conditions. Moreover, the majority of human-associated microbes are not readily cultivable—a situation that raises questions about the best methodology for such quantitation. Initial back-of-the-envelope calculations performed in the 1970s suggested that there were roughly tenfold more bacteria in the body than there were human cells. This rather astounding estimate suggested that humans are really only ~10% “human” and that by far the greatest part of the holobiont is represented by microbes. This stark numerical discrepancy has prompted some to question “who parasit izes whom.” However, it has been suggested that there are “only” ~1.3 times more bacteria in the body than there are human cells and thus that humans are ~56% “bacterial.” Of note, this study does not include the numbers of viruses (known to generally be approximately tenfold more abundant than other microbes), fungi, or Archaea. Given these additional microorganisms, the notion that microbes constitute >90% of the cells present in a human body is likely correct. These ratios are even starker when one considers the genetic potential of human

Firmicutes Actinobacteria Bacteroidetes Proteobacteria Fusobacteria Tenericutes Spirochaetes Cyanobacteria Verrucomicrobia TM7 Central carbohydrate metabolism Cofactor and vitamin biosynthesis Oligosaccharide and polyol transport system Purine metabolism ATP synthesis Phosphate and amino acid transport system Aminoacyl tRNA Pyrimidine metabolism Ribosome

Aromatic amino acid metabolism cells versus that of commensal organisms. In contrast to the ~20,000 genes in the human genome, the estimated total number of genes in the microbiota (which together constitute the microbiome)—i.e.,

> 2,000,000—indicates that the human genome contributes <1% to the total genetic potential of the overall holobiont. Most microbiome studies have focused almost exclusively on the bacterial component; much remains to be learned about the functional interplay of bacteria, viruses, fungi, and Archaea and how these other classes of microorgan isms impact human health. In terms of overall diversity, >10,000 different bacterial species are present in the human microbiota; the intestines alone contain >1000 species. At any given time, the body of any given individual harbors 500–1000 bacterial species, with 100–200 bacterial species in the gut alone. If one considers different strains of the same bacterial species, which may be functionally different from one another, the diversity of the microbiota is probably at least an order of magnitude greater. Although marked diversity exists at the strain and species level, only limited bacterial phyla are generally found in the human microbiota at any given body site (Fig. 484-3). ■ ■INFLUENCES ON THE MICROBIOTA An individual's specific microbial configuration is dynamic and is quickly altered in response to subtle changes in the microenviron ments in which the bacteria reside. On a day-to-day basis, these changes usually reflect alterations in the relative abundance of the various microbes. However, some exposures have a greater effect on

Nares Buccal mucosa GI/Stool KEY Actinobacteria Bacteroidetes Fusobacteria Proteobacteria Firmicutes Other FIGURE 484-3 Different anatomic sites harbor very different microbiomes. The figure indicates the relative proportion of sequences determined at the taxonomic phylum level at six anatomic sites. (Data for stool, vagina, nares, buccal mucosa, and supragingival plaque are from the Human Microbiome Project; data for the skin are from EA Grice et al: Topographical and temporal diversity of the human skin microbiome. Science 324:1190, 2009.) the microbiota and can shift the microbial population to a new equi librium via the loss of specific species and/or the acquisition of others; this new microbial equilibrium can be associated with either health or a disease state (Fig. 484-4). Identification of the factors that influence the microbiota's composition is critical to an understanding of what leads to and controls intra- and interindividual variation. Moreover, an understanding of the influences on the microbiota will facilitate the Healthy state 2 Unstable Healthy state 1 Disease state Stable Current microbial state FIGURE 484-4 A stability landscape of the human microbial ecosystem. A stable state, illustrated as a depression in the landscape, can be associated with either a healthy state or a disease state. The topology of an individual's landscape reflects that person's genetics, age, diet, medications, medical history, and lifestyle. The position of the green ball represents the current microbial state. Clinical changes (e.g., administration of antibiotics, development of disease) can influence both the current state and the overall topology.

design and proper interpretation of microbiota studies. While it is clear that the microbiota can be altered through these various mecha nisms, it is not yet clear whether these changes are

biologically significant.

Supragingival plaque CHAPTER 484 Genetics Studies of monozygotic and dizy gotic twins have revealed that host genetics have a small but statistically significant effect on the microbiota's composition. Notably, some taxa, such as Christensenella species, are more heritable than others. A cross-sectional study of >1000 healthy individuals who have distinct ancestral origins and a relatively shared com mon environment confirmed a weak associa tion between host genetics and the microbiome but highlighted that environmental factors are more prominent modulators of the microbi ome. That said, the host's genetic contribution to the microbiota, albeit small, may be mean ingful. Studies in mice have demonstrated that genetic variation in the major histocompatibil ity complex, a specific set of immune-related genes, leads to changes in the microbiota that alter susceptibility to an autoimmune disease. These studies offer a proof of concept for the notion that the genetic predisposition observed for certain diseases may actually be mediated by indirect alterations in the microbiota. Skin The Human Microbiome in Health and Disease Vagina Age Burgeoning evidence now indicates that microbial exposure may begin in utero: bacterial DNA from bacteria typically associ ated with the oral microbiota has been identi fied in otherwise healthy placentas, in amniotic fluid obtained at early stages of gestation, and in meconium of term newborns. Although some controversy persists about whether these results reflect contamination and/or the pres ence of nonviable bacteria, they raise the possibility that human expo sure to the microbial world begins before birth. The delivery mode (vaginal vs cesarean section) and the method of feeding (breast milk vs formula, timing of solid food introduction) are major determinants of an infant's early microbiota. After birth, the infant's microbiota goes through a stereotyped succession process; with increases in bacterial diversity and functional capacity, the child's microbiota resembles that of an adult by the age of 2–3 years. Cross-sectional studies that have examined the microbiota across the entire age spectrum have revealed a general stability of the fecal microbiota after 2–3 years of age; how ever, the microbiota of the elderly (persons >80 years of age) demon strates notable differences from those of their younger counterparts, with increases in Bacteroides and Eubacterium species and decreases in the bacterial family Lachnospiraceae. Although there has been sig nificant interest in defining microbial features that predispose towards longevity, there has been poor concordance of findings between stud ies, potentially due to very different populations being studied. Diet Diet is a strong determinant of human health. The impact of diet is mediated, in part, by its effects on the composition of the gut microbiota. This makes intuitive sense, as the human diet provides nutrients needed not only by our own cells but also by the microbes living in the alimentary tract. In young children, this dietary influence is marked by major shifts (e.g., a decrease in Bifidobacterium species) in the intestinal microbiota that occur at weaning and with the introduc tion of solid food. In adults, long-term dietary patterns are associated with relatively stable microbial compositions. However, drastic changes in short-term macronutrient availability cause rapid (within 1 day) and reproducible fluctuations in the fecal microbiota that reflect the bio logic processes needed to degrade and metabolize the nutrients in the new diet. For example, vegetarian diets are associated with a microbiota

that has an increased ability to metabolize plant polysaccharides (e.g., Roseburia species, Eubacterium rectale, Ruminococcus bromii), while animal-based diets result in an increased abundance of bile-tolerant organisms (e.g., Alistipes, Bilophila, and Bacteroides species). At the completion of dietary interventions and the resumption of the indi vidual's normal dietary pattern,

the microbial communities revert back to their previous states, probably because the individual resumes their typical diet. Taken together, dietary studies confirm that the microbiota is highly adaptable and varies in relation to changes in the diet. Of note, virtually all these studies have focused on how the diet influences the fecal microbiota, with emerging evidence showing that it may similarly influence the microbiota at some nonintestinal sites. Drugs Virtually all drugs have the capacity to change the microbiota by altering the chemical landscape in which the microorganisms live (e.g., statins, bile acid sequestrants), modulating the host's ability to recognize and react to microbes (e.g., immunosuppressants) and/or directly interfering with the microbiota's constituents (e.g., antibiotics). These potential effects have made critical interpretation of microbiota studies much more difficult. A prominent study that claimed to iden tify a fecal microbiota signature associated with type 2 diabetes was later found actually to have identified a signature for patients taking metformin instead; the effects of this drug on the microbiota were far greater than the effects of the disease itself. These results highlight the importance of controlling for clinical variables in microbiota studies.

