The Microbiome in Rheumatic Diseases and Infection

Gaafar Ragab
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Editors
Gaafar Ragab
Faculty of Medicine
Cairo University
Cairo
Egypt

T. Prescott Atkinson
University of Alabama
Birmingham, AL
USA

Matthew L. Stoll
University of Alabama
Birmingham, AL
USA

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It has long been an axiom of mine that the little things are infinitely the most important

—Sherlock Holmes in A Case of Identity by Sir Arthur Conan Doyle.

This book is dedicated to our wives: Samia, Miriam, and Janice.

Foreword

It is fitting to now have a book dedicated entirely to the role of the microbiome in our rheumatic diseases. After all, the study of the infectious origins of rheumatoid arthritis alone is an exercise in exploration of the modern bestiary. From 30,000 feet I would like to draw attention to observations that may help to open our minds on this topic before entering this valuable new addition to the literature which bridges the fields of rheumatic and immunologic diseases and microbiology.

First, while I am genuinely excited at the exploration of the interface of rheumatic disease etiology, pathogenesis, and natural history and the human microbiome, I am more basically left in wonder by how our microbiome shapes our relationships not just with diseases but with our sum total of experiences with the natural world. Recognize that we are not humans at all but exist as superorganisms or holobionts who have been imprinted with a remarkable spectrum of microbial entities whose own interests may not coincide with ours at any given time. Furthermore and even more remarkable is that our DNA is about 8–10% of viral origin (i.e., endogenous retroviral elements) that has created a host-parasite co-evolutionary dynamic affecting everything from our integrated host defenses to our behavior. Based on this remarkable fact alone it is imperative that we increasingly dedicate our precious resources to furthering our understanding of these relationships and how they contribute to enhancing health and causing disease.

Second, I would like to remind us that the study of the microbiome and its relationship to health and diseases is a long road and that reductionist aspirations to find a microbial culprit that causes a given disease or to therapeutically manipulate the microbiome through microbial supplements or dietary change are likely to be unrewarding, at least for now. Study of the microbiome in many ways, including the massive global efforts to categorize it such as the Human Microbiome Project and the Earth Microbiome Project, remind me in many ways of the excitement and effort poured into the Human Genome Project which in the end taught us relatively little about specific human diseases but opened up a Pandora's box of ever new questions to be addressed. While we hope that some clarion concepts may arise from such reductionist approaches to the microbiome, we may find ourselves in the same situation, facing an ever increasing and complex set of questions to address if we are to move forward. To do so I urge us all to think expansively and consider how our microbiome interacts with other networks such as our food supply, our environment, and our society.

So with these humbling caveats which hopefully remind us of our small vantage point in our complex world I welcome you to *The Microbiome in Rheumatic Diseases and Infection.*

Leonard H. Calabrese
Department of Rheumatic and Immunologic Diseases
College of Medicine of Case Western Reserve University
Cleveland, OH
USA

Contents

List of Contributors

Daniel M. Altmann Department of Medicine, Hammersmith Hospital, Imperial College London, London, UK

Miika Arvonen Department of Pediatrics, Kuopio University Hospital, Kuopio, Finland

John W. Baddley Department of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL, USA

Medical Service, Birmingham VA Medical Center, Birmingham, AL, USA

Thomas Bardin INSERM U1132, Rheumatology Department, Hôpital Lariboisière, University Paris Diderot, Paris, France

Anne Barton Division of Musculoskeletal and Dermatological Sciences, The University of Manchester, Manchester, UK

Meriem Belheouane Max-Planck-Institute for Evolutionary Biology, Plön, Germany

Institute for Experimental Medicine, Christian-Albrechts-University of Kiel, Kiel, Germany

Dimitrios P. Bogdanos Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

Rosemary J. Boyton Department of Medicine, Hammersmith Hospital, Imperial College London, London, UK

Giorgio Bozzi Infectious Diseases Unit, University of Milan, L. Sacco Hospital, Milan, Italy

Paul A. Bryant Department of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL, USA

Patrice Cacoub Sorbonne Universités, UPMC Univ Paris 06, UMR 7211, and Inflammation-Immunopathology-Biotherapy Department (DHU i2B), Paris, France

INSERM, UMR_S 959, Paris, France

CNRS, FRE3632, Paris, France

AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Department of Internal Medicine and Clinical Immunology, Referral Center for Rare Autoimmune Systemic Disorders, Paris, France

Sara Zandonella Callegher Rheumatology Clinic, Academic Hospital Santa Maria della Misericordia, Udine, Department of Medical and Biological Sciences, Udine, Italy

Luca Cantarini Research Center of Systemic Autoinflammatory Diseases, Behçet's Disease and Rheumatology-Ophthalmology Collaborative Uveitis Center, Department of Medical Sciences Surgery and Neurosciences, University of Siena, Siena, Italy

Madhura Castelino Department of Rheumatology, NIHR Manchester Musculoskeletal Biomedical Research Unit, The University of Manchester, Manchester, UK

Cloe Comarmond Sorbonne Universités, UPMC Univ Paris 06, UMR 7211, and Inflammation-Immunopathology-Biotherapy Department (DHU i2B), Paris, France

INSERM, UMR_S 959, Paris, France

CNRS, FRE3632, Paris, France

AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Department of Internal Medicine and Clinical Immunology, Referral Center for Rare Autoimmune Systemic Disorders, Paris, France

Randy Q. Cron University of Alabama at Birmingham, Birmingham, AL, USA

Heleen Cypers Department of Rheumatology, Ghent University Hospital, Ghent, Belgium

Mario M. D'Elios University of Florence, Florence, Italy

Maria I. Danila University of Alabama at Birmingham, Division of Clinical Immunology and Rheumatology, Birmingham, AL, USA

Carina Dehner Department of Immunobiology, Yale School of Medicine, New Haven, CT, USA

Anne Claire Desbois Sorbonne Universités, UPMC Univ Paris 06, UMR 7211, and Inflammation-Immunopathology-Biotherapy Department (DHU i2B), Paris, France

INSERM, UMR_S 959, Paris, France

CNRS, FRE3632, Paris, France

AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Department of Internal Medicine and Clinical Immunology, Referral Center for Rare Autoimmune Systemic Disorders, Paris, France

Salvatore De Vita Rheumatology Clinic, Academic Hospital Santa Maria della Misericordia, Udine, Department of Medical and Biological Sciences, Udine, Italy

Dirk Elewaut Department of Rheumatology, Ghent University Hospital, Ghent, Belgium

Unit for Molecular Immunology and Inflammation, VIB Inflammation Research Center, Ghent University, Ghent, Belgium

Esraa M. Eloseily Assuit University Children's Hospital, Assiut, Egypt

Jean-François Emile EA4340 and Pathology Department, Versailles University and Ambroise Paré Hospital, Boulogne, France

Guliz Erdem Nationwide Children's Hospital Medical Center, The Ohio State University, Infectious Diseases Section, Columbus, OH, USA

Steve Eyre Division of Musculoskeletal and Dermatological Sciences, The University of Manchester, Manchester, UK

Polly J. Ferguson University of Iowa Carver College of Medicine, Iowa City, IA, USA

Massimo Galli Infectious Diseases Unit, University of Milan, L. Sacco Hospital, Milan, Italy

Saviana Gandolfo Rheumatology Clinic, Academic Hospital Santa Maria della Misericordia, Udine, Department of Medical and Biological Sciences, Udine, Italy

Andrea Giacomelli Infectious Diseases Unit, University of Milan, L. Sacco Hospital, Milan, Italy

Hadi Goubran College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Saskatoon Cancer Centre, Saskatoon, SK, Canada

Loïc Guillevin Department of Internal Medicine, Hôpital Cochin, Assistance Publique–Hôpitaux de Paris, Université Paris Descartes, Paris, France

Ahmet Gül Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Rheumatology, Istanbul, Turkey

Hazem Hamza Virology Laboratory, National Research Centre, Cairo, Egypt

Department of Immunology, Interfaculty Institute of Cell Biology, Eberhard Karls University Tübingen, Tübingen, Germany

Julien Haroche Department of Internal Medicine, French Reference Center for Rare Auto-immune and Systemic Diseases, Institut E3M, AP-HP, Pitié-Salpêtrière Hospital, Paris, France

Université Pierre et Marie Curie, University Paris 6, Paris, France

Saleh Ibrahim Lübeck Institute of Experimental Dermatology, University of Lübeck, Lübeck, Germany

College of Medicine and Sharjah Institute for Medical Research, University of Sharjah, Sharjah, United Arab Emirates

Ana Izcue Institute of Molecular Medicine, RWTH Aachen University, Aachen, Germany

Traci Jester Department of Pediatrics, Division of Gastroenterology, University of Alabama at Birmingham, Birmingham, AL, USA

Maria Kaparakis-Liaskos La Trobe University, Melbourne, VIC, Australia

Edward L. Kaplan University of Minnesota Medical School, Department of Pediatrics, Minneapolis, MN, USA

