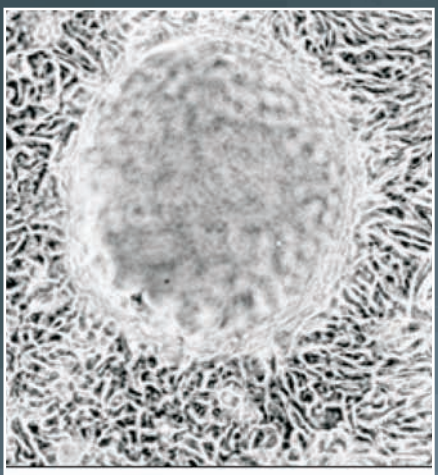

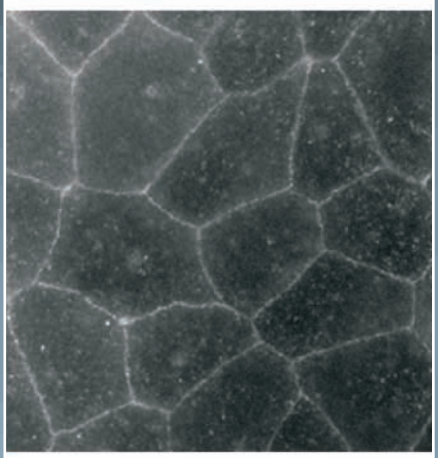

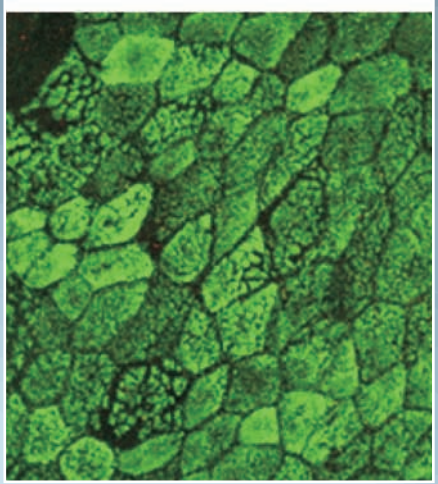

Intestinal Microbiota in Health and Disease

Modern Concepts

Editors:
Eduardo J. Schiffrin
Philippe Marteau
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CRC Press
Taylor & Francis Group
A SCIENCE PUBLISHERS BOOK

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business
A SCIENCE PUBLISHERS BOOK

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Version Date: 20140122

International Standard Book Number-13: 978-1-4822-2677-5 (eBook - PDF)

Preface

Mucosal surfaces are home to a complex microbiota, which rapidly colonizes the tissues at birth and bestows important nutritional, metabolic and protective functions which benefit the host. The numerous and diverse microbial communities residing at the vast intestinal mucosal surface have a particularly important role to play in human health, any *dysbiosis* of this microbiota being an underlying factor in a variety of clinical diseases. As such, the intestinal microbiota is the subject of intense and rapidly advancing research. Recent progress in molecular microbiology, bioinformatics and ecology in particular, are providing new insight into the role of the intestinal microbiota in health and revealing hitherto unexplored means to prevent or treat certain diseases.

Through this book, we sought to provide the reader with an appraisal of the most recent advances in the field. To this end, we have invited recognized experts from the academic and medical communities and from the pharmaceutical and food industry, to provide a treatise on a range of topics that address host-microbe interactions in the gut and the consequences of these interactions for the host.

The opening chapters of the book discuss how microbes interact with host cells in specific intestinal niches, become established members of the intestinal microbiota and impact the development and function of a healthy immune system. The next chapters describe how pathogens overcome host defences and exploit the underlying mechanisms to infect the host. Chapters in the second half of the book then address the characteristics and the mechanisms leading to *dysbiosis* of the microbiota and how this culminates in the development of inflammatory bowel disease, obesity, infection or stress. In this context, authors also discuss how manipulation of gut ecology and the use of probiotic microorganisms are credible therapeutic strategies to treat diseases and conditions affecting both the inside and the outside the gastrointestinal tract.

We are confident that the authors have provided a balanced review of the field and that the contents of this book will offer new information to students and experienced researchers alike.

We sincerely thank each author for the outstanding quality of their respective contributions and we trust that you, like us, will find the contents of this book informative, fascinating and a pleasure to read.

