Univerzita Karlova
Přírodovědecká fakulta

Studijní program: Biologie
Studijní obor: B-BI

Anna Pavličová

Imunomodulační vlastnosti mikrobiálních komponent
Immunomodulatory properties of microbial components

Bakalářská práce

Školitel:
MUDr. Miloslav Kverka Ph.D.

Praha, 2024

Poděkování

Chtěla bych v první řadě velmi poděkovat mému školiteli, MUDr. Miloslavu Kverkovi, Ph.D. za vstřícný a ochotný přístup při vedení této práce a za cenné rady a podněty k zamyšlení, které mi poskytl. Dále děkuji všem členům Laboratoře buněčné a molekulární imunologie za vytvoření přátelského a inspirativního pracovního prostředí a také všem, kteří mě při psaní této práce podporovali.

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, dne 30.4. 2024

Anna Pavličová

Abstract

The human body is inhabited by a complex composition of microorganisms. Particularly significant is the intestinal microbiota due to its profound effect on the development and function of the immune system. The ability of the host to defend against pathogens is kept by two important, interrelated components – the mucosal barrier and immune system. The barrier is composed of several layers (gut microbiota, mucus, epithelium, and immune cells) joined into one functional unit. Through its diverse array of structural components and metabolites, the gut microbiota interacts with the epithelium and mucosal immune system, confers to its induction, and modulates immune responses. Disruption of this interplay can contribute to pathogenesis of several diseases.

Key words – Microbiota, Mucosal immunity, Dysbiosis, Microbial components, Immunomodulation

Abstrakt

Lidské tělo je osídleno komplexní sestavou mikroorganismů. Zvláště významnou roli hraje střevní mikrobiota, která má zásadní vliv na vývoj a funkci imunitního systému. Obrana proti patogenům je zajištěna dvěma vzájemně propojenými složkami – střevní bariérou a imunitním systémem. Střevní bariéra se skládá z několika vrstev (střevní mikrobioty, hlenu, epiteliálních a imunitních buněk) spojených do jednoho funkčního celku. Prostřednictvím rozmanité škály strukturních komponent a metabolitů střevní mikrobiota interaguje s epitelem a slizničním imunitním systémem, přispívá k jeho indukci a moduluje imunitní odpovědi. Narušení těchto mechanismů může přispívat k patogenezi řady onemocnění.

Klíčová slova – Mikrobiota, Slizniční imunita, Dysbióza, Mikrobní složky, Regulace imunity

List of abbreviations

APC – Antigen presenting cell

AhR – Aryl hydrocarbon receptor

ATB – Antibiotics

CD – Crohn's disease

cTreg – Colonic regulatory T cells

DC – Dendritic cell

GALT – Gut associated lymphoid tissue

GF – Germ-free

GIT – Gastrointestinal tract

GPCR – G-protein coupled receptor

HDAC – Histone deacetylase

IBD – Inflammatory bowel disease

IEC – Intestinal epithelial cell

Ig - Immunoglobulin

IL – Interleukin

ILC – Innate lymphoid cells

IRF3 – IFN-regulated factor-3

LP – Lamina propria

LPS – Lipopolysaccharide

MAPK – Mitogen-activated protein kinase

(M)IS – (Mucosal) Immune system

NF-κB – Nuclear factor kappa-light-chain-enhancer of activated B cells

PAMP – Pathogen associated molecular pattern

PP – Peyer's patches

PSA – Polysaccharide A

PRR – Pattern recognition receptor

SAA – Serum amyloid A

SCFA – Short-chain fatty acid

SFB – Segmented filamentous bacteria

SPF – Specific pathogen free

Th – T helper cell

TJ – Tight junctions

TLR – Toll-like receptor

TNF – Tumor necrosis factor

UC – Ulcerative colitis

Table of contents

1 Introduction

The gut microbiota plays an important role in the development and regulation of the immune system. This involvement in host's immunity functioning emphasizes the importance of the microbiota in the development and mediation of a wide range of diseases. The aim of this thesis is to describe the main mechanisms, how interaction between microbiota and host's immune system prevents or promotes diseases, with particular emphasis on the colonization resistance, gut barrier function and immunomodulatory components of the microbial origin.

First part of the thesis is dedicated to the gut microbiota. It briefly describes its composition, its functions and the individual merits of different approaches of studying it. It also discusses numerous factors that influence the microbial composition in the different parts of the gastrointestinal tract, ranging from inner to environmental factors, such as genetic makeup, age, immune system capacity, mode of delivery and diet.

The next part focuses on description of relevant parts of the mucosal immune system. The ability of the host to defend against pathogens is kept by two important, interrelated components – the mucosal barrier and immune system. By interfering with ability of the pathogenic microbes to colonize the host and by stimulating the development of mucosal barriers and immune system reactivity, the commensal microorganisms protect the host from infection. This mechanism is referred to as "colonization resistance". The gut mucosa not only facilitates the absorption of nutrients, but also prevents potentially harmful substances from entering the organism or harmless environmental antigens from overwhelming the immune system. A malfunction of this barrier can disrupt the regulation of the immune system, leading to inflammation and various associated diseases. The individual factors involved in maintaining the homeostasis of the barrier (microbiota, mucous, epithelium and immune cells) and their interactions are described in this part and the consequences of breaching the barrier homeostasis are also mentioned.

The last part focuses on the interactions between the gut microbiota and the mucosal immune system, using well-documented examples. Firstly, it describes immunomodulatory molecules of microbial origin and subsequent signaling pathways involved in the activation of immune system. This chapter covers both well-known structural components, such as lipopolysaccharides or flagellins, as well as metabolites, such as short-chain fatty acids, indole and bile acid metabolites. The consequences include induction and maturation of immune cells and production of both anti- and pro-inflammatory cytokines. Secondly, it focuses on the role of microbes in inflammatory bowel disease (IBD), mainly addressing changes in microbial composition, sensing and translocation and their implications for development of IBD. It also briefly summarizes possible ways of microbiota-targeted therapy of IBD.

2 Microbiota

Microbiota is a complex composition of microorganisms inhabiting the human body, that consists of Archea, bacteria, fungi and viruses (Qin et al., 2010). The term microbiome was originally coined for a collection of microbial genes in a particular niche but is commonly used interchangeably with the term microbiota. The microorganisms inhabit all surfaces of the human body, including internal surfaces, with the largest microbiota of the human body residing in the gut (Sender, Fuchs, & Milo, 2016). The human gastrointestinal tract harbors estimated $10^{13\text{-}14}$ microorganisms (Ley, Peterson, & Gordon, 2006). Approximately 400 bacterial species can be found in the gut of a single individual, based on the findings of 16S rRNA analysis of fecal and mucosal samples (Eckburg et al. 2005). Concentration of microorganisms increases along the length of the GIT from 10^{2}-10^{3} in duodenum to 10^{11}-10^{12} organisms/gram of luminal content in caecum and colon. The most abundant component of the gut microbiota are bacteria, with the phyla *Firmicutes* and *Bacteroidetes* accounting for the vast majority, followed by *Actinobacteria* and *Proteobacteria* (Arumugam et al., 2011; Eckburg et al., 2005).