Martin Kriegel Department of Immunobiology, Yale School of Medicine, New Haven, CT, USA

Axel Künstner Max Planck Institute for Evolutionary Biology, Plön, Germany

Group for Medical Systems Biology, Lübeck Institute of Experimental Dermatology (LIED), University of Lübeck, Lübeck, Germany

S. Louis Bridges Jr. Division of Clinical Immunology and Rheumatology, Comprehensive Arthritis, Musculoskeletal, Bone and Autoimmunity Center, University of Alabama at Birmingham, Birmingham, AL, USA

Philip M. Murphy Laboratory of Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Anoma Nellore Department of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL, USA

Derek Pearson College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Saskatoon Cancer Centre, Saskatoon, SK, Canada

T. Prescott Atkinson Division of Pediatric Allergy, Asthma and Immunology, University of Alabama at Birmingham, Birmingham, AL, USA

Luca Quartuccio Rheumatology Clinic, Academic Hospital Santa Maria della Misericordia, Udine, Department of Medical and Biological Sciences, Udine, Italy

Gaafar Ragab Rheumatology and Clinical Immunology Unit, Department of Internal Medicine, Faculty of Medicine, Cairo University, Cairo, Egypt

Philipp Rausch Max Planck Institute for Evolutionary Biology, Plön, Germany

Institute for Experimental Medicine, Christian-Albrechts-University of Kiel, Kiel, Germany

Catherine J. Reynolds Department of Medicine, Hammersmith Hospital, Imperial College London, London, UK

Donato Rigante Institute of Pediatrics, Università Cattolica Sacro Cuore, Fondazione Policlinico Universitario “A. Gemelli”, Rome, Italy

Hussien Rizk Department of Cardiology, Faculty of Medicine, Cairo University, Cairo, Egypt

Rossana Rocco Nephrology Unit, University Hospital, Parma, Italy

Ester Roffe Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

William Ruff Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA

David Saadoun Sorbonne Universités, UPMC Univ Paris 06, UMR 7211, and Inflammation-Immunopathology-Biotherapy Department (DHU i2B), Paris, France

INSERM, UMR_S 959, Paris, France

CNRS, FRE3632, Paris, France

AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Department of Internal Medicine and Clinical Immunology, Referral Center for Rare Autoimmune Systemic Disorders, Paris, France

Lazaros I. Sakkas Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

Julia Spierings Department of Rheumatology and Clinical Immunology, University Medical Centre Utrecht, Utrecht, The Netherlands

Matthew L. Stoll Department of Pediatrics, Division of Rheumatology, University of Alabama at Birmingham, Birmingham, AL, USA

Konstantinos Thomas Joint Rheumatology Program, Clinical Immunology-Rheumatology Unit, 2nd Department of Medicine and Laboratory, National and Kapodistrian University of Athens, School of Medicine, Hippokration General Hospital, Athens, Greece

Augusto Vaglio Nephrology Unit, University Hospital, Parma, Italy

Jacob M. van Laar Department of Rheumatology and Clinical Immunology, University Medical Centre Utrecht, Utrecht, The Netherlands

Femke C. van Rhijn-Brouwer Department of Rheumatology and Clinical Immunology, University Medical Centre Utrecht, Utrecht, The Netherlands

Department of Nephrology and Hypertension, University Medical Centre Utrecht, Utrecht, The Netherlands

Dimitrios Vassilopoulos Joint Rheumatology Program, Clinical Immunology-Rheumatology Unit, 2nd Department of Medicine and Laboratory, National and Kapodistrian University of Athens, School of Medicine, Hippokration General Hospital, Athens, Greece

Antonio Vitale Research Center of Systemic Autoinflammatory Diseases, Behçet's Disease and Rheumatology-Ophthalmology Collaborative

Uveitis Center, Department of Medical Sciences Surgery and Neurosciences, University of Siena, Siena, Italy

David R. Webb The Department of Integrated Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, USA

Arthur Weinstein MedStar Washington Hospital Center, Washington, DC, USA

Georgetown University, Washington, DC, USA

Exagen Diagnostics, Vista, CA, USA

W. Winn Chatham University of Alabama at Birmingham (UAB), Division of Clinical Immunology and Rheumatology, Birmingham, AL, USA

Wayne Young Food Nutrition and Health Team, AgResearch, Palmerston North, New Zealand

Riddet Institute, Massey University, Palmerston North, New Zealand

High-Value Nutrition, National Science Challenge, Auckland, New Zealand

About the Editors

Gaafar Ragab graduated from the Faculty of Medicine, Cairo University, 1976, where he got his degrees in Internal Medicine: MSc, 1980, and PhD, 1985. He served his faculty as a Resident, Assistant Lecturer (1980), Lecturer (1985), Assistant Professor (1990), and Professor (1995) till now. He spent a sabbatical year 1989 at the UAB, USA, in the Department of Clinical Immunology and Rheumatology. He headed the Clinical Immunology and Rheumatology Unit affiliated to the Internal Medicine Department (2010–2015). He was Chief of the Internal Medicine Department's Research Committee (1995–2013). He is Fellow of the American College of Rheumatology (ACR) since 1989. He is a Co-founder of the Egyptian Society of Immunology and Rheumatology (EGYSIR) which he headed as its President from 2010 to 2013. He was chosen as President of the Egyptian League Against Rheumatism (ELAR) annual meeting in Alexandria, 2013. He is a member of the Advisory Board of the Egyptian Society of Internal Medicine (ESIM) and its journal and served as President of ESIM annual meeting, Cairo, 2014. He is the associate editor of the *Journal of Advanced Research* (JARE), the interdisciplinary publication of Cairo University, in charge of its medical branch. He won the prize of honor, Cairo University, for the great efforts in international publications, for the year 2008. He is a member of the Egyptian National Committee for the management of Hepatitis C Virus (the extrahepatic manifestation) as well as the International Study Group of Extrahepatic Manifestations Related to Hepatitis C Virus infection (ISGEHCV). He is also a member of the Geographical Variation in Rheumatoid Arthritis Group (GEO RA).

T. Prescott Atkinson is a native of Alabama, USA, born and raised in Montgomery. He attended Tulane University (1971–1975) in New Orleans, Louisiana, and then completed 6 years of active duty in the U.S. Navy. After his military tour of duty, he entered the Medical Scientist Training Program (MD-PhD) at Emory University (1981–1987). It was during his PhD work at Emory University that he became interested in immunologic cell signaling, and he carried this area of interest in immunology into Fellowship training at the National Institutes of Health as an NIAID Clinical Fellow in Allergy/Immunology (1987–1992). After moving to the University of Alabama at Birmingham in 1992 as an Assistant Professor of Pediatrics, he began seeing pediatric rheumatology and primary immunodeficiency patients in clinic, and benefited immensely from weekly review conferences/patient discussions with Dr. Max Cooper, one of the giants in the field of immunology. This is an area in which he still maintains an active clinical interest, and over the years, principally in collaboration with groups at the NIH, he has continued to pursue studies on the molecular etiology and clinical characteristics of this extraordinarily diverse group of disorders. He has also become interested in the role of chronic infection, particularly with mycoplasmas, in chronic diseases such as asthma and arthritis, particularly in patients with primary immunodeficiencies and rheumatic disorders. He is a former member and Chair of the American Board of Allergy and Immunology. Currently he is Professor and Director of the Division of Pediatric Allergy, Asthma, and Immunology at UAB.

Matthew L. Stoll is a pediatric rheumatologist who earned his MD and PhD at Upstate Medical University (Syracuse, NY) in 2001, followed by completion of residency in pediatrics at the Long Island Jewish Medical Center in 2004 and fellowship in pediatric rheumatology at the Children's Hospital Boston in 2007. At the completion of his fellowship, he took a staff position at the University of Texas at Southwestern Medical Center (Dallas, TX), where he earned an MSCS degree. In 2011, he moved to the University of Alabama at Birmingham, where he remains. During his fellowship, Dr. Stoll developed an interest in the clinical epidemiology of spondyloarthritis, publishing on age-based subgroups of juvenile psoriatic arthritis as well as risk factors for sacroiliitis in patients with pediatric spondyloarthritis. He subsequently turned his attention to the links between spondyloarthritis and inflammatory bowel disease and reported on the use of fecal calprotectin as well as intestinal MRI to identify subclinical intestinal inflammation in children with spondyloarthritis. His current work focuses on the role of the intestinal microbiota in the pathogenesis of pediatric spondyloarthritis.