Eduardo J. Schiffrin, Medical Director
Philippe Marteau, Professor
Dominique Brassart

Contents

1

Commensal Intestinal Microbiota and Mucosal Immune System Development and Function

Katarina Radulovic[1] and *Jan Hendrik Niess*[1,2,*]

Introduction

Instead of living as one individual organism, different species coexist in complex ecological niches constantly influencing each other. Humans are no exception from the symbiotic way of living since every healthy human individual coexists with an enormous number of microorganisms. The mutually dependent "life together" of two or more species is called symbiosis (Black 1996). Symbiotic relations of humans and microbial species always have positive outcomes for at least one member and this includes the relationships of mutualism and commensalism. Mutualism is a "win-win" situation in which both members benefit from the relationship (Black 1996). In commensalism the situation is "win-zero" since one member of the relationship benefits without helping or harming the other one (Black 1996). When considering the interactions between microbial communities,

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some authors also include the relations with the negative outcome to one member into the symbiosis (Faust and Raes 2012). Such relations include parasitism ("win-lose" situation) amensalism ("zero-loose" situation) and competition ("loose-loose" situation) between the microbes (Faust et al. 2012; Faust and Raes 2012).

Humans start their development in a sterile intrauterine environment, but from the very moment of birth all the epithelial surfaces in direct contact with the environment (skin, respiratory, gastrointestinal and urogenital tract) are colonized by microorganisms (Levy 2007). We are living with these microorganisms in mutualistic or commensal relationships. We refer to the collection of all of these microorganisms as the human microbiome, microflora or commensal microbiota. Rising interest in investigations of how microflora influences the human health, as well as great improvement in microbiological methods have given valuable data about the composition of our microbiological partners and their interactions with the human body. Now we know that healthy individuals remarkably differ in the composition of the microbiota (The human microbiome project consortium 2012b). We also know that in the highly coevolved and bidirectional relationship with the human host, microflora have profound effects on the human health (The human microbiome project consortium 2012a).

This chapter will introduce the influence of microflora on immune system development and function in the gut, summarizing the results obtained on rodents and humans. We will describe the composition of intestinal microflora and the basics of intestinal immune system functioning. We will also discuss how the delicate balance between microflora and underlying immune system cells is maintained and how disturbances in microflora composition influence the function of intestinal immune cell subtypes.

The Composition of Intestinal Microbiota

As the largest body surface directly exposed to the influences of environment, the human intestine is populated with enormous number of microorganisms. More than 400 m^2 of the intestinal epithelial surface is colonized by approximately 100 trillion of microbial cells which is tenfold of the total number of cells in the human body (Backhed et al. 2005; Qin et al. 2010; Stephani et al. 2011). Bacteria are the main type of microbes present in the human gut, but other types like protozoa, fungi and viruses can be found, as well (Macpherson and Harris 2004; O'Hara and Shanahan 2006). The concentration of the bacterial cells gradually increases along the intestinal tract ranging from 10^3 cells/gram (g) of luminal content in the stomach and duodenum, to 10^4 to 10^7 cells/g in the small intestine and up to 10^{11} to 10^{14} cells/g in the colon (Sekirov et al. 2010; Stephani

et al. 2011). Most of the intestinal bacteria are non-cultivable (Kuwahara et al. 2011) which created difficulties for the scientists who investigated them. The recent advance in culture-independent techniques, such as 16S ribosomal RNA sequencing, has made it possible to identify the majority of the bacteria living in the human intestinal tract and also to compare the microflora composition of different individuals and species. Up to 40.000 bacterial species are identified in the human intestine (Frank and Pace 2008) that are distributed in over 50 phyla from which 2 are dominant: Firmicutes and Bacteroidetes (Sekirov et al. 2010; The human microbiome project consortium 2012b). These two phyla represent the core of human intestinal microbiota including more than 80% of all the intestinal bacterial species, but greater inter-individual differences exist on the species level (Tap et al. 2009; The human microbiome project consortium 2012b). Some of the other phyla also represented in the human intestinal microbiome are Proteobacteria, Actinobacteria, Verrucomicrobiota, Fusobacteria and Cyanobacteria (Sekirov et al. 2010; Tap et al. 2009; The human microbiome project consortium 2012b).