Gut microbiota plays an important role in immune system development, resistance against pathogens and regulation of immune responses as well as in nutrition, metabolism, and development of the individual. Because of the extent of metabolic activity and importance in metabolic interactions, the gut microbiota is sometimes referred to as a forgotten organ (O'Hara & Shanahan, 2006). Apart from this, the influence of the microbes on disease modulation is also coming to the forefront of scientific interest, as studies in recent years have shown that a significant number of diseases are closely linked to alterations in gut microbiota known as dysbiosis (see later). This presents a possibility of using modulation of microbiota to influence the response to treatment.

Based on the nature of interaction with the host, the microbes can be divided into three groups – the commensals, with neutral impact, the harmful pathogens and beneficial probiotics (Kverka & Tlaskalova-Hogenova, 2013). It is important to note, that this classification is not strictly given as certain species can have more than one ability, depending on the health state of a particular host. For instance, commensal microbiota regulates the maturation of host mucosal immune system, which creates an effective protective barrier, and they therefore act as probiotics. They produce numerous metabolites via carbohydrate fermentation and vitamin synthesis that can positively affect intestinal permeability and increase defense mechanisms of the epithelial layer (Shimada et al., 2013). Their other important role is competing with pathogens for the niche and resources and thus limiting their numbers. Commensals also produce substances to deter pathogens.

Pathogens, on the other hand, though only present in small quantities compared to commensals, can take advantage of a weakened organism. For example, they might infect individuals who are immunocompromised or have undergone antibiotic treatment, the latter example being particularly evident in *C. difficile* infections (Fordtran, 2006). Other species, such as non-pathogenic segmented

filamentous bacteria (SFB) are capable of producing toxins that breach the intestinal barrier. Their danger lies therefore not in pathogenicity but rather in their ability to allow other species to cross the barrier and infect the host (Stepankova et al., 2007). They must be recognized and eliminated by mucosal immune system, if that fails, it can lead to an abnormal immune response and development of tissue inflammation which further leads to tissue damage and disease development. The term 'pathobiont' was coined for microorganisms, that commonly live as commensals at body sites but can become pathogenic under specific conditions. Usually, their pathogenicity emerges after a disruption of the balance of the inner environment, which yields an opportunity for them to grow more than they do in healthy conditions (Mazmanian, Round, & Kasper, 2008). Furthermore, any commensal might be harmful if the immune system reacts inadequately to its presence, or if translocation through the mucosa is enhanced by, for example, immunodeficiency.

2.1 Composition vs. function

The Human Microbiome Project first elucidated the correlation between taxonomic and functional diversity in microbial communities. This research demonstrated that variability in microbial composition within an individual is rooted in its function, such as defense against pathogens, immune system stimulation and metabolic activities (Huttenhower et al., 2012b). To illustrate, in oral cavity *Streptococcus* species dominate and play a crucial role in preventing pathogen colonization (Aas & Dewhirst, 2005; Dewhirst et al., 2010). Conversely, in the gut *B. fragilis* is responsible for immune system maturation by priming T cells responses through its polysaccharide A (Mazmanian et al., 2005). These examples highlight the variety in microbial species distribution along the length of the GIT, but the microbiota differs in structure also axially and can thus be divided into two main groups, the luminal microbes and the species associated with mucosa (Eckburg et al., 2005). This division reflects the distinct roles and abilities of these two groups. Mucosa-associated species participate in interactions with the host immune system, whereas the luminal species' main role lies in digestion and metabolism (Wu et al., 2020).

Furthermore, metabolic functions performed by the microbiota at specific body sites are remarkably consistent across individuals even though their microbial composition differs (Huttenhower et al., 2012a). Therefore, while human population might not share a distinct core gut microbiome, it likely shares a core of microbial genes, particularly those that participate in metabolic pathways like carbohydrate and amino-acid metabolism (Turnbaugh, Hamady, et al., 2009). Interestingly, differences in gut microbial composition between individuals are greater, than between distinct sites in the gut of one individual (Eckburg et al., 2005). While rare species are highly personalized, most of the common species tend to occupy the same niches and are therefore functionally redundant (they perform the same metabolic actions). For example, gut microflora of lean and obese individuals differs markedly in the distribution ration of *Firmicutes* vs *Bacteroidetes*, but analysis of its function via metabolomics

showed highly similar metabolic pathways. Furthermore, minor species which are often unique for an individual actively contribute to the metabolic function of the microbiota (Ferrer et al., 2013).

There have been attempts to specify the microbial composition and describe distinct types of human gut microbiota, based on the ratio of predominant species. In 2011 a new perspective on the distribution of microbes was introduced, suggesting the stratification of human microflora into enterotypes, based on which an individual would tend to have one of the three described compositional types (Arumugam et al., 2011). Later studies however contradict such a stratification into distinct clusters and propose a rather continuous spectrum, where dominant taxa transition along a gradient, sometimes referred to as enterogradients (Bäckhed et al., 2012; Knights et al., 2014). Microbial composition is dependent on many factors that interact with each other, and the problematics is far too complex to be classified in such simple way.

2.2 Factors with influence on microbial composition

The human gut microbiota is variable, unique in each individual and influenced by many factors including the host's immune system, physiology, diet, genetics and environment (Turnbaugh et al., 2007). Though the microbial composition changes on some level during our whole life, by the influence of external and internal factors, the main composition stabilizes during the first three years of life and remains largely stable thereafter (Koenig et al., 2010). To some degree, the microbial composition is also hereditary, that being ensured by mother-to-child transfer of microbes during delivery (Dominguez-Bello et al., 2010) and exposure to similar diet, including the mother's milk (Gonzalez et al., 2011), and is demonstrated by the fact that family members share a common core microbiome (Turnbaugh, Hamady, et al., 2009). The great diversity in the intestinal microflora protects against instability and changes of its composition (Jeffery, Lynch, & O'Toole, 2016).

The influencing factors that shape intestinal microbiota could be looked at as two groups, depending on where the influence comes from, as discussed below. They could be either internal – to name some: host's inner environment, genetics, immune system capacity, age and health state. The outer influences would be environment, infections, mode of delivery, diet and medicine administration.

2.2.1 Inner factors

The very constitution of the host's body itself presents given conditions for distribution of microbes. For example, the anaerobic environment in the intestine, since presence of oxygen is a strong selective condition for bacterial growth (Albenberg et al., 2014). Another well explored condition is the acidity in the intestines, with pH values typical for each part of the GIT, ranging from highly acidic in the stomach (pH = 2.5), slightly acidic in the small intestine (pH = 6.6) to neutral (pH = 7.0) in the colon and thus gradually rising along the GIT (Evans et al., 1988). Yet another significant factor of the host

physiology is presence of glandular secretions – gastric acid and bile, that affect microbial distribution (Friedman et al., 2018).