Part I

Introductory Chapters

The Microbiome: Past, Present, and Future

1

Matthew L. Stoll

Abbreviations

AS	Ankylosing spondylitis
EEN	Exclusive enteral nutrition
IBD	Inflammatory bowel disease
JIA	Juvenile idiopathic arthritis
RA	Rheumatoid arthritis

Past: Surprising Insights into Today's Microbial World

> All disease starts in the gut.—Attributed to Hippocrates

In the 1670s, Antony van Leeuwenhoek was the first to describe the presence of bacteria, which he described as "animalcules of the most minute size which moved themselves about very energetically [1]." Very little progress was made toward identifying or characterizing bacteria over the next two centuries. Infectious agents had not, it appears, captured the attention of the scientific community until Louis Pasteur promoted the concept that germs can cause transmissible disease, and Pasteur as well as Robert Koch further contributed to the field by developing techniques to culture bacteria [2]. As reviewed in 1911 [1], in the 1870s, two independent groups detected the presence of bacteria in stool. However, much of the work at the time, quite understandably, was focused on isolation of specific organisms associated with devastating diseases. Along those lines, there were some major discoveries at the time, including discovery of the bacteria causing anthrax in the blood of a dead animal accompanied by the demonstration that the disease could be transmitted through injection of the blood into a healthy animal as well as isolation and identification of the bacteria causing such diseases as tuberculosis, bacterial dysentery, and cholera [1]. Of note, the investigator who discovered both *Mycobacterium tuberculosis* in 1882 and *Vibrio cholerae* in 1884, Robert Koch, is still known today for his work proving pathogenicity of these bacteria.

Interest in the intestinal microbiota as a whole did not emerge until early in the twentieth century. Elie Metchnikoff had a rather dismal view of the microbiota, fearing that it released toxins into the systemic circulation that produced senility, and he therefore advocated altering the colonic microbiota [3]. An extreme method of doing so, which gained some attraction in the early twentieth century, was colectomy. There were some adherents to this belief, including Dr. Arbuthnot Lane, who performed colectomy or colonic bypass for a variety of

M. L. Stoll
Department of Pediatrics/Division of Rheumatology, University of Alabama at Birmingham, Birmingham, AL, USA
e-mail: mstoll@peds.uab.edu

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indications [4]. By the 1920s, this procedure had fallen out of favor [3].

A more nuanced view of the intestinal microbiota was offered by Arthur Kendall, who hypothesized that they were typically benign, unless the host is colonized with specific pathogenic agents [1]. That the intestinal microbiota was essential for the health of the host was initially demonstrated in 1915, through studies on germ-free chicks, which showed poor development of the germ-free animals starting at 10 days of life [5]. These observations resulted in the conclusion that "man has a bacterial population in his intestinal tract; that under normal conditions the organisms in the intestinal tract are fairly characteristic and constant; normally they are harmless; [and] *they may be protective* [5]."

In addition to work in germ-free animals, several further lines of current research into the microbiota had their start 100 years ago. One of them is the functional capacity of intestinal bacteria, which today is studied through such tools as shotgun sequencing of microbial DNA and mass spectroscopy of fecal and plasma metabolites. Ford initially noted that bacteria differ in their ability to metabolize carbohydrates and proteins, characterizing bacteria into two categories: fermenters (carbohydrates metabolizers) and putrifiers (protein metabolizers) [6]. Kendall extended these findings, observing that "Food largely determines the type of intestinal bacteria [1]." Specifically, diets rich in carbohydrates resulted in the generation of bacteria with increased capacity to metabolize carbohydrates. Today, it is well recognized that fiber-rich diets result in increased abundance of bacteria capable of metabolizing complex carbohydrates [7]. While carbohydrate and protein metabolism were the focus of attention in the first two decades of the twentieth century, by mid-century, the microbial effects on multiple other endogenous substances were studied, including B-complex vitamins [8–10], vitamin C [11], and cholesterol [12].

Another area of active interest today that had its roots 100 years ago is interest in treating disease through alterations in the intestinal microbiota. While today's efforts, as will be seen throughout this textbook, focus on the treatment of chronic inflammatory diseases, interest in the pre-antibiotic era was in the management of infectious diseases. As discussed above, colectomy was an extreme method of altering the intestinal microbiota, but not the only one. Diet has long been recognized as a very effective means of doing so, beginning with observations from 1911 that bottle-fed and breast-fed infants had substantially different microbial populations, with these studies even showing increased "homogeneity" of the intestinal microbiota in bottle-fed infants [1]. These observations are a precursor to recent findings showing decreased alpha diversity in bottle-fed compared to nursed infants [13]. Torrey as well noted that diet strongly influenced the contents of the microbiota, writing "It has been my experience that the intestinal flora of dogs reacts very promptly and with great uniformity to changes in diet [14]." Kendall proposed using simple sugars to alter the microbiota as a therapeutic tool for bacterial dysentery, thus in effect introducing the first instance of a therapeutic prebiotic [1]. Lane followed therapeutic colectomies in the first decades of the twentieth century with introduction of pure cultures of bacteria, first *Lactobacillus bulgaricus* and later *Lactobacillus acidophilus*, an early use of probiotics [3]. In perhaps the first published fecal microbial transplant, Dalton transplanted *Escherichia coli* from a healthy subject to a child undergoing antibiotic therapy for meningitis, reporting that rectal but not oral administration of the organism resulted in successful uptake and may have contributed to resolution of the illness [15]. In 1955, Winkelstein evaluated *Lactobacillus acidophilus* as a therapeutic agent in 53 subjects with a variety of intestinal disorders, including ulcerative colitis, reporting mixed results [16]. For the most part, however, interest in probiotics remained low until the 1990s [3].

Loss of interest in probiotic therapy as a tool to alter the microbiota may have been due to the development of antibiotics, with penicillin introduced in 1928 and many others to follow. Improved public health measures in developed nations, including vaccinations and improved hygiene, likely also dampened enthusiasm in research into microbial-based therapy of intestinal

infections. In any event, the widespread use of antibiotics spurred interest in the 1940s and 1950s on the effect of these therapies on the contents of the intestinal microbiota [17–20] and subsequently on the development of antibiotic resistance [21]. Another line of research in that era that pertained to antibiotics, which at the time was largely of interest to the agricultural field, were the effects of antibiotic therapy on the growth of livestock. Several studies demonstrated that young animals fed antibiotics demonstrated increased growth [22–24]. Observations that these growth-promoting effects of antibiotics did not occur in germ-free animals [25] and were associated with increased efficiency of absorption of dietary fatty acids [26] resulted in the conclusion that changes in the fecal microbiota mediated the increased weight gain of young animals treated with antibiotics [26]. Although this practice has fallen in disfavor due to concerns of transmission of antibiotic-resistant bacterial pathogens to humans, interest in the effects of antibiotics on growth remains, with a recent study showing that early exposure to antibiotics may be associated with an increased risk of childhood obesity [27].

One final theme that emerged in the 1950s and is germane to this textbook is the association of the intestinal microbiota with autoimmune diseases, including those not intrinsic to the gastrointestinal tract. Perhaps the first such study was published by Seneca, who reported increased total and coliform bacteria in the feces of 15 patients with UC as compared to four healthy controls [28]. Studies in the 1950s evaluated the intestinal microbiota in pediatric celiac disease [29] and acne [30]. Subsequent early studies on the intestinal microbiota were published in Crohn disease in 1969 [31], rheumatoid arthritis (RA) in 1966 [32], and ankylosing spondylitis (AS) in 1978 [33].

Ultimately, all of these efforts were limited by technology. For 100 years following the resurgence of interest in the intestinal microbiota, the only tool available to characterize them was culture, which we know today to be a highly inefficient means to characterize bacteria. It is often cited that only 20% of intestinal bacteria can be cultured [34]. Although this number may be higher [35], many of these bacteria require specialized media, and anaerobic culture is also technically demanding. In 1977, Carl Woese introduced the concept of identifying bacteria according to their ribosomal 16S DNA sequence [36], and 10 years later he published an immense database of bacterial 16S sequences [37]. This permitted use of DNA probes to characterize bacterial communities, and this technology was used in studies of RA [38] and AS [39] to name but two. However, the real explosion in microbial DNA technology had yet to come.

Present: "Democratization of Metagenomics"

> The intestinal tract is a wonderfully perfect incubator and culture medium combined… It must be evident that the direction that this flora takes will not be without influence upon the host.—Arthur Kendall (1911)

The last 10 years has witnessed an explosion of research into the microbiota. A PubMed search of microbiome or microbiota identified nearly 40,000 publications, the vast majority of which are under 10–15 years old. This research has been enabled by advances not only in sequencing technology but primarily in computing power; indeed, a typical smartphone contains more than 100,000 times the computing power of those that launched the lunar mission in 1969. More recently, even the initial sequencing of the Human Genome Project costs over $3 billion and took approximately 13 years, whereas today, the estimated cost of whole human exome sequencing is under $1000 http://www.genome.gov/sequencingcosts/ (accessed December 18, 2017). Due to the lower costs, investigators around the world are able to contribute to the field, a capacity that Jeff Gordon dubbed the "democratization of metagenomics [40]." These efforts around the world have been tremendously supported by massive centralized efforts to catalog the microbiota: the Human Microbiome Project in the United States [41] and Euro-HIT in Europe [42]. Thanks in no small part to these efforts, reference databases contain over

1.4 million bacteria and 53 thousand archaea [43] as of the end of 2016.