Early Life Events Shape the Intestinal Microflora

As already discussed, intrauterine development of humans and all the other mammals, including rodents, is sterile due to efficient maternal immune mechanisms that keep the uterine cavity sterile (Levy 2007). Microbial colonization of the intestine starts at birth and bacteria can be found in faeces within the first few hours of life (Kaplan et al. 2011; Ouwehand et al. 2002). The intestinal flora of newborns is relatively unstable and beside the genotype of the host, efficient colonization depends on many extrinsic factors: the composition of maternal microflora, the mode of delivery, environmental hygiene conditions, and diet and medication (Fanaro et al. 2003; Fouhy et al. 2012). The pattern of initial colonization is very important since it shapes the gut microbiota composition in adulthood. Normally, the first intestinal habitants are the facultative anaerobic bacteria including *Escherichia coli* and the Firmicutes species form the genera *Streptococcus*, *Staphylococcus* and *Enterococcus* (Kaplan et al. 2011; Park et al. 2005). These bacteria take advantage of the oxygen abundance, but at the 1–2 week of age the oxidation-reduction potential decreases and obligate anaerobic bacteria of the genera *Bacteroides*, *Clostridium* and *Bifidobacterium* become dominant (Fanaro et al. 2003; Kaplan et al. 2011; Park et al. 2005; Scholtens et al. 2012). Weaning from the mother's milk to solid food further favours obligate anaerobes, leading to the final stabilisation of microflora composition that at the age of 1 year resembles the microflora of adults (Scholtens et al. 2012; Sekirov et al. 2010). In the adult gut, dominant bacterial genera are *Bacteroides*, *Clostridium*, *Fusobacterium*, *Eubacterium*, *Ruminococcus*,

Peptococcus, *Peptostreptococcus* and *Bifidobacterium*, while *Escherichia*, *Klebsiella*, *Lactobacillus* and *Enterobacter* are less represented (Fanaro et al. 2003; Vedantam and Hecht 2003).

Newborns delivered by caesarean section do not have the contact with maternal microflora during the process of birth and their intestinal microbiota composition highly depends on the environmental bacterial load. In these infants, the establishment of stabile microflora is delayed and instead of *Bifidobacterium*, the frequently isolated bacterial genera are *Klebsiella*, *Clostridium* and *Enterobacter* (Adlerberth et al. 2007; Fanaro et al. 2003; Kaplan et al. 2011). Also the intestine of formula-fed babies is not rich in *Bifidobacteria*, but rather in other bacterial genera such as *Enterobacter*, *Escherichia*, *Clostridium*, *Klebsiella* and *Bacteroides* (Fanaro et al. 2003; Kaplan et al. 2011). Preterm born infants are often hospitalized in the neonatal intensive care units and subjected to medication for a certain time period and this treatment crucially influences the composition of intestinal microbiota. In these infants, bacterial diversity is decreased and *Bifidobacteria* are rare while *Klebsiella*, *Enterobacter* and *Staphylococcus* are predominant (Arboleya et al. 2012; Fanaro et al. 2003; Kaplan et al. 2011).

These early life events and conditions have profound effects on the intestinal microflora composition and therefore on the health status of the individual during the whole life. Namely, an increased prevalence of atopic and allergic diseases in industrialized countries has been attributed to the increased hygiene and vaccination in the neonatal period and during childhood (Macpherson and Harris 2004; Ouwehand et al. 2002; Strachan 1989). Studies showed that children born and raised in farming conditions, rich in microbial antigen load, are protected from the development of asthma (Douwes et al. 2008; Ege et al. 2011) while avoidance of antigens during prenatal and neonatal period can increase the risk of allergy (Falth-Magnusson and Kjellman 1992; Woodcock et al. 2004). The two hypotheses trying to explain these observations, the "hygiene hypothesis" and the "fetal programming hypothesis", both point towards the importance of microbial-derived factors (Kaplan et al. 2011). It is considered that increased hygienic conditions ("hygiene hypothesis") reduce the exposure to microbial antigens, especially in the critical period during the intrauterine and neonatal stage ("fetal programming hypothesis") leading to the aberrant development of the immune system (Kaplan et al. 2011; Macpherson and Harris 2004). In general, the increased risk of atopic diseases is linked with the presence of *Enterobacteria*, *Clostridium*, *Bacteroides* and *Staphylococcus* and reduction of *Bifidobacteria* (Penders et al. 2007). These findings strongly indicate that the balanced composition of intestinal microflora is crucial for the host health.