Genetic makeup of the host proved to be important, as significant associations between microbial composition and genetic variation of the host were found in studies that compared data obtained from large numbers of sequenced human genomes and their associated microbiomes (Blekhman et al., 2015; Lopera-Maya et al., 2022). Another genome-wide associated study (GWAS; a method of screening entire genomes for connection with a particular disease) identified several genetic loci, that were closely linked to differences in gut microbiota composition. In particular variations in the vitamin D receptor-encoding gene proved to affect the microorganisms – upregulation in the receptor gene influenced individual taxa, for instance *Parabacteroides*, while complete loss of function substantially explained microbial diversity among individuals (J. Wang et al., 2016). The claim that microbial taxa are affected by genetic variations is further supported by the fact that monozygotic twins share more of common microbial species than twins dizygotic (Goodrich et al., 2014).

Age is another, not so well explored factor, as with age physiology and processes in the host change and clinical issues related to old age emerge. Gut permeability, disease predisposition and susceptibility to gut pathobionts increase with age, whereas immune strength, metabolism and gut motility are all known to decrease, as reviewed in Nagpal et al., 2018. One particular feature associated with decline in immune system capacity in old age is senescence-associated secretory phenotype (SASP), when senescent cells, especially fibroblasts develop altered secretory activities and produce pro-inflammatory cytokines (e. g. IL-6). That promotes low-grade inflammation which long-term causes senescence of immune cells (X. Li et al., 2023). The alpha diversity (species richness within a single host) is low in infants, increases during life and is again decreased in old age. The beta diversity (diversity among individuals) shows exactly opposite curve, with high values in both infancy and elderly age (Bartosch et al., 2004; Nagpal et al., 2018). On top of that, microbiota in the elderly seems to be less stable compared to younger individuals. Interestingly though, elderly individuals with higher alpha diversity show greater temporal stability of microbial composition (Jeffery et al., 2016). The above-mentioned characteristics of aging body contribute to the change of microbial diversity in the elderly, but it is not fully understood whether these changes are the cause or consequence of senescence (Odamaki et al., 2016).

2.2.2 Environmental factors

Delivery is the first major change in host-microbe interactions, when microorganisms quickly begin to inhabit niches in and on the human body. These first microorganisms are referred to as early colonizers and they influence metabolic and microbiological development, as well as IS maturation. The mode of delivery itself influences the nature of the acquired microbiota (Grönlund et al., 1999). Vaginally delivered newborns harbor microorganisms resembling the mother's vaginal microbiota, while

c-section born infants have microbial composition similar to the one found on skin (Dominguez-Bello et al., 2010). Infants born vaginally are more colonized with bifidobacteria and *Bacteroides*, while c-section born individuals tend to have less convenient microbial composition, as they lack in colonization with these bacteria and are more often colonized with *C. difficile* (Penders et al., 2006). The difference in composition between these two groups however decreases in the first year of life, though c-section born infants show greater overall heterogeneity (Bäckhed et al., 2015). Recently it has been proposed that colonization of human body by microbes begins *in utero*, before delivery, since microorganism have been found present in placenta, umbilical cord and amniotic fluid (Collado et al., 2016; Jiménez et al., 2005; Zhou et al., 2000). A critical re-analysis from last year however pointed out, that presence of distinct microbial species clusters accordingly to the type of delivery and therefore is likely to be caused by contamination. In the wake of these findings, existence of a placental microbiome in typical pregnancies with c-section term delivery was not confirmed, though *in utero* bacterial colonization is known to be associated with complicated pregnancies and pre-term delivery (Panzer et al., 2023).

Diet plays a significant role in microbiota development and its change over time. The colonization of an infant continues via breastfeeding or formula diet, from which the former is preferable in relation to its impact on microbial composition (Penders et al., 2006). Human milk is, apart from a source of varying health promoting and immunomodulating factors including antimicrobial compounds also a source of bacteria for the newborn and so directly influences its microbial composition (Hanson, 1961; Wirt et al., 1992). Estimated number of bacteria in a milliliter of human milk is 10^6 and the dominant species are *Staphylococcus, Streptococcus, Lactobacillus* and *Acinetobacter* (Boix-Amorós et al., 2016; Martín et al., 2003). Diet presents, together with physical contact with other individuals, the main source of microbial exposure in newborns. The infants gut microflora is derived from breastmilk microbiota from 25-30 % and about 10 % of the microbes are acquired from skin (Pannaraj et al., 2017). Nutrition both pre- and post-natal has far-reaching consequences and is one of the main factors driving the early-life programming. The nature of environment during early life can therefore predispose an individual for health risks in adult life, as the fetus undergoes epigenetic changes in response to environmental factors, like nutrition and microbial colonization, during early life stages. These changes then alter metabolism and immune system development (Nauta et al., 2013).

The infant microbiota develops in time and resembles that of an adult, after third year of life (Yatsunenko et al., 2012), but diet influences its composition throughout the whole life since it provides a necessary substrate for the microorganisms to feed upon. Changes in microbial composition can be induced via diet (Turnbaugh et al., 2009) and are detectable as early as one day after the change (David et al., 2014). Diet is acknowledged as the strongest regulating factor of gut microbiota (Faith et al. 2011).

Antibiotics administration significantly changes the composition of gut microflora and affects microbial diversity. This can be used therapeutically in treating some inflammatory diseases where certain bacterial species sustain the inflammation, for instance, ATB are used to treat same cases of IBD (Sartor, 2004). On the other hand, inconsiderate use of ATB can lead to elimination of beneficial commensal species, such as bifidobacteria and *Bacteroides* that prevent pathogen colonization, and can therefore result in higher susceptibility to infection, for example by *C. difficile* (Penders et al., 2006; Theriot et al., 2014). Changes in microbial composition in early life due to ATB treatment have also been linked to higher risk of developing some diseases, like neurodevelopmental disorders (Volkova et al., 2021). Beyond these, many additional factors, including prescription medication use, regular exercise or stress exposure also have an impact on the composition. Moreover, these factors all act together and influence each other. The environmental factors dominate in the significance of influence over the inner factors (Rothschild et al., 2018).

2.3 How to study the microbiota

Various methods are used to study the intestinal microbiota and its interactions with the host. In order to obtain meaningful results, issues related to experimental design, methodology and interpretation of results need to be addressed. Some of these are more general in their nature and include selecting a well-defined population cohort of sufficient size, determining whether the altered microbiota is a cause or a consequence of the disease, defining the normal and dysbiotic microbiota for a particular experiment, to name a few. Others are more related to the method used and include sample collection and storage, extraction of analytes, depth of analysis and selection of proper controls (Kverka & Tlaskalová-Hogenová, 2017).

Regardless of the chosen study approach, certain given limitations must be taken into consideration. For example, a non-negligible part of gut microbes – could be as much as 50 % (Eckburg et al., 2005), cannot be cultivated and so the historically used culture-dependent techniques could not reveal complete image of microbial composition. This was successfully resolved by using cultivation independent techniques, sometimes referred to as “omics”, that have arisen in the past two decades and broaden our view on microbial diversity. These include DNA-focused metagenomics, RNA-focused metatranscriptomics, 16S rRNA sequencing and single-cell sequencing. Another advantage of using these methods is fast evaluation of great number of samples. The point of metagenomics is to study the microbial composition, it involves sequencing and analysis of genetic material of the gut microbiota and allows for taxonomic description of the population. Whereas metabolomics characterizes the functional aspects of the microbiota because it identifies and quantifies the metabolites produced by the microorganisms and provides information about the functional capabilities of the present species.