Much of the human work involving the microbiome consists of identifying differences in the microbiota between patient groups, e.g., those with versus without a particular disease. Such work is open to criticism that these differences are associative, but do not necessarily reflect a causal relationship. That is, the inflammatory milieu associated with a particular disease, or even its treatments, may result in alterations in the microbiota that are challenging to control for using comparison groups of healthy individuals. However, important work in animals and even in humans to some extent has shown the power of the microbiota to shape the disease, as well as the therapeutic potential of alterations of the microbiota.

Multiple animal models of inflammatory disease are attenuated or in some cases accelerated when the animals are raised in a germ-free setting, either in a true gnotobiotic facility or through treatment with broad-spectrum antibiotics. These include models of RA [44], ulcerative colitis [45], and chronic noninfectious osteomyelitis [46]. In each of these models, disease was highly attenuated in the germ-free state, and, furthermore, Koch's postulates of disease causation were partially established by recurrence of the disease when the microbiota were reintroduced into the animals.

A striking example of mediation of disease through the microbiota is the transfer of the obesity phenotype. Turnbaugh et al. studied mice that were genetically programmed to develop obesity based upon mutations in the gene coding for the satiety signal leptin [47]. Obese mice had increased *Firmicutes* in their intestines, findings typical in the obese state. Impressively, transfer of the fecal microbiota to germ-free mice resulted in increased weight gain among mice that received microbiota from obese as compared to lean mice. There were no differences in chow consumption, so this difference reflected increased energy harvest.

Another example is the HLA-B27 transgenic rat model of spondyloarthritis. Typically, transgenic rats develop a spontaneous arthritis, orchitis, and colitis. When raised in a sterile environment, the rats are protected against arthritis and colitis [48]; however, disease recurs when the animals are exposed to a cocktail of bacteria that includes *Bacteroides vulgatus* [49].

Human studies as well demonstrate that the microbiota can impact inflammatory diseases. One interesting illustration of this came from research in infants at risk for type I diabetes mellitus based upon HLA types [50]. The investigators obtained serial fecal specimens from 33 at-risk children from birth through age 3 years, finding that changes in the contents of the fecal microbiota preceded development of clinical disease.

Similarly, a study of adults with newly diagnosed RA showed an expansion of a single organism, *Prevotella copri*, in 75% of newly diagnosed subjects, that was not seen in healthy controls or established patients [51]. The pathogenic nature of this species was further demonstrated by oral gavage of mice, which resulted in colitis.

Finally, the impact of the microbiota on human disease is illustrated by therapeutic responses to treatment, possibilities that are still in their infancy. While antibiotic [52] and probiotic [53] therapy have long been a mainstay of treatment of inflammatory bowel disease, there has been increasing interest in the potential role of fecal microbial transplantation [54]. Additionally, it is clear that dietary manipulation through the use of exclusive enteral nutrition (EEN) can induce remission of inflammatory bowel disease (IBD) as effectively as can corticosteroids [55, 56], and EEN has also been reported to be beneficial in children with juvenile idiopathic arthritis [57]. Although dietary changes can induce rapid shifts in the microbiome [58], it is not clear whether the beneficial effects of dietary changes are mediated through the microbiome or some other mechanism. It remains to be seen whether microbial manipulation will have similar effects in other diseases.

It is not at all surprising that alterations in the microbiota can impact inflammatory diseases. The microbiota is required for normal development of the immune system [59], and the intestinal microbiota in particular represents the largest mass of microbial antigen and adjuvant that is encountered in life, thus setting the stage

for marked effects on systemic and mucosal immune systems [60]. Indeed, antibodies directed against commensal microbial components are present and potentially pathogenic in a variety of autoimmune diseases, including IBD [61], spondyloarthritis [62], and RA [63].

Finally, it bears mentioning that certain microbiota may also be beneficial. Not only are certain bacteria generally considered protective (e.g., *Faecalibacterium prausnitzii* in IBD (Chap. 19)), but there is a body of literature that an entire class of organisms, helminth parasites, may also be protective against allergic or autoimmune diseases. The data in mice were summarized in a recent review [64]. Evidence that parasitic infection may be protective against allergy or autoimmunity is as follows: (a) A meta-analysis determined that current infection with an intestinal parasite was associated with reduced risk of allergic sensitization [65]; (b) worldwide rates of multiple sclerosis and parasitic infestation show an inverse correlation [66]; and (c) in an area endemic for filarial parasites, patients with RA were significantly less likely to be infected as compared to healthy controls [67]; an observational study of multiple sclerosis patients demonstrated that helminth infection was associated with reduced disease progression [68]. It does bear mention, however, that some studies have shown contradictory data with respect to helminth infection and atopic diseases [69–71], and consequently not all investigators have been convinced by the epidemiologic data [72]. Additionally, interventional studies of live parasites in a variety of human autoimmune disorders have generally shown mixed results (Table 1.1).

Table 1.1 Therapeutic trials of parasitic worms

Study	Patient population	Study design	Parasite	Outcome
Allergic rhinitis				
[79]	100 adults	RCT	*Trichuris suis*	No improvement in symptoms
[80]	100 adults	RCT	*Trichuris suis*	No changes in allergic reactivity
Asthma				
[81]	30 adults	RCT	*Necator americanus* larvae	No improvement in airway hyperreactivity
[82]	32 adults	RCT	*Necator americanus* larvae	No improvement in airway hyperreactivity
Inflammatory bowel disease				
[83]	4 adults with CD and 3 with UC	OL, uncontrolled	*Trichuris suis*	6/7 achieved remission for at least part of the study period
[84]	29 adults with CD	OL, uncontrolled	*Trichuris suis*	At week 24, 21/29 (72%) responded; 23/29 (79%) met criteria for remission
[85]	54 adults with UC	RCT	*Trichuris suis*	Favorable response seen in 13/30 (43%) in the treatment group versus 4/24 (15%) controls ($p = 0.04$). Remission occurred in ≤10% in both groups
[86]	36 adults with CD	RCT	*Trichuris suis*	Improvements in symptoms seen in placebo and treatment groups; no comparisons performed
Multiple sclerosis				
[87]	5 treatment-naïve adults	OL, uncontrolled	*Trichuris suis*	Decrease in number of new MRI lesions from 6.6 to 2; no change in self-reported symptoms
[88]	10 adults	OL, uncontrolled	*Trichuris suis*	Increase in number of new MRI lesions from 6 to 21
[89]	16 treatment-naïve adults	OL, uncontrolled	*Trichuris suis*	Nonsignificant improvement in MRI lesions; self-reported improvement in symptoms in 12/16

CD Crohn disease, *MRI* magnetic resonance imaging, *OL* open-label, *RCT* randomized controlled trial, *UC* ulcerative colitis

It is of particular interest that we have come full circle in our understanding that some of the chronic rheumatic diseases may have microbial causes. Over a century ago, C. Fred Bailey proposed that RA was likely caused by toxins elaborated by microorganisms, which potentially resided in the joints, nasopharynx, or gastrointestinal tract [73]. Sulfasalazine was developed as a therapeutic agent on the basis of this assumption that RA is an infectious disease [74]. Indeed, as discussed in the RA chapter (Chap. 15), there have been multiple successful trials of antibiotics in RA, yet by the late twentieth century, the notion that this was an infectious illness was abandoned, and the effectiveness of antibiotics was attributed to intrinsic anti-inflammatory effects of these agents [75]. Yet now, as shall be discussed as well in the RA chapter (Chap. 15), there is substantial evidence that specific microbes and their associated inflammatory properties underlie the disease.

Future: Microbiota-Based Therapeutics or Prevention

> A lack of knowledge of the normal intestinal bacteria and their relations will be a serious handicap in recognizing the abnormal bacteria and their relations… Arthur Kendall (1911)

Much work lies ahead to understand not only the contributory role of the microbiota to the disease but also the extent to which microbial manipulation may have therapeutic potential. As with any medication, this will require well-designed randomized studies to assess safety and efficacy. Many rheumatologists are familiar with the concept of a "window of opportunity" to treat an inflammatory disease. We are also familiar with the idea that the disease process begins long before the first symptom emerges, as illustrated by lupus-associated antibodies being formed years before the clinical onset of disease [76]. For diseases mediated by the microbiota, the window may be long before even the first disease manifestation. We will learn in the juvenile idiopathic arthritis (JIA) chapter (Chap. 17) of evidence that elevated fecal *Bacteroides* in JIA may reflect not intrinsic pathogenicity of this genus but altered immune development on account of it. We are also learning that early childhood events affecting the gut microbiota may influence the risk not only of pediatric autoimmune disease but possibly even adult disease as well. Gordon proposed the concept of microbial prevention, such as administering probiotics to infants immediately after birth, or even to their mothers just before delivery [40]. Probiotic studies involving infants have shown benefit in reducing the risk of type I diabetes [77] and atopy [78]. Thus, the future of microbiota-based therapeutics may prove to be as much of a public health measure as therapeutic measures for individual diseases.