PART 16 Genes, the Environment, and Disease Antibiotics are the most obvious and best-studied class of drugs that modulate the microbiota. Multiple groups have demonstrated that antibiotics exert a considerable effect on the gut microbiota by depleting antibiotic-sensitive strains. What is more surprising is that many strains resistant to the antibiotic tested are also eliminated. For example, treatment with ciprofloxacin, which has little to no activ ity against clinically relevant anaerobes, leads to a loss of roughly one-third of the bacterial taxa in the gut. This broad effect is likely mediated by the depletion of certain "keystone" species that are required for the persistence of other, unrelated species and highlights the intricate microbe–microbe interactions that are fundamental to maintenance of the overall microbial community. While many of the observed antibiotic effects (e.g., loss of specific taxa) are shared across many different individuals, some effects vary greatly among people. For example, studies found that microbiota recovery following anti biotic treatment differed significantly in terms of timing and degree. The microbiota of most healthy people who received ciprofloxacin for 5 days had completely recovered within 4 weeks, whereas micro biologic changes lasted up to 6 months in other individuals. More over, the degree of variation was compounded by repeated antibiotic administration, with fewer individuals reverting to their baseline microbiota after a second course of ciprofloxacin given 6 months after the first. These findings are consistent with those of microbial ecology experiments, which also showed that this type of repeated disturbance leads to less predictable results. Lifestyle Many seemingly innocuous lifestyle decisions can impact the human microbiota. For example, a person's skin and fecal microbio tas are more similar to those of their household members, regardless of genetic relatedness, than to those of residents of different households. The degree of similarity in skin microbiotas is even greater if a dog also lives in the home; in contrast, the presence of a cat or a young child does not accentuate this microbial relatedness. The presumption is that the dog serves as a more effective "vector" for transmitting microbes dur ing its frequent direct contact with adults in the household. The type of setting in which a person lives also impacts the microbiota. Living in a rural or farm setting leads to a different fecal microbiota than living in an urban environment. Similarly, the individual's country of residence affects the microbiota. An analysis of daily fecal samples from an indi vidual who temporarily (i.e., for a couple of months) moved from the United States to Thailand demonstrated a large shift in the fecal micro biota that coincided with arrival in Thailand and a reversion in most respects to the "American" microbial configuration upon return to the United States. Similarly, immigration to the United States

“westernizes” the microbiome of individuals coming from non-Western countries.

These geography-driven changes probably reflect a combination of environmental and dietary differences between locations. Circadian Rhythms Many human biologic processes follow a cir cadian clock; aspects of physiology are tuned by external cues, including the degree and timing of ambient light, temperature, and availability of nutrients. This endogenous biologic clock enables animals to efficiently adapt to changing environmental conditions. Similarly, the microbiota maintains a circadian rhythm that is linked to—and helps entrain—the host’s circadian clock. If circadian oscillations are disrupted in the host, they are similarly disrupted in the microbiota, and vice versa. These bacterial vacillations occur at the level of spatial localization within the intestine, relative species abundance, and bacterial metabolite secre tion. Work in the 1960s showed that mice exhibited daily periodicity of susceptibility to infection with either Streptococcus pneumoniae or E. coli lipopolysaccharide (LPS). Although the fundamental basis for this difference was not known at the time, it is likely to be related, in part, to the microbial circadian clock. Derangements of these microbial oscillations have also been linked to the development of metabolic diseases and may underlie some of the health hazards associated with shift work and jet lag. THE MICROBIOTA AND DISEASE ■ ■THE HYGIENE HYPOTHESIS Over the past few decades, abundant epidemiologic data have revealed an inverse correlation between exposure to microbes and the incidence of autoimmune and/or atopic diseases (Fig. 484-5). This type of epi demiologic correlation led to the proposal of the “hygiene hypothesis” in 1989. Initially, this hypothesis focused on the development of atopic diseases in young children, with the idea that these epidemiologic observations could “be explained if allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally from a mother infected by contact with her older children.”1 In fact, this notion that differences in living conditions and environmental exposures contribute to susceptibility to hay fever (summer catarrh) dates back to at least the early nineteenth century. The hygiene hypothesis has continued to evolve over the past three decades and now posits that inadequacies in microbial exposure—in combination with genetic susceptibilities—lead to a collapse of the normally highly coordinated, homeostatic immune response. At its core, the hygiene hypothesis holds that specific early-life microbial exposures are required to prevent subsequent disease and that the “westernization” of society has led to a decrease in such exposures. This concept is being applied beyond atopic diseases to other inflammatory and autoimmune diseases and is thought to reflect processes that occur in later life as well. ■ ■RELATIONSHIP BETWEEN THE MICROBIOTA

AND SPECIFIC DISEASE STATES The ideas inherent in the hygiene hypothesis—in sum, that micro bial exposure can affect long-term health outcomes—laid the theo retical foundation for translational microbiome studies. While most of the studies described earlier sought to describe how the microbiota responds to specific and often transient influences (e.g., a course of antibiotics, dietary interventions, travel), a multitude of studies have characterized the microbiota in patients with various diseases in the hope that a better understanding of the nature of disease-specific microbial communities will provide insight into disease pathogen esis and potentially uncover novel treatment modalities. Remarkably, virtually all these studies have demonstrated differences between the microbiotas of healthy controls and patients, irrespective of the specific disease process examined. Although it is difficult to generalize across all studies, a couple of general themes have emerged. First, disease states are typically associated with microbiotas that are less diverse than those of healthy individuals. This loss of diversity can be measured 1D. Strachan: BMJ

299:1259, 1989.

Crohn’s disease Incidence of infectious diseases (%) Incidence of immune disorders (%) Rheumatic fever

Hepatitis A

Tuberculosis

Measles Mumps

B A

FIGURE 484-5 There was an inverse relationship between the incidence of select infectious diseases and the incidence of autoimmune disorders during the latter half of the twentieth century. A. Relative incidence of prototypical infectious diseases from 1950 to 2000. B. Relative incidence of select autoimmune disorders from 1950 to 2000. (From JF Bach: The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med 347:911, 2002. Copyright © 2002, Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.) either as a decrease in the number of species (alpha diversity; often measured as the number of operational taxonomic units or amplicon sequence variants, which are the bioinformatic equivalent of species) or as a reduction in the microbial relatedness of the species present (beta diversity). Often, both alpha and beta diversity decrease in the setting of disease. Second, states of inflammation—regardless of site or underlying disease process—are often associated with an increase in the relative abundance of the bacterial family Enterobacteriaceae and a decrease in the relative abundance of Lachnospiraceae. Dissecting Correlation and Causality Given that most of these investigations have been designed as case-control studies, it is difficult to determine whether microbiologic findings are the cause or the effect of the disease. Even studies that examine treatment-naïve patients at the time of initial diagnosis are still confounded by this “chicken or egg” issue. Moreover, prospective, longitudinal clinical studies—still rare in the microbiome field—may simply yield correlations between the microbiome and subclinical disease rather than necessarily proving causality. Experiments in animals—specifically, studies using gnotobi otic mice (GF mice that have been colonized with specified microbial communities)—have been critical in this regard as they allow investiga tion of specific differences in microbial components while controlling for the host’s genetics, diet, and housing conditions. Moreover, human microbes can be transplanted into gnotobiotic mice to permit in-depth mechanistic studies of how these microbial communities affect disease pathogenesis. This marriage of human samples and animal experi ments has facilitated the identification of causal roles played by some microbes in disease pathogenesis; these findings provide a critical proof of concept for the interplay of the microbiota with human health. However, the vast majority of microbiome studies are still at the level of correlation. The next several sections describe the clinical and animal data for many different disease processes. Given the voluminous and rapidly changing nature of this field, it is impossible to cover all of the disease associations known to date; rather, the following discussion represents a combination of the leading exemplars of microbiome data and nascent areas of significant clinical interest. In all cases, the hope is that further study of the role of the microbiota will provide novel diagnostics, new therapeutic modalities, and/or additional insight into disease pathogenesis.