A valuable study approach involves comparing the microbial composition of healthy and ill individuals. For instance, diabetic patients show a significant decrease in *Clostridia* (phylum of

Firmicutes) and increase in *Betaproteobacteria* (*Proteobacteria* phylum) compared to healthy controls (Larsen et al., 2010). Similarly, obesity is associated with changed ratio of *Firmicutes* vs *Bacteroidetes*, with remarkable shift towards the former, as compared to lean individuals that have almost six times more of *Bacteroidetes* (18,9 % to 3,2 % of total sequenced DNA) (Ferrer et al., 2013). Twin studies are an interesting source of information regarding microbiota composition in genetically equal individuals. The composition may be studied directly in humans or via animal models, often used are particularly rodents because of their known similarities to humans as well as relatively easy manipulation, keeping and condition control.

Germ-free (GF) models are often used to demonstrate the influence of microbes on immune, metabolic and physiological processes. They can be used to study the physiology in absence of microbiota or be colonized with diseased individual's microbiome to clarify the causality of altered microbiome and a disease. For instance, colonization of GF mice with microbiome from obese individuals lead to significant gain in weight, conforming the contribution of specific microbial composition to obesity, because of a prevalence of species with increased capacity for energy harvest from the diet (Turnbaugh et al., 2006). The IS in GF models is significantly under-developed, showing both the consequences of microbial influence on IS development, but also that an existence without microbes is possible, both reviewed in Tlaskalová-Hogenová et al., 2011. Gnotobiotic animals are such that are colonized with specific defined microbiota, one example being SPF (specific pathogen free) models, or contain no microorganisms in the case of GF models and these examples together represent *in vivo* models. The advantages of *in vitro* models, like cell cultures and culture systems are better definable conditions, usually lower cost and the generated samples are more readily analyzed, but their drawback is an inability to precisely imitate the complicated physiology of the GIT (Fritz et al., 2013).

For gut microbiota analysis, mainly stool samples are used, representing the microbial diversity of colon and moderately also that of small intestine (Yasuda et al., 2015). Disadvantage worth mentioning is that while these samples are easily collected, they represent mainly the composition of luminal microorganisms in the gut and do not include the variability of mucosa-associated species (Zoetendal et al., 2002). To evaluate adhesive species in the mucosa, mucosal biopsies must be secured, but their use is less frequent due to more complicated and possibly invasive collection (Sun et al., 2021). Technical manipulation with samples, their collection, storage, and processing can, and do influence the obtained data and precautions must be taken to minimize these effects. Changes in the microbiota after the sample collection can be introduced by incorrect manipulation with samples and lead either to decrease in the variability, when some of the species die due to incorrect thawing methods, oxygen exposure etc., or contamination with other species from outer environment (Henderson et al., 2013; Salter et al., 2014).

3 Mucosal immune system

Most pathogens enter the body via mucosal surfaces. To prevent infection, a significant functional part of the immune system (IS), referred to as the mucosal immune system, underlays these mucosal areas. The components of the IS associated with the mucosa – the lymph nodes and other lymphoid tissues and scattered immune cells, are collectively referred to as the mucosa-associated lymphoid tissue (MALT). This chapter focuses specifically on one part of the MALT, the gut associated lymphoid tissue (GALT). The significant role of intestinal mucosa lies within allowing nutrient absorption while preventing potentially harmful antigens of both dietary and microbial origin from being uncontrollably transported via the barrier. The mucosal IS has several mechanisms ensuring the homeostasis between the inner tissues and outer environment that collectively contribute to the intestinal barrier function. Dysfunction of the intestinal barrier leads to dysregulation of the immune system, and further to inflammation and variable illnesses may occur as a result.

3.1 Intestinal barrier

The intestinal mucosal IS possesses unique functional mechanisms, which reflect its specific features. Firstly, the gut epithelium is comprised of a single constitutive layer of intestinal epithelial cells (IECs), of a vast surface of approximately 200 m^2. In the small intestine, the apical membrane of the IECs is coated with villi, that contribute to the large surface which serves its purpose for nutrient absorption, but also interacts in all its volume with microbes. Therefore, the tolerance to dietary antigens as well as to commensal microorganisms must be maintained. This phenomenon is facilitated by an active mechanism of the immune system known as oral tolerance. However, the mucosal IS must still reliably distinguish occasional pathogens and elicit an appropriate response. Proximity of host cells to the luminal antigens ensures the activation of the adaptive part of the immune system via antigen sampling. There are other mechanisms, specific for the mucosal IS, such as lymphocyte homing, that are explained further bellow.

3.1.1 Frontline defensive layers

Gut barrier consists of multiple layers joined into one functional unit. Microbial layer could be accounted for as the first line of the mucosal IS defense. The commensal microbiota plays a significant role by occupying the intestinal niche and competing for resources with pathogens, thus preventing their dissemination. Furthermore, as part of its metabolic activities, the microbes produce metabolites that drive the IS development and promote immune surveillance against pathogens (see later). Moreover, with signaling molecules they produce, used for cell-cell communication known as quorum sensing, the commensals can influence both host's and other microbial cells. Signaling molecules of probiotic *Lactobacillus plantarum*, which alleviate the colonization by *Staphylococcus aureus* by interfering with its quorum sensing and promoting the secretion of antimicrobial peptides by host cells

serve as great example (Oliveira et al., 2023). Altogether, these mechanisms of commensal protection of the host against pathogens contribute to the phenomenon known as colonization resistance.

The next line of defense is a thick layer of mucus, secreted by goblet cells, that provides a physical protection. Mucous layer comprises of highly glycosylated proteins mucins, MUC2 being the major component. One thin transparent layer of mucus is found in the small intestine, whereas in the colon, two layers of mucus are present. The outer layer is loose and may contain microorganisms, while the inner is much denser, because its main role is preventing the microorganisms from direct contact with the epithelium (Atuma et al., 2001; Johansson et al., 2008). The mucus production is influenced by biochemical substances. These molecules can be either produced by host cells, like pro-inflammatory cytokines, which enhance MUC2 production (Iwashita et al., 2003), or of microbial origin, for instance LPS (see later). Secretory immunoglobulin A (sIgA) as well as antimicrobial proteins, like defensins, secreted by the underlying cells are present in the mucus layer and participate in keeping the microbiota at bay (reviewed in Linden et al., 2008). Interestingly, sIgA does not coat all microbes equally, and instead preferentially targets species with potential to induce inflammatory responses, such as colitogenic bacteria (Palm et al., 2014). The mucus layer therefore serves both as physical protection and for display of effector antimicrobial molecules.