References

1. Kendall AI. Certain fundamental principles relating to the activity of Bacteria in the intestinal tract. Their relation to therapeutics. J Med Res. 1911;25(1):117–87.
2. Smith KA. Louis pasteur, the father of immunology? Front Immunol. 2012;3:68.
3. Podolsky SH. Metchnikoff and the microbiome. Lancet. 2012;380(9856):1810–1.
4. Smith JL. Sir Arbuthnot lane, chronic intestinal stasis, and autointoxication. Ann Intern Med. 1982;96(3):365–9.
5. Kendall AI. The Bacteria of the intestinal tract of man. Science. 1915;42(1076):209–12.
6. Ford WW. Classification of intestinal bacteria: (preliminary note). J Med Res. 1901;6(1):211–9.
7. Heinritz SN, Weiss E, Eklund M, Aumiller T, Louis S, Rings A, et al. Intestinal microbiota and microbial metabolites are changed in a pig model fed a high-fat/low-fiber or a low-fat/high-fiber diet. PLoS One. 2016;11(4):e0154329.
8. Jimenez Diaz C, Ales JM, Vivanco F. Symbiotic action of intestinal microbial flora; studies on nicotinic acid, pyridoxine, folic acid, and vitamin B12 synthesis by microbial flora in the enteric tract. Bull Inst Med Res Univ Madr. 1953;6(2–3):105–28.
9. Abdel-Salaam A, Leong PC. Synthesis of vitamin B(1) by intestinal bacteria of the rat. Biochem J. 1938;32(6):958–63.
10. Ellinger P, Abdel Kader MM. The nicotinamide-saving action of tryptophan and the biosynthesis of nicotinamide by the intestinal flora of the rat. Biochem J. 1949;44(3):285–94.
11. Esselen WB, Fuller JE. The oxidation of ascorbic acid as influenced by intestinal bacteria. J Bacteriol. 1939;37(5):501–21.
12. Wainfan E, Henkin G, Rittenberg SC, Marx W. Metabolism of cholesterol by intestinal bacteria in vitro. J Biol Chem. 1954;207(2):843–9.

13. Azad MB, Konya T, Persaud RR, Guttman DS, Chari RS, Field CJ, et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. BJOG. 2015;123(6):983–93.
14. Torrey JC. The regulation of the intestinal flora of dogs through diet. J Med Res. 1919;39(3):415–47.
15. Dalton HW. Implantation of B. coli into the human intestine. Ir J Med Sci. 1951;308:384–6.
16. Winkelstein A. Lactobacillus acidophilus tablets in the therapy of various intestinal disorders: a preliminary report. Am Pract Dig Treat. 1955;6(7):1022–5.
17. Campos JV, Hoenen W, Costa A, Trabulsi L, Pontes JF. Changes in intestinal flora under tetracycline. Gastroenterology. 1958;34(4):625–35.
18. Anderson GW, Cunningham JD, Slinger SJ. Effect of terramycin and certain phenylarsonic acid derivatives on the growth and intestinal flora of Turkey poults. J Nutr. 1952;48(4):539–52.
19. Lipman MO, Coss JA Jr, Boots RH. Changes in the bacterial flora of the throat and intestinal tract during prolonged oral administration of penicillin. Am J Med. 1948;4(5):702–9.
20. Thomas AR, Levine M. Some effects of penicillin on intestinal bacteria. J Bacteriol. 1945;49(6):623–7.
21. Goldberg HS, Goodman RN, Lanning B. Low-level, long-term feeding of chlortetracycline and the emergence of antibiotic-resistant enteric bacteria. Antibiot Annu. 1958;6:930–4.
22. Stern JR, Mc GJ. Antibiotics and early growth of rats fed a soybean oil meal diet. Arch Biochem. 1950;28(3):364–70.
23. Berg LR, Bearse GE, Mc GJ, Miller VL. The effect of removing supplemental aureomycin from the ration on the subsequent growth of chicks. Arch Biochem. 1950;29(2):404–7.
24. Sieburth JM, Gutierrez J, Mc GJ, Stern JR, Schneider BH. Effect of antibiotics on intestinal microflora and on growth of turkeys and pigs. Proc Soc Exp Biol Med. 1951;76(1):15–8.
25. Forbes M, Park JT. Growth of germ-free and conventional chicks: effect of diet, dietary penicillin and bacterial environment. J Nutr. 1959;67(1):69–84.
26. Eyssen H, de Somer P. The mode of action of antibiotics in stimulating growth of chicks. J Exp Med. 1963;117(1):127–38.
27. Saari A, Virta LJ, Sankilampi U, Dunkel L, Saxen H. Antibiotic exposure in infancy and risk of being overweight in the first 24 months of life. Pediatrics. 2015;135(4):617–26.
28. Seneca H, Henderson E. Normal intestinal bacteria in ulcerative colitis. Gastroenterology. 1950; 15(1):34–9.
29. Anderson CM, Langford RF. Bacterial content of small intestine of children in health, in coeliac disease, and in fibrocystic disease of pancreas. Br Med J. 1958;1(5074):803–6.
30. Loveman DE, Noojin RO, Winkler CH Jr. Comparative studies of enteric bacterial flora in acne vulgaris. J Invest Dermatol. 1955;25(3):135–7.
31. Drasar BS, Shiner M. Studies on the intestinal flora. II. Bacterial flora of the small intestine in patients with gastrointestinal disorders. Gut. 1969;10(10):812–9.
32. Mansson I, Olhagen B. Intestinal Clostridium perfringens in rheumatoid arthritis and other connective tissue disorders. Studies of fecal flora, serum antitoxin levels and skin hypersensitivity. Acta Rheumatol Scand. 1966;12(3):167–74.
33. Ebringer RW, Cawdell DR, Cowling P, Ebringer A. Sequential studies in ankylosing spondylitis. Association of Klebsiella pneumoniae with active disease. Ann Rheum Dis. 1978;37(2):146–51.
34. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science. 2005;308(5728):1635–8.
35. Goodman AL, Kallstrom G, Faith JJ, Reyes A, Moore A, Dantas G, et al. Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. Proc Natl Acad Sci U S A. 2011;108(15):6252–7.
36. Woese CR, Fox GE. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc Natl Acad Sci U S A. 1977;74(11):5088–90.
37. Woese CR. Bacterial evolution. Microbiol Rev. 1987;51(2):221–71.
38. Vaahtovuo J, Munukka E, Korkeamaki M, Luukkainen R, Toivanen P. Fecal microbiota in early rheumatoid arthritis. J Rheumatol. 2008;35(8):1500–5.
39. Stebbings S, Munro K, Simon MA, Tannock G, Highton J, Harmsen H, et al. Comparison of the faecal microflora of patients with ankylosing spondylitis and controls using molecular methods of analysis. Rheumatology (Oxford). 2002;41(12):1395–401.
40. Gordon JI. Honor thy gut symbionts redux. Science. 2012;336(6086):1251–3.
41. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207–14.
42. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464(7285):59–65.
43. Schloss PD, Girard RA, Martin T, Edwards J, Thrash JC. Status of the archaeal and bacterial census: an update. MBio. 2016;7(3):e00201–16.
44. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity. 2010;32(6):815–27.
45. Garrett WS, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK, Ito S, et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. Cell. 2007;131(1):33–45.
46. Hubbard TD, Murray IA, Perdew GH. Indole and tryptophan metabolism: endogenous and dietary routes to Ah receptor activation. Drug Metab Dispos. 2015;43(10):1522–35.
47. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut

microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027–31.
48. Taurog JD, Richardson JA, Croft JT, Simmons WA, Zhou M, Fernandez-Sueiro JL, et al. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. J Exp Med. 1994;180(6):2359–64.
49. Dieleman LA, Goerres MS, Arends A, Sprengers D, Torrice C, Hoentjen F, et al. Lactobacillus GG prevents recurrence of colitis in HLA-B27 transgenic rats after antibiotic treatment. Gut. 2003;52(3):370–6.
50. Lahoti TS, John K, Hughes JM, Kusnadi A, Murray IA, Krishnegowda G, et al. Aryl hydrocarbon receptor antagonism mitigates cytokine-mediated inflammatory signalling in primary human fibroblast-like synoviocytes. Ann Rheum Dis. 2013;72(10):1708–16.
51. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, et al. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. Elife. 2013;2:e01202.
52. Wilson L, Arabshahi A, Simons B, Prasain JK, Barnes S. Improved high sensitivity analysis of polyphenols and their metabolites by nano-liquid chromatography-mass spectrometry. Arch Biochem Biophys. 2014;559:3–11.
53. Li S, Pozhitkov A, Ryan RA, Manning CS, Brown-Peterson N, Brouwer M. Constructing a fish metabolic network model. Genome Biol. 2010;11(11):R115.
54. Castagnini C, Luceri C, Toti S, Bigagli E, Caderni G, Femia AP, et al. Reduction of colonic inflammation in HLA-B27 transgenic rats by feeding Marie Menard apples, rich in polyphenols. Br J Nutr. 2009;102(11):1620–8.
55. Sigall-Boneh R, Pfeffer-Gik T, Segal I, Zangen T, Boaz M, Levine A. Partial enteral nutrition with a Crohn's disease exclusion diet is effective for induction of remission in children and young adults with Crohn's disease. Inflamm Bowel Dis. 2014;20(8):1353–60.
56. Soo J, Malik BA, Turner JM, Persad R, Wine E, Siminoski K, et al. Use of exclusive enteral nutrition is just as effective as corticosteroids in newly diagnosed pediatric Crohn's disease. Dig Dis Sci. 2013;58(12):3584–91.
57. Berntson L, Hedlund-Treutiger I, Alving K. Anti-inflammatory effect of exclusive enteral nutrition in patients with juvenile idiopathic arthritis. Clin Exp Rheumatol. 2016;34(5):941–5.
58. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011;334(6052):105–8.
59. Abramowicz S, Susarla HK, Kim S, Kaban LB. Physical findings associated with active temporomandibular joint inflammation in children with juvenile idiopathic arthritis. J Oral Maxillofac Surg. 2013;71(10):1683–7.
60. Saleh M, Elson CO. Experimental inflammatory bowel disease: insights into the host-microbiota dialog. Immunity. 2011;34(3):293–302.
61. Targan SR, Landers CJ, Yang H, Lodes MJ, Cong Y, Papadakis KA, et al. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. Gastroenterology. 2005;128(7):2020–8.
62. Mundwiler ML, Mei L, Landers CJ, Reveille JD, Targan S, Weisman MH. Inflammatory bowel disease serologies in ankylosing spondylitis patients: a pilot study. Arthritis Res Ther. 2009;11(6):R177.
63. Pianta A, Arvikar S, Strle K, Drouin EE, Wang Q, Costello CE, et al. Evidence for immune relevance of Prevotella copri, a gut microbe, in patients with rheumatoid arthritis. Arthritis Rheumatol. 2016;69(5):964–75.
64. Wu Z, Wang L, Tang Y, Sun X. Parasite-derived proteins for the treatment of allergies and autoimmune diseases. Front Microbiol. 2017;8:2164.
65. Feary J, Britton J, Leonardi-Bee J. Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy. 2011;66(4):569–78.
66. Fleming JO, Cook TD. Multiple sclerosis and the hygiene hypothesis. Neurology. 2006;67(11):2085–6.
67. Panda AK, Ravindran B, Das BK. Rheumatoid arthritis patients are free of filarial infection in an area where filariasis is endemic: comment on the article by Pineda et al. Arthritis Rheum. 2013;65(5):1402–3.
68. Correale J, Farez M. Association between parasite infection and immune responses in multiple sclerosis. Ann Neurol. 2007;61(2):97–108.
69. Cooper PJ, Chico ME, Platts-Mills TA, Rodrigues LC, Strachan DP, Barreto ML. Cohort profile: the Ecuador life (ECUAVIDA) study in Esmeraldas Province, Ecuador. Int J Epidemiol. 2015;44(5):1517–27.
70. Lynch NR, Palenque M, Hagel I, DiPrisco MC. Clinical improvement of asthma after anthelminthic treatment in a tropical situation. Am J Respir Crit Care Med. 1997;156(1):50–4.
71. Webb EL, Nampijja M, Kaweesa J, Kizindo R, Namutebi M, Nakazibwe E, et al. Helminths are positively associated with atopy and wheeze in Ugandan fishing communities: results from a cross-sectional survey. Allergy. 2016;71(8):1156–69.
72. Briggs N, Weatherhead J, Sastry KJ, Hotez PJ. The hygiene hypothesis and its inconvenient truths about Helminth infections. PLoS Negl Trop Dis. 2016;10(9):e0004944.
73. Bailey CF. The treatment of chronic rheumatic and rheumatoid arthritis by radiant heat and cataphoresis. Br Med J. 1909;1(2505):13–5.
74. Mayberry J. The history of 5-ASA compounds and their use in ulcerative colitis – trailblazing discoveries in gastroenterology. J Gastrointest Liver Dis. 2013;22(4):375–7.
75. O'Dell JR, Elliott JR, Mallek JA, Mikuls TR, Weaver CA, Glickstein S, et al. Treatment of early seropositive rheumatoid arthritis: doxycycline plus methotrexate versus methotrexate alone. Arthritis Rheum. 2006;54(2):621–7.
76. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development

of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med. 2003;349(16):1526–33.
77. Uusitalo U, Liu X, Yang J, Aronsson CA, Hummel S, Butterworth M, et al. Association of early exposure of probiotics and islet autoimmunity in the TEDDY study. JAMA Pediatr. 2016;170(1):20–8.
78. Zhang GQ, Hu HJ, Liu CY, Zhang Q, Shakya S, Li ZY. Probiotics for prevention of atopy and food hypersensitivity in early childhood: a PRISMA-compliant systematic review and meta-analysis of randomized controlled trials. Medicine (Baltimore). 2016;95(8):e2562.
79. Bager P, Arnved J, Ronborg S, Wohlfahrt J, Poulsen LK, Westergaard T, et al. Trichuris suis ova therapy for allergic rhinitis: a randomized, double-blind, placebo-controlled clinical trial. J Allergy Clin Immunol. 2010;125(1):123–30 e1–3.
80. Bourke CD, Mutapi F, Nausch N, Photiou DM, Poulsen LK, Kristensen B, et al. Trichuris suis ova therapy for allergic rhinitis does not affect allergen-specific cytokine responses despite a parasite-specific cytokine response. Clin Exp Allergy. 2012;42(11):1582–95.
81. Feary J, Venn A, Brown A, Hooi D, Falcone FH, Mortimer K, et al. Safety of hookworm infection in individuals with measurable airway responsiveness: a randomized placebo-controlled feasibility study. Clin Exp Allergy. 2009;39(7):1060–8.
82. Feary JR, Venn AJ, Mortimer K, Brown AP, Hooi D, Falcone FH, et al. Experimental hookworm infection: a randomized placebo-controlled trial in asthma. Clin Exp Allergy. 2010;40(2):299–306.
83. Summers RW, Elliott DE, Qadir K, Urban JF Jr, Thompson R, Weinstock JV. Trichuris suis seems to be safe and possibly effective in the treatment of inflammatory bowel disease. Am J Gastroenterol. 2003;98(9):2034–41.
84. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. Trichuris suis therapy in Crohn's disease. Gut. 2005;54(1):87–90.
85. Summers RW, Elliott DE, Urban JF Jr, Thompson RA, Weinstock JV. Trichuris suis therapy for active ulcerative colitis: a randomized controlled trial. Gastroenterology. 2005;128(4):825–32.
86. Sandborn WJ, Elliott DE, Weinstock J, Summers RW, Landry-Wheeler A, Silver N, et al. Randomised clinical trial: the safety and tolerability of Trichuris suis ova in patients with Crohn's disease. Aliment Pharmacol Ther. 2013;38(3):255–63.
87. Fleming JO, Isaak A, Lee JE, Luzzio CC, Carrithers MD, Cook TD, et al. Probiotic helminth administration in relapsing-remitting multiple sclerosis: a phase 1 study. Mult Scler. 2011;17(6):743–54.
88. Voldsgaard A, Bager P, Garde E, Akeson P, Leffers AM, Madsen CG, et al. Trichuris suis ova therapy in relapsing multiple sclerosis is safe but without signals of beneficial effect. Mult Scler. 2015;21(13):1723–9.
89. Fleming J, Hernandez G, Hartman L, Maksimovic J, Nace S, Lawler B, et al. Safety and efficacy of helminth treatment in relapsing-remitting multiple sclerosis: results of the HINT 2 clinical trial. Mult Scler. 2017. https://doi.org/10.1177/1352458517736377.