Gastrointestinal Diseases Given that the intestines harbor the largest number and greatest diversity of organisms in the body, much work has focused on how the microbiota impacts gastrointestinal diseases. Even though the luminal surface area of the gastrointestinal tract is 30–40 square meters (~90% of which is contained within the small intestine) and features marked anatomic and functional differ ences that result in many discrete macro- and micro-ecosystems, stool is often used as a surrogate for the intestinal microbiota given the

relative ease of collecting samples. A few studies that have compared the microbial profile in stool with the mucosa-adherent organisms present in biopsy samples have demonstrated that stool is, in fact, a rea sonable proxy for biopsy samples; how ever, the relative microbial “noise” present in stool can sometimes overwhelm the “signal,” making biopsy samples more informative for some scientific questions. The key issue is to ensure that the biopsy samples evaluated represent relatively similar intestinal regions, as there are significant differences between the organ isms present in the crypt and the tip of the villus and between microbes found in the ascending versus the descending colon. Newer technologies (e.g., smart capsules) are being developed that will allow for noninvasive sampling of microbial com munities along the length of the gastroin testinal tract, which will provide new insight into regional differences in host–microbiota interactions.

Multiple sclerosis CHAPTER 484 Type 1 diabetes The Human Microbiome in Health and Disease Asthma OBESITY Obesity is a worsening epidemic throughout the world, and multiple studies have linked the composition of the intestinal microbiota to the development of obesity in animal models and in humans. Indeed, many of the initial translational microbiome studies performed in mice at the beginning of the twenty-first century focused on obesity. Gnotobiotic mouse studies have demonstrated the gut microbiota impacts host metabolism—resulting in body weight and adiposity changes—through several different mechanisms: the micro biota impacts the amount of energy extracted from the diet, promotes small-intestinal absorption of dietary fatty acids, regulates expression of lipid metabolism genes in the intestines, and induces hepatic lipo genesis and synthesis of triglycerides. Consistent with these findings, GF mice are resistant to diet-induced obesity, which establishes the requirement of the microbiota in the development of obesity. Over the past ~15 years, numerous human studies examining the relationship between the microbiome and obesity have been completed, all with mixed results. Although initial studies suggested obesity was associ ated with a lower ratio of the relative abundance of Bacteroidetes to Firmicutes, this has not held up in subsequent studies. Beyond this ratio of major bacterial phyla, obesity was linked to a microbiome with a lower alpha diversity. A meta-analysis of 10 studies including nearly 3000 individuals revealed an apparent lack of relationship between the Bacteroidetes/Firmicutes ratio and obesity, though there is ~2% lower diversity associated with obesity that is statistically significant but of unclear biologic significance. This finding highlights a problem com mon to microbiome studies: i.e., there is no sense as to what magnitude of change is biologically meaningful. Ultimately, although murine stud ies have indicated a causal link between the microbiota and obesity, the human data are less convincing, and their significance may be limited because the studies primarily examined only high-level taxonomic information rather than also assessing differences in bacterial products or metabolites. The rise in obesity has elicited a plethora of ideas about the type of diet that might be most successful in leading to sustained weight loss. However, it has become clear that the same dietary ingredient can have highly diverse effects on blood glucose measurements in different peo ple and that this effect is mediated largely by the microbiome. These observations suggest that the “optimal” diet needs to be individualized

in the context of the person’s microbiome, which itself may continue to change over the course of the diet. Moreover, the microbiota may also influence dietary preferences, which suggests important feedback loops between the microbiome and diet. MALNUTRITION Representing the other end of the metabolic spec trum from obesity, malnutrition is also linked to an altered micro biome. Analysis of Malawian twin pairs (≤3 years of age) who were

discordant for kwashiorkor—a severe form of malnutrition—revealed that kwashiorkor is associated with a microbiologically “immature” fecal microbiota that resembles that of a chronologically younger child. Transplantation of the fecal microbiota from these discordant twins into gnotobiotic mice that were fed a diet similar in composition to a typical Malawian diet established that the kwashiorkor-associated microbiome is causally related to poor weight gain. Subsequent studies demonstrated these same general trends in malnourished Bangladeshi children. Investigators were able to identify five bacterial species (Faecalibacterium prausnitzii, Ruminococcus gnavus, Clostridium nex ile, Clostridium symbiosum, and Dorea formicigenerans) that—when administered together as a “cocktail” to mice colonized with a kwashiorkor-associated microbiome—were able to prevent growth impairments. Moreover, children with moderate acute malnutrition fed therapeutic food purposefully designed for its ability to alter the microbiota in defined manners have improved growth. These results demonstrate that rationally designed modulation of the microbiota may lead to improved health outcomes.

PART 16 Genes, the Environment, and Disease INFLAMMATORY BOWEL DISEASE Ulcerative colitis and Crohn’s dis ease, the two predominant forms of inflammatory bowel disease (IBD), are chronic gastrointestinal inflammatory conditions that differ in their locations and patterns of inflammation (Chap. 337). The following observations have led to the suggestion that IBD is the result of an immune response to a dysbiotic microbiota in a genetically suscep tible individual: genes account for only ~20% of susceptibility to IBD (and many of the relevant genes are related to host–microbe interac tions), antibiotic treatment reduces the clinical severity of disease, and relapses of Crohn’s disease are prevented by diversion of the fecal stream. While the microbiota clearly is not the only driver of disease, it is considered to be an important element. Accordingly, numerous animal and clinical studies have been designed to tease out the nature of the relationship between the microbiota and IBD. Most of these studies have focused on comparing the microbiome’s composition in IBD patients with that in healthy controls, concen trating on microbial diversity and specific bacterial taxa that are associated with health or disease. Unfortunately, few, if any, results have been universally obtained, probably because of differences in study design, inclusion criteria, and methodology (e.g., the use of stool, rectal swabs, or biopsy samples; the choice of sequencing prim ers; the analysis pipeline). Even with these differences among studies, patients with IBD typically have reduced alpha and beta diversity in their fecal microbiotas. Moreover, Clostridium clusters IV and XIVa, which are polyphyletic and encompass several different bacterial families, are generally reduced in patients with IBD. F. prausnitzii is a notable example from Clostridium cluster IV that is often under represented in the stool of patients who have Crohn’s disease, with more mixed results in biopsy samples. The bacterial family Lachno spiraceae, which is largely contained in Clostridium cluster XIVa, and other butyrate-producing organisms are also reduced in the stool of patients with IBD. Some of these species produce butyrate by using acetate generated by other members of the microbiome, and some of these acetate-producing species are similarly reduced (e.g., Rumino coccus albus). These complex interactions and dependencies among bacterial species pose unique challenges to definitive ascertainment of the cause–effect relationships

between microbes and disease. Even before researchers were able to assess the entire microbiome at once, they often noted that patients with Crohn’s disease had a higher rep resentation of adherent invasive E. coli in the ileal mucosa, an obser vation consistent with the increased abundance of Enterobacteriaceae seen in sequencing-based microbiome studies. Beyond bacteria, burgeoning evidence supports a role for Caudovirales bacteriophages in IBD pathogenesis, though these findings may merely reflect the underlying dysbiosis related to the loss of bacterial diversity in IBD. Moreover, dysregulation of the fungal component of the microbiota (the mycobiota) alters the mucosal immune system and is linked to IBD disease severity. It is still unclear whether any of these microbial associations reflect the cause of IBD or merely serve as biomarkers of disease.