3.1.2 Epithelial layer – cellular barrier integrity

Intestinal epithelial cells (IECs) have, apart from their role in nutrition, a significant role in immune responses in the gut and may be considered a part of intestinal innate immunity. Adaptive immune mechanisms are only involved after this layer of the barrier is breached. The function of IECs lies in interactions with the luminal microbiota as well as in production and transport of antimicrobial components into the gut lumen. The IECs respond to microbial stimuli and decide the nature of the response, based on recognizing commensals from pathogens. Primarily, they provide the segregation of microbes from the host, and maintain tolerance to harmless antigens, but can induce immune response. They are involved in the process of antigen uptake and transport known as antigen sampling.

There are multiple types of IECs with various properties which collectively contribute to the maintenance of the mucosal barrier. The most common have villous apical side, absorptive function and are called enterocytes. Goblet cells produce mucus, that is secreted into the gut lumen and creates a physical barrier. Paneth cells (PCs) have secretory function and their products, antimicrobial peptides (AMPs), e.g. defensins serve as innate immunity response to luminal microbiota. Secreted granules with AMPs are embedded within the mucus layer and their mechanisms of action is creating pores in the bacterial walls. PCs can directly sense microbial components and metabolites by their PRR receptors and react by upregulated secretion of AMPs (Ayabe et al., 2000). They also respond to cytokines, IL-17 is the major inducer of antimicrobial peptides production at epithelial barriers (Brabec et al., 2023). PCs are not present in the colon. Tuft cells serve as sentinels of the luminal

content and upon activation by microbes (and particularly helminths) secrete immunomodulatory molecules, such as IL-25, which leads to differentiation of goblet cells and enhanced mucus production (Nadjsombati et al., 2018). Therefore, both mucus and defensin secretions, though produced continuously, are enhanced upon bacterial stimuli.

M cells are involved in direct immune surveillance of the mucosa through the process of antigen sampling. They are predominantly found in the small intestine epithelium. They have short microvilli, referred to as microfolds (therefore “M cells”) and take up luminal contents via phagocytosis or pinocytosis, enabling them to take up whole microbes, as well as their components. By endocytic vesicles, the antigens then move through the cytosol of the M-cell and are exocytosed on its basolateral side – this process is called transcytosis. M cells primarily overlay the areas of PPs and LP lymphoid follicles and are in close proximity to DCs which take up the exocytosed antigen and migrate to lymphoid tissues to present it to adaptive immunity cells (Neutra & Kraehenbuhl, 1992). Some pathogens, including *Salmonella typhimurium* may exploit the M cell mediated transcytosis to enter the host's inner environment (Jones, Ghori, & Falkow, 1994).

Transcytosis is a way by which the antigens can pass the barrier transcellularly, as explained above, but the passage between cells is restricted. In the sake of providing the uncontrolled dietary and microbial antigens from passing through the epithelium, the IECs are held together near their apical sides by tight junctions (TJ) consisting of proteins like occludin and claudins. The TJs form a selectively permeable membrane, only allowing passage of small soluble molecules, ions and water. The disruption of TJs leads to increased paracellular permeability, luminal molecules can easily pass the barrier and continuously activate the IS which causes inflammation and tissue damage. This promotes the development of several intestinal and systemic diseases, for instance inflammatory bowel disease (IBD) (Clayburgh et al., 2004). The intestinal epithelium is differentiated into crypts, with stem cells at their base, from which all IECs originate and migrate from to apical side, as the cells on the top die due to apoptosis. Thus, the IECs are renewed, the cell turnover being approximately 4-5 days. An exception are the Paneth cells, which stay at the base of the crypts, because they have a protective role to the stem cells. Their lifespan is approximately two months (Yuan Liu & Chen, 2020). In the small intestine, but not in the colon, villi are present on the top of the crypts.

IECs express pattern recognition receptors (PRR), such as TLR (Toll-like receptors), NLR (NOD-like receptors) and RLRs (RIG-I-like receptors), which sense conserved microbial structures known as pathogen-associated molecular patterns (PAMPs) and play a crucial role in maintaining homeostasis by mediating immune responses to pathogens while also inducing hypo-responsiveness to commensal microbiota. Signaling via TLRs modulates the barrier function in IECs, since it promotes the integrity of the barrier by inducing tightening in the TJ proteins and enhanced IECs proliferation (Cario, Gerken, & Podolsky, 2004; Peterson & Artis, 2014). PRRs activation also triggers the induction of

inflammatory cytokines via signaling pathways, for instance NF-κB. These cytokines stimulate the maturation of cells involved in adaptive immunity and increase the production of defensins and sIgA. The key in deciding on the nature of response lies in the polarized expression of the TLRs. When apical-based receptors are activated by commensal luminal microbiota it promotes inhibition of NF-κB pathway. Conversely, the stimulation of the basolateral TLRs by microbial components indicates that the barrier was breached and leads to NF-κB activation (Peterson & Artis, 2014).

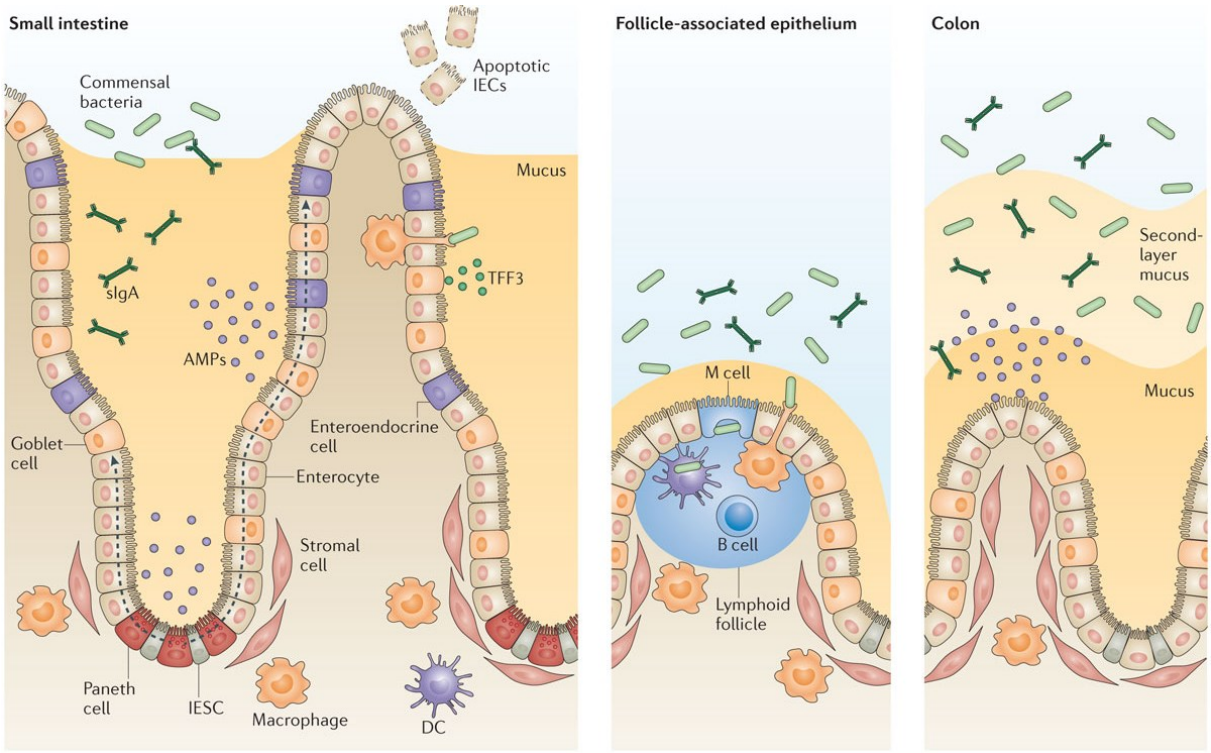

Figure 1: ***The IECs barrier in the small and large intestine*** *(Peterson & Artis, 2014).*