Mutualism in the Intestine

Life in symbiosis with intestinal microorganisms brings mutual benefits for both partners. The gastrointestinal tract is rich in molecules that can serve as nutrients for the microorganisms and therefore represent a secure habitat in which microbes can survive and multipy (Costello et al. 2012; Sekirov et al. 2010). In return, the genome of gut microbiome, which contains ≥ 100 times the number of genes in the human genome, endows us with the functional features that we have not had to evolve ourselves (Backhed et al. 2005). Germ free (GF) animals provide an excellent model system for studying the contribution of microflora to various body functions. These animals are born and bred in special isolators that are ventilated with sterile filtered air and by using sterile bedding, food and water for their breeding in order to make them completely devoid of microorganisms (Macpherson and Harris 2004). Studies on GF animals showed that intestinal microbiota have nutritive, metabolic, protective and immunogenic functions (Fanaro et al. 2003; Rossi et al. 2011).

The intestinal flora assists in the digestion of otherwise indigestible energy substrates (e.g., plant-derived pectin, cellulose). In the line with this, it is shown that GF animals need about 30% more calories than conventional (CV) animals to keep a stable body weight (Wostmann et al. 1983). Through the production of short-chain fatty acids (SCFA), microflora stimulates the proliferation and differentiation of the intestinal epithelial cells (IECs) assuring the efficient nutrient absorption (O'Hara and Shanahan 2006). A prominent characteristic of GF animals is the pronounced enlargement of the caecum which can be explained by the accumulation of the luminal content, in particular mucus and urea that are normally degraded by microflora (Gustafsson et al. 1970; Juhr and Ladeburg 1986). The metabolic role of microbiota is also seen in the production of 1–20% of the total plasma level of lysine, the indispensable amino acid (Metges 2000) and regulation of the iron and copoper metabolism (Reddy et al. 1965a; Reddy et al. 1965b). Furthermore, intestinal microflora represents an important source of the vitamins that cannot be produced by the host body (Rossi et al. 2011). Vitamin K and the vitamins of B group are mainly produced by the bacterial genera *Bacteroides* and *Eubacterium* (Hill 1997; Rossi et al. 2011). Through the competitive occupation of the attachment sites and the consumption of nutrients, microflora protect the host from the pathogen invasion (Sekirov et al. 2010). The protection role of microflora is also achieved by the active interactions with the host immune system. The fact that lethal oral dose of the *Listeria monocytogenes* in GF mice is one million fold less than in CV animals (Zachar and Savage 1979) shows the importance of the microflora in host protection.

The Intestinal Immune System Coexist with the Enteric Microflora

Despite the symbiotic nature of the host-microbiota relationship and numerous beneficial effects of the intestinal microflora, presence of such a high non-self antigen burden in the intestinal lumen represents an immense challenge to the underlying mucosal immune system. Approximately 80% of all body's immune cells are located in the intestine (Smith and Garrett 2011). These cells are organized in the complex network of the intestinal immune system which faces a specific problem: it has to preserve the beneficial enteric microbiota without starting an immune response against them, but at the same time it has to efficiently eliminate pathogenic agents that can invade the body through the intestinal route. Closely associated together, microbiota and intestinal immune system interact in a bidirectional manner to ensure the maintenance of homeostasis. On one hand, the immune system evolved various adaptations to control the composition and distribution of the microflora without threatening the existence of these beneficial microorganisms. On the other hand, microflora has profound influence on the development and functioning of the immune system. Therefore, microflora and the intestinal immune system should be viewed and studied as a single entity.

The past few decades have seen some major advances in understanding the complex host-microbiota relationships due to the development of new model systems and methods in both microbiology and immunology. As already mentioned, the development of new culture-independent microbiological methods that rely on PCR, sequencing and DNA microarray techniques was a break-through in understanding the microflora composition and features. Still, further advance of the culture methods, especially of the anaerobic bacterial species, would be helpful for the development of the tools for manipulation of the intestinal microbiota. Much has been learned about host-microbial interactions from the experiments with GF animals that are shown to be a very suitable model for detailed investigation of how microflora interact with the host cells and regulate the functions of various host organ systems. Furthermore, GF animals served as the basis for development of gnotobiotic animals that become the important tool for investigation of host-microbiota interactions. Gnotobiotic animals are former GF animals colonized with the single microbioal specie or a defined mixture of species (Gordon and Pesti 1971). Nowadays, almost all the animal strains can be bred in GF and gnotobiotic conditions allowing the studies of how different genetic alterations in the host genome influence the host-microbiota symbiosis. Experiments of monoassociation of GF mice with the single bacterial species can explain the role of these specific strains in the immune system development and functioning. However, in normal