Studies of antibiotic-treated mice and gnotobiotic mice colonized with IBD-associated microbiotas have been useful in confirming that the microbiota affects colitis severity. Several bacterial species have been identified as either promoting colitis in mice (e.g., Klebsiella pneumoniae, Prevotella copri) or protecting against it (e.g., Bacteroi des fragilis, Clostridium species); however, these organisms do not always correlate with the taxa identified as differentially abundant across multiple clinical studies. In contrast, IgA-coated commensal organisms isolated from patients with IBD promote more severe colitis in mice than either IgA-uncoated bacteria from patients with IBD or IgA-coated bacteria from healthy controls. These data suggest that functional categorization of the microbiota based on immune rec ognition (e.g., IgA coating) may be a useful approach for identifying pathogenic organisms. Cardiovascular Disease Inflammation helps drive the pathogen esis of atherosclerosis, and it has long been postulated that microbes are involved in the atherosclerotic process. Early work demonstrated that patients with cardiovascular disease have higher titers of antibody to Chlamydia pneumoniae than control patients, that C. pneumoniae is present within atherosclerotic lesions, and that C. pneumoniae can both initiate and exacerbate atherosclerotic lesions in animal models. This type of analysis has been extended to other bacteria, such as Porphy romonas gingivalis, with the idea that multiple different bacteria may play some role in the pathogenesis of atherosclerosis. Studies have demonstrated clinical correlations between serum levels of trimethylamine N-oxide (TMAO) and atherosclerotic heart disease. Animal studies have confirmed that transfer of the gut micro biota from atherosclerosis-susceptible strains of mice to atheroscle rosis-resistant animals leads to increased serum levels of TMAO and a dietary choline-dependent increase in atherosclerotic plaques; this observation confirms the role of the gut microbiota in the generation of TMAO and atherosclerosis. Given that red meat, eggs, and dairy products are important sources of carnitine and choline (both precur sors of TMAO), it is not surprising that levels of TMAO are higher in omnivores than in vegans. The gut microbiota converts carnitine into the intermediary metabolite γ-butyrobetaine, which it further metabo lizes—in a diet-dependent fashion—into trimethylamine (TMA); hepatic flavin-containing monooxygenases then transform TMA into TMAO. Moreover, treatment of atherosclerosis-susceptible strains of mice with a structural analogue of choline that inhibits the first enzymatic step in TMAO formation leads to decreased circulating TMAO levels and, more importantly, restrains macrophage foam-cell formation and atherosclerotic lesion development. In a study of >4000 patients, plasma TMAO levels were also predictive of incident throm bosis risk (myocardial infarction, stroke). Gnotobiotic animals were used to demonstrate that this risk was dependent on the microbiota; although eight bacterial taxa were identified as being associated with both plasma TMAO levels and thrombotic risk, organisms with cholineutilization genes that represent the first step of TMAO production were not more abundant in animals at greater risk for thrombosis. This discrepancy highlights the complexity of the microbiota and suggests that other aspects of the overall dynamics of the

microbial community may be in play. Oncology Studies exploring the link between the microbiota and cancer have demonstrated that specific members of the microbiota can affect treatment efficacy in both a positive and a negative manner. For example, therapy with antibody to programmed cell death ligand 1 (anti-PD-L1) has proven highly effective for many different can cers (Chap. 78); however, a significant proportion of patients do not respond even when their tumors have high PD-L1 expression levels. Three groups have independently performed clinical studies—some times coupled with gnotobiotic mouse experiments to verify causal relationships—to demonstrate that specific bacteria can potentiate checkpoint blockade inhibition in melanoma, non-small-cell lung cancer, and renal cell carcinoma. Intriguingly, these groups identified different bacteria (Bifidobacterium, Faecalibacterium, and Akkermansia species) as being associated with the anticancer effects, even when the same

oncologic process was being studied. The biologic factors driving these differences are not yet clear but may relate to differences in adjunc tive therapies, geography, and/or other as-of-yet unidentified factors. Although these seemingly disparate findings raise concern about the generalizability of microbiome studies, it may be that identifying rel evant bacterial species—as opposed to their bioactive molecules—does not offer sufficient granularity for comparison across studies. The clinical relevance of the microbiota in this process was highlighted by proof-of-concept clinical trials demonstrating that fecal microbiota transplantation (FMT)—the “transplantation” of stool from one indi vidual into another—led to clinical benefit in a few patients who previ ously did not respond to anti-PD-1 therapy after they received stool from patients who had previously responded to anti-PD-1 therapy, findings which still require confirmation in larger clinical trials. In a separate set of studies, the efficacy of therapy with antibody to cytotoxic T lymphocyte–associated antigen 4 (anti-CTLA-4) was associated with T-cell responses specific for either Bacteroides thetaio taomicron or B. fragilis. In particular, administration of B. fragilis to GF or antibiotic-treated mice restored the normally absent anticancer response to anti-CTLA-4 therapy. While these examples demonstrate potentiation of anticancer therapies by the microbiota, other therapies can be antagonized. Some cancers, such as pancreatic ductal adenocar cinoma, contain intratumoral bacteria, particularly Gammaproteobac teria, that can metabolize the chemotherapeutic agent gemcitabine and thereby contribute to the drug resistance of these tumors. In addition, the gut microbiota can increase the half-life of irinotecan, a chemotherapeutic agent commonly used in treating rhabdomyosar coma and colorectal cancer, by converting an inactive metabolite back to the active form, which leads to increased drug toxicities. Overall, these examples highlight the microbiota’s critical impact—both direct and indirect—on the efficacy and safety profile of drugs. Many other notable examples have been described (e.g., involving cyclophospha mide, digoxin, levodopa, and sulfasalazine), and many more likely remain to be discovered. Using advances in computational tools for sequence decontamina tion and batch effect correction, reanalysis of data repositories gener ated by The Cancer Genome Atlas (TCGA) Research Network has identified microbial signatures within tumor genome sequences that predicted clinical outcomes in cancer, although these findings have been questioned given potential errors in the computational pipe line. This ongoing controversy highlights the complexity within the bioinformatic pipelines, the requirement for detailed reference data bases, and dealing with samples that have an overall low abundance of microbes. Additional work is required to validate these signatures in prospective cohorts and to understand the biology underlying microbe–cancer interactions within the tumor milieu. The application of microbiome science to hematopoietic stem cell transplantation (HSCT) is an area of expanding interest, particularly given the significant morbidity and mortality related to

graft-versus-host disease (GVHD). In light of studies in the 1970s showing that GF mice developed less frequent and less severe gut GVHD than wild-type mice, clinicians began to use antibiotics to decontaminate the gut of patients undergoing HSCT. This decontamination approach yielded mixed results, probably because of differences in the antibiotic regimens used. The natural history of patients undergoing allogeneic HSCT includes a substantial loss of diversity in the fecal microbiota and intestinal domination (≥30% abundance in the fecal microbiota) by Enterococcus species and other pathogens, with a higher bacterial diversity at time of neutrophil engraftment associated with lower mortality. Moreover, a retrospective analysis of ~850 patients undergoing allogeneic HSCT revealed that receipt of imipenem-cilastatin or piperacillin-tazobactam for neutropenic fever was associated with increased GVHD-related mortality at 5 years; this observation suggested that specific bacteria may help protect against GVHD-related mortality. More detailed anal yses revealed an association between the abundance of Blautia species and protection against GVHD and mortality, though this correlation is still being examined with regard to its causal relationship. Despite sig nificant interest in examining these microbial relationships with HSCT, little has yet been studied in the context of solid organ transplantation,

which likely represents the next frontier of transplantation-related microbiome investigation.

Autoimmune Diseases The dramatic rise in the incidence of many autoimmune diseases over the past few decades has been far more rapid than can be explained simply by genetic factors (Fig. 484-5). It is increasingly thought that environmental triggers, including the microbiome, are partially responsible for the development of these autoimmune diseases. CHAPTER 484 TYPE 1 DIABETES Type 1 diabetes (T1D) is an autoimmune disorder characterized by T cell–mediated destruction of insulin-producing pancreatic islets (Chap. 415). There is a clear genetic predisposition for the disease: ~70% of patients with T1D have human leukocyte antigen (HLA) risk alleles. However, only 3–7% of children with these risk alleles actually develop disease, an observation that sug gests a role for other environmental factors. Studying a prospec tive, densely sampled, longitudinal cohort of at-risk, HLA-matched children from Finland and Estonia, investigators detailed changes in the microbiota prior to development of disease. Although only 4 of the 33 children studied developed T1D within the time frame of the study, a marked decrease of ~25% in alpha diversity occurred after seroconversion but before disease diagnosis. The low number of cases in this study unfortunately precluded identification of any specific disease-associated taxa. A follow-up study compared the microbiomes of a larger cohort of these high-risk northern European children with those of low-risk Russian children who lived in geographic proximity. Bacteroides species were more abundant in the high-risk group than in the low-risk group, particularly at early ages. This difference was postulated to be associated with an altered structure of the bacterial LPS to which children were exposed at a young age. It was further suggested that Bacteroides-derived LPS was not able to provide the immunogenic stimulus necessary to prevent T1D. These two studies offer attractive—though logistically complicated—options for future clinical investigations aimed at exploring the role of the microbiome. The first approach—longitudinally following individuals who are at high risk for a given disease—may provide insight into host–microbe relation ships by mapping temporal changes in the microbiome with disease onset. An important caveat with this type of study, though, is that the associations identified may reflect preclinical disease rather than specifically indicating causality for any observed changes. The second approach illustrates how careful selection of study participants may offer an opportunity to uncover more meaningful associations that can