3.1.3 The immune professionals

The specialized IECs responsible for the antigen uptake and transport are commonly referred to as follicle associated epithelium (FAE), because they overlay the lymphoid follicles of GALT. The FAE is rich in M cells, whereas in the conventional epithelium absorptive enterocytes dominate. It is also more greatly infiltrated by B and T cells, DCs and macrophages (Neutra & Kraehenbuhl, 1992). These follicles, together with scattered lymphocytes, represent the immunological level of the barrier and they are placed in the lamina propria (LP). The LP is a connective tissue underlying the epithelium, which houses, apart from immunocompetent cells, also blood and lymphoid vessels and nerve endings.

This subchapter will briefly summarize the multiple types of lymphoid tissues of the GALT, associated immune cells and their roles in regulating the immune response in the gut. The GALT can be roughly divided into induction and effector sites. The organized tissue, consisting of various lymphoid follicles like Peyer's patches (PPs), colonic patches or isolated lymphoid follicles (ILFs) presents, together with mesenteric lymph nodes (MLNs) the induction sites, where naïve cells of adaptive immunity mature.

Effector part of the GALT is on the other hand diffused, constituted by scattered lymphocytes in the LP (A. M. I. Mowat, 2003). The organized lymphoid structures generally increase in abundance along the GIT in correlation with the increasing number of microorganisms present, an exception being PPs which can only be found in the small intestine.

PPs are clusters of lymphoid follicles that consist of areas of T cells and B cell follicles. They have a subepithelial dome (SED) structure rich in lymphocytes, that is close to the overlying epithelium with which they interact. Antigen presenting cells (APCs) – dendritic cells (DCs) and macrophages are localized in the SED of the PPs and are in contact with M cells. After the APCs retrieve the antigens from M cells, they migrate to the GALT structures where they present the antigen to naïve T and B lymphocytes resulting in their maturation. The process of antigen sampling by M cells therefore significantly contributes to the activation of adaptive immunity response since the antigens activate T cells and are a significant stimuli for the IgA production in plasma B cells (Rios et al., 2016). The antigen sampling is also crucial for the maturation of PPs, as they only start forming germinal centers (GCs) in response to foreign antigens taken up by M cells (Rios et al., 2016). GALT are primary sites for induction of commensal microbiota-specific responses by Th cells (Nakamura et al., 2020).

Another mechanism worth mentioning is lymphocyte homing, which involves the ability of effector lymphocytes to preferentially return to the same tissue where they were initially activated, in this case, the lamina propria. This mechanism ensures that antigen-specific cells primed in the GALT eventually find their way back to the mucosal tissues. Upon interaction with the antigen and maturing in the GALT, both effector T cells and IgA producing plasma cells (mature B cells) undergo a change in their chemokine receptors and adhesion molecules and therefore gain the ability to recirculate back into the LP. By bloodstream they then move back to the mucosa, which is ensured by a homing molecule α4β7 integrin and its ligand, mucosal addressin cell-adhesion molecule 1 (MAD-CAM1), expressed in the LP and PPs (Berlin et al., 1993). T cells are present in both LP and in the epithelial layer, while plasma cells locate preferentially in the LP.

While the effector cells maturation in PPs, followed by migration of these cells to LP, is responsible for local immunity in the gut, this mechanism does not provide the explanation of systemic priming and tolerance to intestinal antigens (A. M. I. Mowat, 2003). For systemic hyporesponsiveness to ingested antigens, known as oral tolerance, the effector cells must be primed in another significant lymphoid tissue associated with the gut, the MLNs (Worbs et al., 2006). Also, for successful induction of oral tolerance, the presentation of antigens by DCs is essential, and the cell-bound antigens reach the MLNs via afferent lymphatic vessels (Worbs et al., 2006). The hyporesponsiveness of the T cells to orally administered antigens is ensured by either their deletion (apoptosis of the antigen-specific cell) or anergy (unresponsiveness to the antigen), depending on the dose of the antigen (Macpherson &

Smith, 2006). Particularly Foxp3+ T regulatory cells, primed in the MLNs and with an imprinted homing capacity, play a vital role in mediating oral tolerance (Coombes et al., 2007).

Both the active immune response and tolerance are dependent on the constant luminal antigen sampling from the gut lumen. After the antigens are transcytosed by M cells, DCs take up the antigen and bring it to the GALT structures, where they present it to naïve T and B lymphocytes. The DCs are additionally also able to sample the luminal content on their own, they interact with TJ proteins and can stick out protrusions between the IECs (Rescigno et al., 2001). Another cell type with this ability are macrophages, specifically CX3CR1+ macrophages, that reside in the intestinal lamina propria and directly sample luminal bacteria by forming transepithelial dendrites (TEDs). These soluble antigens are then transferred via gap junctions to DCs (the CD103+ subset), which migrate to MLNs and present it to naïve T cells (Mazzini et al., 2014). An additional way by which may soluble antigens get to CD103+ DCs residing in the LP is by goblet cells-associated antigen passages (GAPs) (McDole et al., 2012). Furthermore, enterocytes can also process some dietary antigens and present them at their basolateral side to CD4+ T cells since they express MHC II molecules on their surface, which contributes to local tolerance (Mayer & Shlien, 1987).

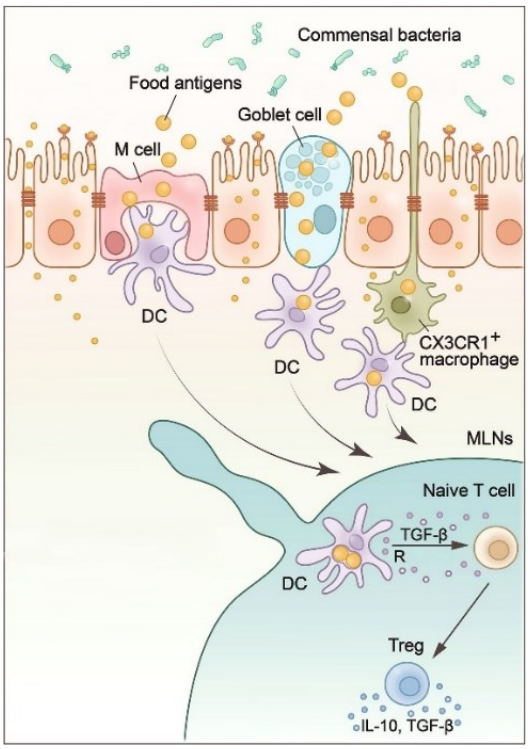

Figure 2: ***Distinct ways of antigen passage through the epithelium*** *(adopted from Xiong, Xu, Chen, & Ma, 2022 and modified).*