2 Methods for Microbiota Analysis: Sample Collection and Laboratory Methods

Saleh Ibrahim and Meriem Belheouane

Abbreviations

CD	*Clostridium difficile*
CD	Crohn's disease
CDI	*Clostridium difficile* infection
DGGE	Denaturing gradient gel electrophoresis
FACS	Flow cytometry (FCM) fluorescence-activated cell sorting (FACS)
FISH	Fluorescence in situ hybridization
GC-MS	Gas chromatography-mass spectrometry
MAR	Microautoradiography
OTU	Operational taxonomic unit
RTF	Reduced transport fluid
SIMS	Secondary ion mass spectrometry
SIP	Isotope-labeled substrates
TGGE	Temperature gradient gel electrophoresis
T-RFLP	Terminal restriction fragment length polymorphism

S. Ibrahim
Lübeck Institute of Experimental Dermatology, University of Lübeck, Lübeck, Germany

College of Medicine and Sharjah Institute for Medical Research, University of Sharjah, Sharjah, United Arab Emirates

M. Belheouane (✉)
Max-Planck-Institute for Evolutionary Biology, Plön, Germany

Institute for Experimental Medicine, Christian-Albrechts-University of Kiel, Kiel, Germany
e-mail: belheouane@evolbio.mpg.de

Introduction

We live in a world dominated by microbes [1]. In fact, various environments, including multicellular organisms, are inhabited by a myriad of complex and diversified microbial assemblages. The complete set of microorganisms that resides in a given habitat is referred to as "microbiota" and combines diverse microbial species such as bacteria, viruses, and fungi. Through this chapter, we will be mainly focusing on the bacterial communities that are associated with several human body sites.

Microbiota Research: From Culture- to Molecular-Based Methods

Until early in the twenty-first century, studies of microbiota were traditionally addressed using culture-dependent methods. Culture of pure microbial colonies using selective and diverse culture media (solid, semisolid, and liquid), which take advantage of the distinctive metabolic properties of the microorganisms, has enabled isolation, identification, and characterization of several microbial species, ultimately defining treatments against many pathogenic microbes [2, 3]. Nowadays, culture methods continue to be an approach in exploring microbial diversity [4–7] and are central for identifying pathogenic organisms from clinical specimens. However, numerous microbial species show fastidious growth

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requirements which render their isolation and identification extremely challenging. In fact, culturable bacteria in laboratory conditions represent solely a tiny fraction of the entire bacterial diversity, and the unculturable species play essential roles in community functioning such as synthesizing and degrading key components [8]. Besides missing the unculturable members of the community, differences in growth requirements across different species potentially lead to biases in describing the relative abundances of the taxa within a mixed community. Indeed, bacteria with less fastidious growth requirements likely overcompete the more challenging species, thus providing an inaccurate estimation of the real relative abundances of the species within a community. Fortunately, over the last decades, methods of microbiota investigation have tremendously improved, allowing deep, detailed, and complete characterization of the microbial components in a given environment (Table 2.1). Specifically, with the introduction of the bacterial 16S rRNA genes described by Pace et al. [9] that harbor hypervariable and much conserved regions which permit the identification and reconstruction of the bacterial phylotypes phylogeny, the strong advancement of molecular-based approaches, and sequencing technologies, the field of microbiome research has massively expanded and host-microbiota interactions became a central interdisciplinary area of research in health and disease.

High-Throughput Sequencing of 16S rRNA Genes and Whole Community Profiling

With the aim to identify and quantify the relative abundances of microbial species, the 16S rRNA genes are amplified, commonly using primers that target one or two hypervariable regions such as V1–V2, V3–V4, or V6 regions. The primers for each sample contain a unique barcode sequence which allows merging several samples together in one sequencing run. Substantially, PCR products are pooled together at identical concentration and sequenced using high-throughput sequencing technology such as the Illumina platform [10, 11]. Of note, primer choice is crucial and might impact the detection of certain microbial species and thus impact the downstream analyses. Indeed, the ability to discriminate between diverse species is essential in clinical investigations. In this line, previous studies reported that the choice of the V1–V3 region is valuable in discriminating between common skin resident bacteria especially the *Staphylococcus* species [12, 13]. Deep sequencing of 16S rRNA genes offers phylogenetic and quantitative data, including for unknown species; however, phylogenetic definition depends on available databases, and the technique suffers from PCR biases and remains relatively expensive and laborious.

Whole community approaches or the so-called "omics" are advancing the characterization of microbial assemblages by addressing several community aspects. The Human Microbiome Project Consortium [14] employed metagenomics approach, which is based on the massive and parallel sequencing of the entire genomes of microbial communities associated with several human body sites. This technique takes advantage on genomics, sequencing tools, as well as bioinformatics analyses to define the genetic content of all community members and infer their functions [15]. The study reported higher stability among individuals at the level of bacterial metabolic pathways, whereas the structural disparities assessed via sequencing the 16S rRNA genes were substantial. Similarly, Oh et al. [16] applied metagenomics technique to the skin microbiota and reported that the microbial functional diversity varied along the different skin sites.

While metagenomics reveal the potential functions of the complete collection of microbes, it does not define the actual physiological or metabolic status of the community members. Metatranscriptomics provide further information about the current activity state. In fact, this method which requires RNA isolation identifies the relative expression of genes in a community, without characterizing the actual or direct enzymatic activity. Recently, Maurice et al. [17] defined the active part of gut-associated microbial communities in human using metatranscriptomics and revealed that the gut harbors a distinctive set of

Table 2.1 Description of various methods employed in microbiota research

Method	Description	Data provided
Culture	Isolation of bacteria on selective culture media and growth conditions	Identification and characterization of metabolic properties of the bacteria
Direct and high-throughput sequencing of 16S rRNA	Amplification of a hypervariable region of the 16S rRNA and massive parallel sequencing of the amplicons	Phylogenetic identification and quantification of bacteria of also unknown sequences
qPCR	Amplification of 16S rRNA with fluorescence labeled, primers or probes	Phylogenetic identification and quantification of species of known sequences
Cloning of the 16S rRNA	Amplification of full-length 16S rRNA gene using broad primers, cloning, and Sanger sequencing	Phylogenetic identification of bacteria
Microbiota array	Amplification of full-length 16S rRNA gene with degenerate primers; amplicons hybridize to an array that contains a set of specific probes	Phylogenetic identification and quantification of bacteria species of known sequences
Gram staining	Staining of bacteria cells based on the composition of the cell wall	Detection, localization, visualization, and sorting of bacteria species
Immunofluorescence	Binding of an antibody, linked to a fluorophore, and a specific bacterial antigen, e.g., lipopolysaccharide which generates fluorescence signal	Detection, localization, identification, and visualization of bacterial structure for bacteria of known sequences
Fluorescence in situ hybridization (FISH)	Fluorescence-labeled probes target the 16S rRNA genes of total and specific bacteria taxa	Phylogenetic identification, localization, visualization, and quantification of microbial presence and activity of known sequences
Microautoradiography (MAR)	Substrate absorption is quantified using radioactive-labeled substrates	Determination of the physiological status of a single cell
Temperature gradient gel electrophoresis (TGGE) and denaturing gradient gel electrophoresis (DGGE)	Gel separation of 16S rRNA PCR products using temperature or chemical denaturation	Comparative and quantitative assessment of bacterial profiles
Terminal restriction fragment length polymorphism T/RELP	16S RNA is amplified using fluorescence-labeled primers; amplicons are digested with restriction enzymes and separated by gel electrophoresis	Quantitative assessment of bacterial profiles
Flow cytometry (FCM) fluorescence-activated cell sorting (FACS)	Cell sorting based on cell properties including metabolic activity, cell damage, growth rate, gene content, and transcription levels	Definition of cell categories within a community based on the chosen sorting criteria, e.g., highly active vs. dormant cells
Mass spectrometry	Stable isotope labels of bacterial components such as peptides	Quantifies the actual metabolic activity within a single cell
Whole community profiling "omics"	Massive parallel sequencing of whole genome, transcriptome within a community	Phylogenetic identification, quantification, and reconstitution of functions, activity, and metabolic properties of the collection of microbes within a community
Single-cell omics	Whole genome, transcriptome, or proteome sequencing of a single cell, e.g., single-cell RNA sequencing (scRNA-seq)	Definition of gene content, function, activity and metabolic status of a single microbial cell

active species compared to the present species defined on the DNA level. Ultimately, metaproteomics provide information about the actual enzymatic functions that are expressed in a community [18]. Erickson et al. [19] took advantage of the improvements in protein isolation and preparation techniques reviewed by Xiong et al. [20] and combined shotgun metagenomics and metaproteomics methods to characterize and identify potential functional signatures of human gut

microbiota in the context of Crohn's disease. This pioneering study reported novel differences in microbial communities between healthy and diseased individuals that include several genes, proteins, and pathways. An additional technique includes metabolomics, which focuses on the metabolome, i.e., the entire collection of metabolites such as hormones, and signaling molecules which belong to a given sample (e.g., cell, organism, and community). This method aims to define the metabolic profile by identifying, characterizing, and quantifying the metabolites of interest, as well as describing the biochemical pathways of metabolites. Antharam et al. [21] investigated the contribution of specific gut microbes to fecal metabolites in *Clostridium difficile*-associated gut microbiome. The researchers employed gas chromatography-mass spectrometry (GC-MS) and 16S rRNA deep sequencing, to analyze the metabolome and microbiome of fecal samples of patients suffering from *C. difficile* infection and from healthy controls. This study identified 63 human gut microbes with cholesterol-reducing activities, thus supporting a potential role of microbial components in host lipid metabolism. Overall, mass spectrometry quantifies the actual metabolic activity. This technique combines stable isotope labels and Raman microspectroscopy or secondary ion mass spectrometry (SIMS) [22, 23]. In addition, nuclear magnetic resonance spectroscopy technology is also employed to characterize the metabolic profile of the microbial communities. Mass spectrometry methods are powerful in terms of coverage, sensitivity, and quantification to characterize the metabolic properties of the cells including uncultured microorganisms and associate the structure and function in complex microbial assemblages. To date, these techniques remain fairly expensive.