subsequently be experimentally verified. The Human Microbiome in Health and Disease RHEUMATOID ARTHRITIS Similar to many other autoimmune dis eases, rheumatoid arthritis (RA) is a multifactorial disease that comes to clinical attention after an environmental factor triggers symptoms in an individual with preexisting autoantibodies. Multiple lines of evidence support the notion that RA pathogenesis is reliant on the microbiota, including the findings that GF mice do not develop symptoms in several RA models and that antibiotic treatment of mice mitigates against RA development. Several taxa (e.g., Bacteroides spe cies, Lactobacillus bifidus, and segmented filamentous bacteria) have been implicated in promoting RA in murine models, and analysis of the fecal microbiota of patients with newly diagnosed RA has indicated that P. copri is a biomarker of disease. That this association with P. copri does not exist for chronic, treated RA or for psoriatic arthritis suggests some specificity for new-onset RA. A major limitation of this approach is that the identified association is shown to be a biomarker of disease (and, in this case, potentially of response to treatment), but no added insight is gained into a possible causal relationship between P. copri and RA. In fact, many of the patients with new-onset RA had no Prevotella detected, and several of the healthy controls had significant levels of Prevotella. The lack of a strict concordance between the presence (or absence) of a specific taxon and a given disease state argues against a possible causal role. MULTIPLE SCLEROSIS Epidemiologic studies of twin pairs and atrisk individuals moving between high- and low-risk geographic areas indicate that genetics plays a minor component in multiple sclerosis

(MS) susceptibility relative to environmental factors. For example, in monozygotic twin pairs in which one sibling has MS, the other sibling also develops MS in only ~30% of cases. Although MS is a disease of the central nervous system (CNS), there is growing evidence of a link between MS and the microbiota, specifically that of the gut. In murine models of MS, GF and antibiotic-treated animals displayed reduced disease incidence and severity, and gnotobiotic mice harboring the fecal microbiota of individuals with MS—but not that of healthy controls—had increased disease activity. Clinical studies have bioin formatically associated numerous microbial changes with the presence of MS, including prior infection with Epstein-Barr virus. Importantly, a causal role has not yet been established for any of these microbes in MS pathogenesis. Although work relating the microbiome to MS is ongo ing, it has opened the door to exploring this link with other neurologic diseases. Animal studies have linked the microbiota with Parkinson’s disease, Alzheimer’s disease, and autism, and there are clinical data assessing fecal microbiomes in relation to a variety of neurologic con ditions. It is not yet clear how the gut microbiota is communicating with the CNS—i.e., whether communication takes place via bacterial metabolites that travel in the bloodstream and cross the blood-brain barrier, via migration of whole organisms into the CNS, or via feedback through the vagus nerve. Emerging data suggest that a subset of entero endocrine cells in the intestinal epithelium is synaptically connected to the CNS, which may provide another means for the gut microbiota to impact neurologic function. Although our understanding of this brain-gut axis is still in its infancy, research in this area has elicited tremendous excitement as a tractable approach to potential treatments for these challenging diseases.

PART 16 Genes, the Environment, and Disease Atopic Diseases The incidence and prevalence of allergic diseases continue to steadily increase, as do more severe clinical presentations. Life-threatening food allergies are now such a public health issue that nut-free classrooms are the norm in many cities. The development of allergic diseases often follows a stereotyped progression that begins with atopic dermatitis (AD) and continues, in order, with food allergy, asthma, and allergic

rhinitis. The microbiome has been linked to all of these conditions and has the potential to modulate effects anywhere along this spectrum. ATOPIC DERMATITIS The skin is the largest organ in the body, and its different anatomic sites (e.g., antecubital fossa, volar forearm, alar crease) represent distinct ecologic niches and harbor unique microbial communities. Moreover, given that the skin serves as a critical inter face between the body and the external environment (e.g., microbes), it must be able to respond to unwanted microbes with an adequate immune response. AD is an inflammatory skin disorder involving immune dysfunction and a dysbiotic skin microbiota that is typically marked by greater abundances of Staphylococcus aureus and reduced bacterial diversity. Effective treatment of AD does not require com plete elimination of S. aureus but is associated with restoration of the normal level of diversity. It is likely that this increase in bacterial diver sity reestablishes normal immune homeostasis in the skin; specific members of the skin microbiota have been shown to induce protective skin-restricted immune responses. Coagulase-negative staphylococci (CoNS; primarily S. epidermidis and S. hominis) obtained from lesional and nonlesional skin of patients with AD were functionally screened and compared to CoNS from healthy controls; AD-lesional CoNS were much less often able to produce antimicrobial peptides (lantibiotics) directed against S. aureus. To demonstrate that these lantibioticproducing CoNS were biologically relevant, they were incorporated into a lotion and applied to the arms of patients with AD. Surprisingly, a single application of the probiotic-laced lotion led to a decrease in the abundance of S. aureus recovered; no such decrease was observed when lantibiotic-negative strains were used. The authors of this study did not specifically comment on the clinical improvement of the AD lesions. Nevertheless, this is one of a limited number of studies that is beginning to extend microbiome-related findings into clinical trials. ASTHMA Asthma is characterized by the clinical triad of airflow obstruction, bronchial hyperresponsiveness, and inflammation in the

lower respiratory tract. Although the long-standing dogma was that the lungs are sterile, there is now convincing evidence for a constant ebb and flow of bacteria within the lower airways. In healthy states, the mucociliary escalator continually eliminates these bacteria soon after they land in the airways; in disease states (e.g., cystic fibrosis, chronic obstructive pulmonary disease), these bacteria establish long-term colonization of the airways and influence disease pathogenesis. In asthma specifically, both fecal and airway microbes have been linked to clinical outcomes. Early studies of the microbiome’s influence on asthma used culturebased methods to assess the hypopharyngeal microbiota of asymp tomatic 1-month-old infants. Intriguingly, in one study, early-life colonization with S. pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, or a combination of these organisms—but not S. aureus— was significantly associated with persistent wheeze and asthma at 5 years of age. Eosinophilia and total IgE levels at 4 years of age were also increased in children who were neonatally colonized with these organisms. Although this study examined a focused set of bacteria, it laid the experimental groundwork indicating that early-life microbial exposures influence subsequent development of asthma. A later lon gitudinal investigation of the fecal microbiota in a general-population birth cohort of >300 children demonstrated that lower abundances of the genera Lachnospira, Veillonella, Faecalibacterium, and Rothia at 3 months of age were associated with an increased risk for development of asthma. The fact that these bacterial changes were no longer appar ent when the children were 1 year of age is consistent with the notion that microbial exposures early in life are important to disease patho genesis later in life. Transplantation of stool samples from 3-month-old children at risk for asthma into gnotobiotic mice resulted in significant airway inflammation in a murine model of asthma; pre-

and postnatal exposure of mice to a four-species cocktail (F. prausnitzii, Veillonella parvula, Rothia mucilaginosa, and Lachnospira multipara) inhibited airway inflammation, with a marked reduction in neutrophil num bers in bronchoalveolar lavage fluid. These data suggest that early-life modulation of the microbiome may be an effective strategy to help prevent asthma, though the specific logistics (e.g., strains, dose, timing of exposure, patient selection) remain to be clarified. Infectious Diseases The increased susceptibility of antibiotictreated mice to infection with a wide range of enteric pathogens was initially observed in the 1950s and led soon thereafter to the concept of colonization resistance, which holds that the normal intestinal micro biota plays a critical role in preventing colonization—and therefore disease production—by invading pathogens. Seminal work in the 1970s demonstrated that this protection is largely reliant on anaerobic gram-positive organisms, and the subsequent half-century has been spent trying to identify the specific microbes involved. Although much of the work relating the microbiota to infection has focused on enteric pathogens, the intestinal microbiota has also been clearly linked to bacterial pneumonia in mouse models, and changes in the microbial composition of the gut have been causally related to changes in the severity of disease. Although this gut-lung axis clearly exists in animals, its relevance in humans is still unclear. Several groups are beginning to study the human lung microbiome in the context of pneumonia and tuberculosis. Moreover, the relationships between the microbiota and both systemic infections (e.g., HIV infection, sepsis) and the response to vaccination are starting to be explored. ENTERIC INFECTIONS Clostridioides difficile infection (CDI) represents a growing worldwide epidemic and is the leading cause of antibioticassociated diarrhea (Chap. 139). Roughly 15–30% of patients who are successfully treated for CDI end up with recurrent disease. The strong association between antibiotic exposure and CDI initially raised the idea that the microbiota is inextricably linked to acquisition of disease, presumably because of the loss of colonization resistance. Consistent with the epidemiologic data, characterization of the fecal microbiota of patients with CDI revealed that it is a markedly less diverse, dysbiotic community. FMT using stool from a healthy individual was success fully used in the 1950s to treat four patients with severe CDI and has since been demonstrated in numerous studies to be an effective