Various populations of T cells are included in the mucosal IS. They are either present in the organized lymphoid tissues where they eventually mature (after activation by an antigen) or scattered across the LP as well as embedded between the epithelial cells. The intraepithelial lymphocytes (IELs) are in direct contact with enterocytes and luminal antigens and are mainly comprised of T cells. The

intestinal T cells can also develop and mature in the thymus. Furthermore, it was recently shown, that some thymic T cells are primed by intestinal microbial antigens and develop into microbiota-specific T cells, this process being possible because of intestinal DCs that migrate to thymus with intestinal antigens (Zegarra-Ruiz et al., 2021). Upon specific stimuli, naïve T cells undergo differentiation into distinct phenotypes, like Th1, Th2, Th17 and Treg, that engage in a range of immune responses. Treg cells regulate immune responses and prevent excessive inflammation, thereby contributing to immune tolerance. T helper cells, on the other hand, are involved in active immune responses, they induce tissue inflammation, and each type focuses on different pathogens. Th1 cells are associated with intracellular pathogens defense, Th2 exert their action against helminth infections and are included in allergic responses and Th17 cells play role in mucosal defense and tissue repair (Shale, Schiering, & Powrie, 2013). They are also capable of producing cytokines, that stimulate other cells. For example, Th17 secrete IL-17 and IL-22, which promote IECs proliferation, IgA production by plasma cells and mucus and AMPs secretion and overall enhance the intestinal barrier function. Some T cells that reside in the LP function as effector memory cells.

Many immune cells in the LP, mainly CD4+ T cells, produce various cytokines that influence other cells and events that take place in the intestinal mucosa. Key anti-inflammatory factors include IL-10 and TGF-β, which limit the expansion of effector cells and in turn induce proliferation of Tregs, thereby promoting immune tolerance. Conversely, pro-inflammatory factors like interferon-γ (IFN-γ) and IL-4 have the opposite effect – they induce the differentiation of T helper cells and strengthen the intestinal barrier. It is important to bear in mind that these factors greatly influence each other, often acting as antagonists and so this description provides only a simplified summary of their actions.

B cells are primed in lymphoid follicles and patches. Particularly germinal centers (GCs) of PPs are potent sites of B cells differentiation, where they are exposed to antigens presented by DCs and also receive help from T cells. They are as well as T cells capable of preferential homing to intestinal mucosa, the receptor responsible for the homing specificity is primed by retinoic acid (RA) secreted by intestinal DCs (Mora et al., 2006). The mature stadium termed plasma cells, reside in the LP and produce sIgA, which is transferred into the gut lumen. There it serves the purpose of neutralizing bacteria by blocking their adhesion properties, so they can be cleared away with mucus. This non-inflammatory process of promoting homeostasis with intestinal microbiota is called immune exclusion (Stokes, Soothill, & Turner, 1975).

Antigen presenting properties of mononuclear phagocytes (DCs and macrophages) were mentioned, together with the special ability of DCs to imprint the lymphocyte homing phenotype. But these innate immunity cells have other roles in the intestinal mucosa too, for instance tissue repair and immune surveillance. Macrophage mediated phagocytosis of both dead or damaged self-cells and microbial cells is essential for intestinal homeostasis. They are also capable of producing cytokines, like

anti-inflammatory IL-10 and pro-inflammatory IL-1β that promote the function of intestinal Foxp3+ T reg and Th17 cells, respectively. Eosinophiles and mast cells are also present in the mucosal tissues where they serve regulatory roles in maintaining the gut barrier integrity (summarized in A. M. Mowat & Agace, 2014).The role of innate immunity cells in the maintenance of barrier function and stimulation of adaptive part of IS is therefore indispensable. Malfunction or absence of these mechanisms leads to serious health issues. For instance, impaired regulation of microbiota by intestinal DCs contributes to IBD pathogenesis (Bates & Diehl, 2014).

4 Interactions between mucosal immune system and microbiota

The composition and diversity of gut microbiota is a result of its coevolution with the host. Similarly, the mucosal immune system develops together with microbial colonization (Ley et al., 2006). Careful and precise balance must be maintained at the mucosal sites, which constitute the place of contact between the outer environment and the body itself, to recognize and eliminate pathogens but maintain tolerance to abundant harmless species. Since the immune system has developed together with colonization by microbes, its coexistence with the host has evolved into a mutually beneficial relationship. In this homeostatic arrangement the host provides a habitat and nutrients for the microorganisms and is in turn provided with defense against pathogens ensured by both the physical presence of the commensals and by their metabolites, which serve as potent immunomodulatory stimulants and on top of that as an additional source of nutrients. Inadequate reaction to microbes, both an exaggerated response to commensals and an insufficient one to pathogenic species, poses a danger for the host organism. Incorrect response in these cases is often a base for autoimmune diseases as it can promote inflammation and cause imbalance in the microbial composition.

An ideal state between the host and its intestinal microbiota is homeostasis – a state of equilibrium from which both sides benefit. As a result of inner or outer factors including genetic predisposition, immunodeficiency, or ATB administration, which were discussed above, the composition of the intestinal microflora can be changed. The depletion of beneficial species, accompanied with an increase in species whose effects are largely non-beneficial or even harmful (with pathogenic nature, lacking useful metabolites or even producing toxic metabolites) and an overall unwelcome change in the microbiota composition is called dysbiosis. It is associated with a change in distribution and an overall loss of microbial diversity. Dysbiosis has been linked to many diseases, including IBD, obesity and diabetes (Postler & Ghosh, 2017). It is important to mention that in many cases, it is not clear if the dysbiosis is a cause or a consequence of the disease, likely because often a self-perpetuating cycle emerges, that leads to chronic inflammation. Dysbiosis leads to compromised gut barrier function and exacerbated inflammatory response. Consequently, a self-sustaining loop is created wherein inflammation leads to further dysbiosis and gut barrier dysfunction.

Some of the significant interaction between mucosal IS and intestinal microbiota, such as maintaining tolerance to commensals or priming of the IS by microbial components were already mentioned above. This main chapter of the thesis focuses on describing mechanisms and molecules underlying these interactions. Firstly, it characterizes several well-known microbial structures and metabolites that are involved in these interactions and explains the recognition of the components together with consequent signaling pathways. It also mentions the ensuing effects, including induction and maturation of immune cells and production and distribution of cytokines. In its last part the chapter underscores the significance of the microbiota-host interactions in the context of human inflammatory diseases, particularly emphasizing the implications of alterations in microbe composition observed in conditions such as IBD. Role of microbes in IBD is discussed, aiming at comparison of distinct phenomenons connected to microbiota in health and in disease. The discussed topics are the features behind the recognition of (and distinguishing between) commensals and pathogens by the mucosal IS, communication between host and microbial cells and microbial translocation, this all with special focus on how it is altered in Crohn's disease and ulcerative colitis.