In short, whole community approaches are focusing on a global characterization of the microbial species within a community; nonetheless, these techniques remain relatively costly, while the process of data analyses is laborious and time costly. Of note, annotations of the various databases (e.g., reference genomes, transcripts) continue to expand, to improve the accuracy of study's conclusions [24].

Beyond the 16S rRNA High-Throughput Sequencing

While sequencing of the 16S rRNA phylogenetic marker revolutionized the field of microbiome research, this approach provides a subset of information on the microbial assemblages, and additional techniques are valuable in providing supplementary pieces of information on several community aspects.

Quantitative PCR

Real-time PCR is frequently employed to identify and quantify microbial taxa, while quantification is based on the measure of fluorescent signals from primers or probes; identification is based on the use of specific primers that are commonly designed for the 16S rRNA gene [25, 26]. The specific primers target various taxonomical levels such as genus or species. Real-time PCR is sensitive and accurate, yet it is subject to PCR biases and targets solely taxa of known sequences. It is frequently used to confirm findings obtained through deep sequencing of the 16S rRNA gene.

Cloning of the 16S rRNA Genes

In this technique, 16S rRNA genes are amplified using broad-range primers; then PCR products are purified and cloned. A high number of colonies are randomly picked and processed for Sanger sequencing, and phylogenetic identification is performed using a classification database tool [27, 28]. This method provides phylogenetic data based on the full length of the 16S rRNA gene; however, it suffers from PCR and cloning biases and remains laborious and relatively expensive.

Microbiota Array

The microbiota array requires the amplification of full-length 16S rRNA gene with degenerate primers. PCR products hybridize to an array that comprises a set of specific probes whereby the

specificity of the probes allows the identification of the taxa, while quantification of the bacterial taxa is achieved through the assessment of fluorescence signal [29, 30]. Nevertheless, cross hybridization is likely to occur, and unknown and very low abundant microbes are challenging to detect.

Staining-, Histology-, and Microscopy-Based Methods

Spatial localization of microbes is critical in the characterization of microbial assemblages. Accordingly, Nakatsuji et al. [31] investigated whether microbial species localize in deep sections of the skin and combined several staining techniques. Gram staining was employed to visualize and localize the bacterial structure across various skin layers. This technique discriminates bacteria based on the chemical and physical properties of their cell walls through detection of peptidoglycan, a structure present in Gram-positive bacteria [32]. Moreover, immunofluorescence was used to target particular bacterial structures. This technique is based on the specificity of an antibody to its antigen, e.g., lipopolysaccharide, whereby the specific binding triggers fluorescent signal that permits the visualization of the target species [33]. These techniques allow the detection, localization, and visualization of bacterial components and demonstrated that commensal bacteria are also localized in deep layers of the skin. Similarly, fluorescence in situ hybridization (FISH), which requires RNA isolation and labeled probes with fluorescent dyes such as cyanine 3 (Cy3) and/or cyanine 5 (Cy5), aims to define, localize, and quantify the 16S rRNA gene content. Broad and specific probes are employed separately or in combination to assess total and specific microbial abundance. Namely, the Eub338 targets the 16S rRNA of most but not all bacteria and defines the total bacterial abundance [34], while probes for specific taxa, for instance, Alf968 for α-proteobacteria [35] and Bet42a for β-proteobacteria [36], allow the detection of uniquely these taxa. Cottrell and Kirchman [37] quantified the relative abundances of major bacterial species inhabiting an estuary, while Earle et al. [33] quantified taxa abundances in different sections of the mouse gut. Both studies combined FISH and high-resolution microscopy. Of note, high-resolution microscopy and image analysis permit the description of relevant cell properties such as volume and size.

In addition to identifying, localizing, and quantifying the relative abundances of distinct taxa within a mixed community, FISH can be combined with microautoradiography (MAR), a technique employed to define the physiological state of a single cell. MAR is based on quantifying substrate absorption using radioactive-labeled substrates; for example, it can identify cells specifically uptaking radiolabeled leucine. Thus, MAR defines the metabolic state of the cell [38], while FISH provides phylogenetic data, and the two procedures can be used in tandem to identify which bacteria are metabolizing a specific compound of interest. Overall, these techniques are sensitive and accurate, though they do not define unknown species.

Electrophoresis-Based Methods

Methods that apply electrophoresis include the terminal restriction fragment length polymorphism (T-RFLP), which is based on fluorescently labeled primers that amplify 16S rRNA genes, whereby restriction enzymes digest the amplicons and the fragments are separated by gel electrophoresis. Sizes of every sample's terminal fragments are defined via sequencing and fluorescence intensity [39]. Similarly, temperature gradient gel electrophoresis (TGGE) and denaturing gradient gel electrophoresis (DGGE) use either a temperature or chemical gradient, respectively, to denature the sample during the migration process on an acrylamide gel. At last, sample specific profiles are generated during migration [40]. Zoetendal et al. [41] compared the composition of the active and present bacteria in human fecal samples by applying temperature gradient gel electrophoresis of 16S rRNA genes. Terminal restriction fragment length polymorphism and gradient gel electro-

phoresis provide only quantitative data, and henceforth are outdated methods.

Flow Cytometry (FCM)

Flow cytometry is a great tool that permits fast and simultaneous analysis of millions of cells. The microbial cells are held in suspension and exposed to a strong source of light, so that fluorescence signals for every single cell are collected and recorded [42]. Flow cytometry sorts cells based on different characteristics such as size, shape, intracellular content, or membrane integrity [17]. For example, cell damage, or whether a cell is deceased, can be investigated by examining the membrane integrity using exclusion dyes (PI, EtBr, TOPRO dyes). Furthermore, the enzymatic activity is assessed via quantifying the esterase activity, while nucleic acid content, to define cell activity levels, is measured using nucleic acid dyes such as SYBR Green or SYTO 13. An additional example includes substrate usage, which is quantified through isotope-labeled substrates (SIP). Recently, Peris-Bondia et al. [43] investigated the active fraction of human gut microbiota by measuring the nucleic acid content using Pyronin-Y, a fluorescent dye for total RNA, to sort the cells into categories based on the activity levels. A recently developed technique involves sorting intestinal bacteria based upon adhesion to mucosal IgA, with IgA^+ bacteria demonstrating greater ability to mediate colitis [44]. Flow cytometry performs high-throughput analysis, yet it does not define the phylogeny or the localization of the microbial cells.

Single-Cell Approaches

Single-cell approaches are valuable for characterizing various properties of the cell within a mixed microbial community. Based on cell sorting, these methods deeply describe intra- and intercellular variations of several properties including metabolic activity, cell damage, growth rate, gene content, and transcripts levels.

Several approaches of single-cell analyses have been developed and applied on microbial assemblages. These methods, which include whole-genome sequencing, transcriptomics, proteomics, and metabolomics, do not require prior culture and thus potentially reveal new genomes of unculturable species [45, 46]. Single-cell analysis was applied on an unculturable bacterium inhabiting the human oral cavity which belongs to the TM7 phylum, through which the whole-genome amplification and sequencing permitted the identification of thousands of genes and disclosed several microbial functional pathways. Ultimately, the collected genetic information likely contributes to understanding the culture requirements of this bacterium [47]. In the future, these methods will continue to improve to achieve deeper cell phenotyping, particularly when combinations of these analyses are performed within a single cell (e.g., genome sequencing, transcriptomics) [48–51].

Combination of Various Methods in Microbiota Studies

While using one method is common in investigating the microbiota, the approach of integrating several methods is also frequently employed. Indeed, combination of various methods is advantageous and allows the definition of complementary pieces of information on diverse community aspects and/or confirms each method outcome. Shankar et al. [52] characterized the gut microbiota of human patients suffering from *C. difficile* infection (CDI) by combining microbiota array, high-throughput Illumina sequencing of the 16S rRNA genes, and fluorescence in situ hybridization (FISH). Precisely, the microbiota array provides data on the phylogeny and abundances of microbial taxa, while the FISH localizes and also identifies the present taxa. Xu et al. [53] combined RNA sequencing and metabolomics to identify the pathogens in a clinical specimen in the context of esophageal squamous cell carcinoma, while Yu et al. [54] combined 16S rRNA sequencing and metabolomics to characterize association between gut microbial phenotypes and depression. On the other hand, previous