therapy for recurrent CDI, with clinical cure in 85–90% of patients (as detailed below). Thus, FMT for recurrent CDI has become the “poster child” for the idea that microbiome-based therapies can transform the management of many diseases previously considered to be refractory to medical therapy. Although FMT is agnostic as to the underlying mechanism of protection, work is ongoing to identify specific microbes and host pathways that can protect against CDI. Studying mice with differential susceptibilities to CDI due to antibiotic-induced changes in their microbiota, investigators identified a cocktail of four bacteria (Clostridium scindens, Barnesiella intestihominis, Pseudoflavonifractor capillosus, and Blautia hansenii) that conferred protection against CDI in a mouse model. Intriguingly, treatment of mice with just C. scindens offered significant, though not complete, protection in a bile acid– dependent manner. Clinical data from patients who underwent HSCT also associated C. scindens with protection from CDI, an observation that suggests the possibility of translating these findings from mice to humans. This study provides another example of the identification of relevant bacterial factors through examination of microbial differences in populations that differ in disease risk. Microbiome-related changes associated with Vibrio cholerae infec tion include a striking loss of diversity (largely due to V. cholerae becoming the dominant member of the microbiota) and an altered composition that rapidly follows the onset of disease. These changes, which occur in a reproducible and stereotypical manner, are reversible with treatment of the disease. This recovery phase involves a microbial succession that is similar to the

assembly and maturation of the micro biota of healthy infants. In addition to V. cholerae, streptococcal and fusobacterial species bloom during the early phases of diarrhea, and the relative abundances of Bacteroides, Prevotella, Ruminococcus/Blau tia, and Faecalibacterium species increase during the resolution phase and mark the return to a healthy adult microbiota. Analysis of these microbial changes occurring in patients with cholera and in healthy children led to the selection of 14 bacteria that were transplanted into gnotobiotic mice, which were then challenged with V. cholerae. Bioin formatic analysis of specific taxa changing during cholera determined that Ruminococcus obeum restrained V. cholerae growth. Subsequently, this relationship was experimentally confirmed, and the R. obeum quo rum-sensing molecule AI-2 (autoinducer 2) was found to be respon sible for restricting V. cholerae colonization via an unclear mechanism. These studies highlight the potential for use of microbiome-based therapies to prevent and/or treat infectious diseases. Moreover, they suggest that temporal analysis of longitudinal microbiome data may be an effective strategy for identifying microbes with causal relationships to disease. VIRAL INFECTIONS One long-standing maxim for management of infectious diseases is that antibiotics are only to be used for treatment of bacterial infections. Studies using mouse models have demonstrated, however, that a variety of viruses require the bacterial component of the microbiota for pathogenesis. Moreover, antibiotic therapy, which has bacteria-independent effects on the host, leads to reduced disease severity in some animal models of viral infection, though the clinical relevance of this is not yet clear. In addition to being required for some viral infections to proceed, commensal bacteria have also been shown to play a critical role in inducing type I interferons, which represent a potent defense mechanism against many viruses, and for modulating cellular physiology in ways that inhibit viral replication. HIV INFECTION The augmentation of HIV pathogenesis by some viral, bacterial, and parasitic co-infections suggests that a patient’s underlying microbial environment can influence the severity of HIV disease. Moreover, it has been hypothesized that the intestinal immune system plays a significant role in regulating HIV-induced immune activation; this seems particularly likely since the intestines are an early site for viral replication and exhibit immune defects before peripheral CD4+ T-cell counts decrease. Several studies of HIV-infected indi viduals have identified substantial differences in the HIV-associated fecal microbiota that correlate with systemic markers of inflammation. Curiously, these microbial changes do not necessarily normalize with antiretroviral therapy; this finding suggests that the microbiota may

have some “memory” of the previously high HIV loads and/or that HIV infection helps reset the “normal” microbiota. This memory-like capacity of the microbiota has been demonstrated in animal models in the context of other infections and in response to dieting.

Given that the majority of new HIV transmission events follow het erosexual intercourse, there has been significant interest in examining the relationship between the vaginal microbiota and HIV acquisition. A longitudinal study of South African adolescent girls who under went high-frequency testing for incident HIV infection facilitated the identification of bacteria that were associated with reduced risk of HIV acquisition (Lactobacillus species other than L. iners) or with enhanced risk (Prevotella melaninogenica, Prevotella bivia, Veillonella montpellierensis, Mycoplasma, and Sneathia sanguinegens). In mice inoculated intravaginally with Lactobacillus crispatus or P. bivia, the latter organism induced a greater number of activated CD4+ T cells in the female genital tract, a result suggesting that the increased risk of HIV acquisition associated with P. bivia may be secondary to the increased presence of target cells. In a separate study, the composi tion of the

vaginal microbiota was shown to modulate the antiviral efficacy of a tenofovir gel microbicide. Although tenofovir reduced HIV acquisition by 61% in women who had a Lactobacillus-dominant vaginal microbiota, it reduced HIV acquisition by only 18% in women whose vaginal microbiota comprised primarily Gardnerella vaginalis and other anaerobes. This difference in efficacy was due to the ability of G. vaginalis to metabolize tenofovir faster than the target cells can take up the drug and convert it into its active form, tenofovir diphosphate. These findings illustrate how microbial ecology can be an important consideration in choosing effective treatment regimens. CHAPTER 484 The Human Microbiome in Health and Disease RESPONSE TO VACCINATION Second only to the provision of clean water, vaccination has been the most effective public health interven tion in the prevention of serious infectious diseases. Its effects are mediated by antigen-specific antibodies and, in some cases, effector T-cell responses. Although vaccines are clearly effective on a popula tion scale, the magnitude of the immune response to vaccines can vary among individuals up to a hundredfold. Although many factors (e.g., genetics, maternal antibody levels, prior antigen exposures) can affect vaccine immunogenicity, the microbiota is now recognized as another important factor. Several cohort studies have associated dif ferences in the fecal microbiota with altered vaccine responses, and the nasal microbiota is thought to contribute to the IgA response to live, attenuated influenza vaccines. These correlations based on clinical data have been partially confirmed in animal studies. The best example is the demonstration that the responses to nonadjuvanted viral subunit vaccines (inactivated influenza and polio vaccines) are reliant on the microbiota, whereas the responses to live or adjuvanted vaccines (live attenuated yellow fever, Tdap/alum, an HIV envelope protein/ alum vaccine) are not. A causal role for the microbiome influencing vaccine-induced immunity in humans was demonstrated by com paring microneutralization titers following the inactivated influenza vaccine in individuals treated with or without antibiotics, although an antibiotic-dependent effect was only present in subjects who had low levels of preexisting immunity to influenza. These data suggest that the microbiota may serve as an adjuvant for certain vaccine types and in naïve populations. Incorporation of specific commensal bacteria and/ or their products that improve vaccine responses into vaccine formula tions may increase overall vaccine efficacy. MECHANISMS OF MICROBIOME-MEDIATED EFFECTS As highlighted in the examples above, numerous associations have been made between the microbiome and various disease states. These correlations have often been established at broad taxonomic levels, with little or no insight into causality. Given that most clinical studies of these relationships have a fairly small sample size (often <100) and are simultaneously comparing numerous variables (i.e., each of the bacterial species in the microbiota is effectively a different feature being compared), many of these studies may not be adequately powered and therefore may yield false-positive results. Testing of these correlations

in animal models of disease has been critical in demonstrating a causal relationship between microbes and specific phenotypes. Because microbiome-wide association studies typically result in a long list of bacterial taxa that are correlated with a disease, it has been challenging to know which organism to test further in mechanistic studies. More over, even if a specific bacterial species is identified in these analyses, there is potentially enough strain-to-strain variation that the “func tional” isolate may need to be recovered from the individuals studied; a publicly available representative of the species may not confer the same phenotype. Despite all these difficulties, a handful of specific microbes have now been linked to disease effects; some examples have been mentioned above. The next layer of challenges relates to identification of the specific mechanisms that underlie these causal relationships. Although the microbiota modulates most facets of host

physiology, its impact on the immune system is the best-studied mechanism and helps explain its role in many diseases, particularly those that stem from misdirected immune responses.

PART 16 Genes, the Environment, and Disease ■ ■REGULATION OF THE IMMUNE SYSTEM The microbiota is required for the proper development, education, and maintenance of the immune system, a finding underscored by the fact that GF animals have an immature and underdeveloped immune system. Moreover, given that the microbiota has co-evolved with its host, a host-specific microbiota is critical for normal maturation of the immune system: gnotobiotic mice colonized with the microbiota from healthy humans have a small-intestinal immune system indistinguish able from GF mice. This impact on immune ontogeny begins in early life, with maternal transfer of microbially-targeted antibodies and microbe-derived metabolites augmenting neonatal immune develop ment. Some of these early-life microbial exposures must occur during a time-sensitive window, after which subsequent exposures fail to redress the initial deficiency. Examples of these "original sins" are limited in number, but they can have long-lasting physiologic consequences that extend into adult life. In contrast, most host–microbe–immune interactions occur on an ongoing basis throughout life, with microbial perturbations (e.g., antibiotic use, changes in diet) disrupting this homeostatic immunity and potentially altering disease susceptibility. The microbiota impacts virtually all aspects of host immunity, including its different arms (i.e., innate, adaptive), its varied anatomic niches (e.g., intestinal, skin, lung, bone marrow, CNS), and its overall immunologic tone and responsiveness. Not only does the micro biota influence the development and education of immune cells, but it also plays a critical role in modulating epithelial cell responses that contribute to immune defenses and disease pathogenesis. While the microbiota as a whole is known to drive these varied responses, not all microbes have the same immunomodulatory effects. Indeed, a broad screen of >50 taxonomically diverse commensal bacteria in GF mice demonstrated that most have the capacity to modulate the immune system, with very few bacterial taxa being immunologically quiescent; however, the immunomodulatory effects were often not detected when the same bacterium was administered to a mouse with a normal microbiota, which highlights the functional redundancy within the microbiota. Interestingly, bacterial taxonomy did not correlate with effects on the immune system, a finding that suggests the bioactive molecules may be unique rather than evolutionarily conserved. Given that all the tested bacteria express various canonical ligands (e.g., LPS, peptidoglycan, flagellin) for pattern recognition receptors, such as Tolllike receptors, commensal bacteria either modulate the immune system via a different class of products or their "canonical ligands" have unique structural motifs that trigger distinct signaling pathways—or combina tion of pathways—that result in education of the immune system. Efforts are ongoing to define cognate relationships between spe cific commensal bacteria and their immunomodulatory effects, and approaches are being developed to define specific bacterial factors that are responsible for the phenotypic changes. Complicating factors are that many organisms, particularly those in the phylum Firmicutes, are not readily genetically tractable and that many of the phenotypes are not easy to assess with high-throughput screening. The use of mass spectrometry to detect and profile tens of thousands of different

metabolites present in different bodily fluids has offered the promise of deeper insight into microbially mediated processes that underlie dis ease susceptibility. However, the fact that the overwhelming majority of these metabolites are not annotated, coupled with the sheer volume of data generated, has so far limited the general utility of these untargeted approaches. The few immunomodulatory bacteria and their bioactive molecules that have been identified serve as

useful archetypes for how the microbiome influences the immune system and, more generally, host physiology. These commensal-derived products can generally be categorized as endobiotic microbial structures, modified dietary nutri ents, and modified host-derived metabolites. Endobiotic Microbial Immunomodulatory Molecules B. fragilis polysaccharide A (PSA) is perhaps the best-studied commensal-derived molecule that has been demonstrated to influence disease outcomes in mouse models. PSA—one of at least eight capsular polysaccharides expressed by B. fragilis—has a unique zwitterionic structure that incor porates both a positive and a negative charge within each repeating unit. Studies in which mice have been treated either with isogenic strains of

B. fragilis that differ in PSA expression or with purified PSA have shown that PSA confers protection—prophylactically and therapeutically— against experimental colitis and MS. PSA is recognized by Toll-like recep tor 2 on antigen-presenting cells, particularly plasmacytoid dendritic cells, and—in the setting of inflammation—induces interleukin 10 (IL-10)–

producing regulatory T cells (Tregs) that help restrain inflammation. B. fragilis is also the source of an immunomodulatory glycosphin golipid that, if present during neonatal life, decreases the number of colonic invariant natural killer T (iNKT) cells and improves outcomes in a model of colitis in adulthood. It is not clear whether these glyco sphingolipids activate or inhibit iNKT cells; results have been discor dant, probably because different glycosphingolipid species have been tested. A chemical synthesis approach confirmed that B. fragilis glyco sphingolipids have distinct immunomodulatory functions depending on their specific structure. There are an increasing number of commensal-derived polysac charides and other large molecules that have been shown to modulate the immune system and/or disease outcomes. Advances in bacterial genetics have facilitated the identification of structural features that contribute to some of these host–microbiota relationships. It is likely that our general understanding of structure–function relationships of commensal-derived products will continue to grow in the coming years, mirroring what has occurred in microbial pathogenesis studies over the past several decades. Modified Dietary Nutrients As described above, the human diet provides nutrients for the gut microbiota, which can metabolize them into new, bacteria-derived compounds. Perhaps the best example of this is fermentation of undigested dietary fibers into short-chain fatty acids (SCFAs). Several groups have demonstrated that SCFAs, the intestinal levels of which are largely determined by bacterial metabolism, are important for the induction of Tregs, though there is not agreement on which specific SCFA (propionate, acetate, or butyrate) is most relevant. Wild-type mice colonized with bacteria known to induce colonic Tregs have elevated cecal levels of SCFAs. Colonization with any of three Bacteroides species (B. caccae, B. massiliensis, and B. thetaiotaomicron) increases levels of acetate and propionate, whereas colonization with Parabacteroides distasonis or a mix of 17 human-derived Clostridium species elevates levels of all three SCFAs. In all of these cases, though, the SCFAs inhibit histone deacetylase, with a consequent increase in Foxp3 expression. Notably, microbe-induced SCFA production has not been shown to be critical for Treg induction by any of these organisms. In contrast, there appears to be no correlation between SCFA levels and Treg numbers in mice monocolonized with various Treg-inducing bacterial species. Taken together, these data suggest important hetero geneity in the mechanisms underlying Treg development and do not rule out the possibility of other, redundant mechanisms for Treg induc tion. In addition to effects on Tregs, SCFAs also promote the epithelial barrier, impact cell proliferation (directionality depends on the specific cell type and SCFA), regulate host metabolism, and provide an energy source to colonocytes.

Although SCFAs represent the best-studied molecules that the microbiota generate from diet, there are many other physiologically important examples. The microbiota metabolizes tryptophan into various products (e.g., kynurenine, indole, and its derivatives) that influence immune function, metabolic diseases, viral infections, and neuronal function, among other things. Desaminotyrosine produced by Clostridium orbiscindens confers protection from influenza by inducing type I interferon activity. Modification of unsaturated fatty acids (e.g., linoleic acid) into different isomers regulates specific T-cell subsets embedded in the small-intestinal epithelium. These examples represent an important proof of concept that diet plays an important role in the functional output of the microbiota, not just its composition. Modified Host-Derived Molecules Bile acids are produced in the liver but then are metabolized by intestinal bacteria to form deconjugated and secondary bile acids. These microbially produced bile acid profiles act through complex signaling pathways to balance the metabolism of lipids and carbohydrates and to affect immune responses. Therefore, bile acids are now being investigated as micro bial metabolites that are critical to maintaining human health. As mentioned above, C. scindens helps protect mice against CDI through a bile acid–dependent process. Alterations in bile acid profiles due to underlying microbial dysbiosis have also been associated with hepatic and colonic inflammation, hepatic cellular carcinoma, colorectal can cer, and impaired gut motility. Almost all of these relationships have been documented at the level of correlation and, at best, reflect a partial change in phenotype in the setting of bile acid sequestrants (e.g., chole styramine). Work is ongoing to determine causal relationships between bacterial metabolism of bile acids and changes in host physiology, though the most definitive evidence is that microbe-produced bile acid metabolites influence colonic Treg homeostasis. In addition to bile acids, the gut microbiota can metabolize many other host-derived molecules, thereby regulating their levels and downstream effects. Taurine enhances NLRP6 inflammasome–induced colonic IL-18 secretion, while histamine, spermine, and putrescine suppress IL-18 secretion; the levels of all of these host-derived metabo lites can be regulated by the microbiota. Inosine, the deamination product of adenosine, produced by Bifidobacterium pseudolongum enhances efficacy of checkpoint blockade inhibitors in mouse models. While these examples represent the tip of the iceberg, many more examples of bacterial metabolites will undoubtedly be linked to health and disease given the thousands of different bacterial metabolites pres ent throughout the body. However, the clinical relevance of any of these bacterial metabolites remains unknown. MOVING MICROBIOME SCIENCE FROM BENCH TO BEDSIDE The numerous microbiome–disease associations identified thus far have generated a great deal of hope that understanding the relevant microbe–host interactions will open the door to unlimited therapeu tic applications. Microbiome-based therapies offer several potential benefits. Patients often view such treatment as more “natural” than conventional drug therapy and are therefore more likely to comply with it. Biologically, microbiome-based therapies are more likely to address one of the root causes of disease (microbial dysbiosis) rather than sim ply affecting the downstream sequelae. Finally, a given microbiomebased therapy may serve as a “polypill” that is effective against several different diseases stemming from similar microbial changes. Despite tremendous interest in therapeutically exploiting the microbiome, there have thus far been few clinical successes along these lines. The most successful therapeutic application of microbiome science has been the use of FMT, particularly for CDI. As mentioned earlier, FMT involves “transplanting” stool from one individual to a diseased patient, with the idea that the donor microbiota will correct whatever derangement may exist in the ill patient and therefore will alleviate symptoms. Fundamentally, this notion is agnostic as to the specific microbial dysbiosis and holds that any “healthy” microbiota will be curative, though some are now using donor stool from patients with a desired phenotype rather

than any healthy individual. The idea of

FMT dates to at least the fourth century, when traditional Chinese doctors used a “yellow soup” (fresh human fecal suspension) to suc cessfully treat food poisoning and severe diarrhea. The continued use of FMT through the centuries for the treatment of diarrheal illnesses in both humans and animals, along with the growing appreciation of the importance of the microbiota, laid the groundwork for using FMT to treat CDI. Since the first major prospective trial assessing FMT for recurrent CDI in 2013, most of the numerous studies of FMT for CDI have demonstrated remarkable efficacy, with an average clinical cure rate of ~85%. The donor stool can be fresh or frozen (use of the latter allows biobanking of samples from a limited number of prescreened donors) and can be administered via nasogastric tube, nasoduodenal tube, colonoscopy, enema, or oral capsules; the cure rate is slightly higher with lower-gastrointestinal administration than with uppergastrointestinal treatment. The optimal screening, preparation, and concentration of infused donor stool have not yet been determined, and there have been cases of antimicrobial-resistant pathogens trans mitted by FMT that have led to mortality. The most common adverse effects of FMT include altered gastrointestinal motility (with constipa tion or diarrhea), abdominal cramps, and bloating, all of which are generally transient and resolve within 48 h. Although controlled stud ies of the use of FMT in immunosuppressed patients do not yet exist, meta-analyses of case reports and case series have found no serious FMT-related adverse events in >300 immunocompromised patients.

CHAPTER 484 The Human Microbiome in Health and Disease The successful use and the favorable short-term safety profile of FMT for CDI have led to its expanded application for other indications. As of July 2024, >500 trials (listed at ClinicalTrials.gov) were investigat ing the efficacy of FMT for a range of indications, including CDI, IBD (ulcerative colitis and Crohn’s disease), obesity, eradication of multi drug-resistant organisms, anxiety and depression, cirrhosis, and type 2 diabetes. The few published studies regarding indications other than CDI have generally included small sample sizes and have offered mixed results. In contrast to the successes in CDI, the results have been more varied for patients with IBD, which is perhaps the second best-studied indication. It is not clear whether these discrepancies are due to hetero geneity in recipients (e.g., in terms of underlying disease mechanisms or endogenous microbiotas), the donor material, and/or the logistical details of FMT administration (e.g., route, frequency, dose). However, these results demonstrate that—under the right circumstances— modulation of the microbiota can be an effective therapy for IBD. Although FMT offers an important proof of concept that microbi ome-based therapies can be effective, treatment is difficult to standard ize across large populations because of variability among stool donors and among the endogenous microbiotas of recipients. In addition, FMT is fraught with safety concerns, and its mechanisms of action are unclear. That said, there are now two microbiome-derived therapies conceptually analogous to FMT that are approved by the U.S. Food and Drug Administration (FDA) for treatment of recurrent CDI. FMT likely represents the first generation of microbiome-based therapies; subsequent generations will include the use of more refined bacte rial cocktails, single strains of bacteria, or bacterial products and/or metabolites as the therapeutic intervention. The field of probiotics has a complicated history: many different strains have been tested against a multitude of diseases. Several meta-analyses have combined results across bacterial strains and/or disease indications and have generally concluded that the data are not yet convincing enough to support the use of the tested regimens. It should be noted that the tested organisms have generally been chosen based on their presumed safety profile

rather than in light of a plausible biologic link to disease. The hope is that more focused, mechanistic microbiome studies will identify specific commensal organisms—and their underlying mechanisms of action—that are involved in disease pathogenesis and that will serve as the basis for the next wave of rationally chosen probiotics, a few of which are currently in clinical trials. The main hurdle in this endeavor has been identifying specific microbes that are causally related to protection from disease. Future therapeutic strategies might include administering a beneficial microbe/microbial product; targeting a deleterious microbe/microbial pathway; or modulating the microbial ecology, potentially by impacting keystone species.

PERSPECTIVE The medical view of microbes has changed radically, moving from the early-twentieth-century notion that we are engaged in a constant struggle with microbes—an “us-versus-them” mentality that focused on the necessity of eradicating bacteria—to the current understanding that we live in a carefully negotiated state of détente with our commen sal organisms. Instead of holding a simple view of microbes as enemies to be eliminated with antibiotics, scientists are increasingly recognizing the critical role these organisms play in maintaining human health; loss of these host–microbe interactions in the increasingly sterile environment typical of Western civilization may have predisposed to the increased incidence of autoimmune and inflammatory diseases. The field of microbiome research has made great strides over the past decade in cataloguing the normal microbiota and is now beginning to identify clinically actionable microbe–host relationships.

PART 16 Genes, the Environment, and Disease The explosion of “–omics” technologies (e.g., metagenomics, meta transcriptomics, metabolomics) has enabled the generation of vast amounts of data, but it is not yet clear how best to integrate data sets in order to gain useful insights into host–microbe relationships. The use of FMT has demonstrated that modulation of an individual’s micro biota can effectively treat certain diseases; however, models with which to predict specifically how a microbiota will change after modulation— and what potentially untoward effects these changes might have—are still lacking. Implicit in this limitation is our ignorance about what microbial configuration is optimal and how a given microbiota should be rationally altered to obtain an ideal outcome.

Despite initial hyperbolic hype and a few false starts, microbiome research now stands at the forefront of an ability to treat the fundamen tal basis of many diseases. As the field continues to mature, it will need to move beyond correlations and address causation. The identification of causal microbes and their mechanisms of action will create a “micro bial toolbox” from which relevant bioactive strains can be chosen on a per-patient basis to correct specific underlying microbial dysbioses. In the near future, our knowledge base regarding the microbiome and its relationship to health and disease will be robust enough that this information can be applied in making important treatment decisions. ■ ■FURTHER READING Amato KR et al: The human gut microbiome and health inequities. Proc Nat Acad Sci 118:e2017947118, 2021. Goodrich JK et al: Conducting a microbiome study. Cell 158:250, 2014. Human Microbiome Project Consortium: Structure, function and diversity of the healthy human microbiome. Nature 486:207, 2012. Schmidt TSB et al: The human gut microbiome: From association to modulation. Cell 172:1198, 2018. Shalon D et al: Profiling the human intestinal environment under physiological conditions. Nature 617:581, 2023. Stefan KL et al: Commensal microbiota modulation of natural resistance to virus infection. Cell 183:1, 2020. Walker AW, Hoyles L: Human microbiome myths and misconceptions. Nature Microbiol 8:1392, 2023.

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