4.1 Key immunomodulatory structures and pathways

The molecules of microbial origin that stimulate the immune system or otherwise confer to the interactions between intestinal microbiota and the host could be divided into two groups for better comprehension. First, there are structural components, mainly constituents of the bacterial cell wall, and of flagella and other structures. The latter group comprises the metabolites produced by the gut microbes, either by processing food intake or by converting primary metabolites of the host into secondary substances. The following subchapter covers the most significant examples of these immunomodulatory molecules, together with the signaling pathways they initiate and receptors responsible for their recognition.

4.1.1 Structural components

Pattern recognition receptors (PRRs) that are expressed on innate immunity cells and on intestinal epithelial cells recognize pathogen associated molecular patterns (PAMPs) – highly conserved molecular structures that are typical for microorganisms. There are several classes of PRRs, transmembrane Toll-like receptors (TLRs) are expressed on the surface of cells and on intracellular membranes, whereas NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs) are intercellular and detect structures that infiltrate the cells (Wicherska-pawłowska et al., 2021). The microbial sensing by PRRs, particularly TLRs, triggers several signaling cascades within cells. Among these cascades, the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), MAPK (mitogen-activated protein kinase), and IRF (interferon regulatory factor) pathways are prominently involved. Activation of these pathways leads to the increased production of pro-inflammatory cytokines (tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), IL-1 and IL-6) which play

a crucial role in immune responses to microbial pathogens (Kawai and Akira 2011). The most prominent structural components, together with receptors that sense them and signaling pathways they initiate are summarized in Figure 3.

Lipopolysaccharide (LPS) is a structural component of the outer cell wall of gram-negative bacteria. It is also known as endotoxin, is toxic to human and sudden increase of LPS in serum can trigger septic shock. Therefore, the immune system must be able to recognize even small doses of LPS at the beginning of a bacterial infection. LPS structure consists of a hydrophobic anchor – lipid A, which serves as the actual endotoxin, a core oligosaccharide and a distal polysaccharide also known as O-antigen (Raetz & Whitfield, 2002). LPS has an important immunomodulatory role, its release induces pro-inflammatory cytokine production and recruitment of macrophages and neutrophils. It is usually released upon destruction of the bacterial wall, during cell lysis, but also in outer membrane vesicles (OMVs), which constitute a normal physiological activity of bacterial vesicle trafficking.

Signaling receptor of lipid A is TLR4 (Hoshino et al., 1999; Poltorak et al., 1998). Another protein, MD-2 is present in LPS signaling and interacts with TLR4 and confers its responsiveness to LPS (Shimazu et al., 1999). LPS binding protein (LBP) promotes the recognition of LPS by the LPS receptor complex, that consists of dimerized TLR4 and MD-2 by binding LPS to CD14 co-receptor (Hailman et al., 1994; Ryu et al., 2017). The activation of TLR4 first induces an early response, which is dependent on an adaptor molecule MyD88, and activates the NF-κB and MAPK signaling pathways. A later response uses TRIF (TIR-domain-containing adapter-inducing interferon-β) and TRAM (TRIF-related adapter molecule) adaptor molecules and leads to late activation of NF-κB and IRF3. MyD88, TRIF and TRAM are all adaptor molecules that are recruited by TLRs upon recognition of PAMPs (Kawai & Akira, 2011). Activation of the pathways leads to upregulation in cytokine and chemokine production. The activation of NF-κB leads to immediate production of pro-inflammatory cytokines, primarily IL-1, IL-6, IL-8 TNF and IFN-β (Medzhitov et al., 1997). In IECs, activation of NF-κB by LPS enhances mucus production (J. D. Li et al., 1998). These interactions stimulate the differentiation of CD4+ cells, mostly into Th1 and Th17. Furthermore, LPS stimulated B cells produce more IL-6 and TNF-α (Rahhal et al., 2007). Additionally, TLR2 also responds to LPS, but is less specific and recognizes other ligands, like membrane lipoproteins too (Lien et al., 1999).

Polysaccharide A (PSA) is primarily recognized by APCs via TLR2. It is produced for instance by *Bacteroides fragilis* and induces systemic response of T cells (Mazmanian et al., 2005). PSA promotes development of CD4+ cells to Foxp3+ Treg that secrete anti-inflammatory IL-10 in the gut. Role of PSA in Treg development was demonstrated with use of GF mice, in which Treg numbers in the intestine are lower, and their ability to produce IL-10 is significantly decreased, but both is restored upon colonization with *B. fragilis* (Round & Mazmanian, 2010). Importantly, PSA also suppresses

production of IL-17 and thus dampens intestinal inflammation (Mazmanian et al., 2008). It therefore contributes to the establishment of mucosal homeostasis by modulating immune responses in the gut.

Direct contact of bacteria with intestinal epithelial cells is usually considered a thread for the host's health, but there are some species, whose contact is beneficial and promotes immune system responses. Segmented filamentous bacteria (SFB) are a group of gram-positive bacterial species, with characteristic filamentous appearance, genomically related and functionally similar to clostridia (Prakash et al., 2011). SFB contribute to maturation of immune system mostly via direct adhesion to IECs, particularly to PP epithelium. Mutants unable to adhere to IECs do not induce intestinal Th17 development (Atarashi et al., 2015). The adhesion of SFB to IECs leads to serum amyloid A (SAA) production, which stimulates CD11c+ to produce IL-6 and IL-23, and other APCs to produce IL-1β, which all drive the differentiation of CD4+ to Th17 (Atarashi et al., 2015; Ivanov et al., 2009).

Colonization with SFB leads to maturation of CD4+ Th17 cells producing IL-17, development of lymphoid tissue and an enhanced IgA production (Klaasen et al., 1993; Lécuyer et al., 2014). SFB interactions provide further protection against pathogens but can also lead to autoimmunity in susceptible individuals. The enhancement of IgA and IL-17 production, together with IL-17 receptor signaling retrospectively regulates SFB colonization (Kumar et al., 2016). Additionally, IL-17 promotes the gut barrier integrity via TJ enhancement as it regulates occludin (Lee et al., 2015). Th17 cells responses induced by SFB improve intestinal permeability defects and protect against infections by pathogenic species such as *Toxoplasma gondii* or *Salmonella typhimurium* (Edelblum et al., 2017).

The exact process and bacterial components behind SFB influence on Th17 maturation are still under research. SFB antigenic proteins were found to be transferred to IECs by endocytic vesicles containing bacterial cell-wall associated proteins in the process of microbial adhesion-triggered endocytosis (MATE) (Ladinsky et al., 2019). Protein **flagellin** was proposed to be the inducer of SFB recognition by IECs, but it is not yet clear, if it is flagellin that participates in the MATE process (Y. Wang et al., 2019). Flagellin is a component of bacterial flagella and is recognized by TLR5, leading to pro-inflammatory response. Upon stimulation by flagellin, LP DCs expressing TLR5 induce differentiation of B cells into IgA producing plasma cells as well as Th17 development (Uematsu et al., 2008, 2006).

Another immunomodulatory molecules are **peptidoglycans (PGN)** present in both gram-positive (thick PGN layer) and gram-negative (thin layer) bacteria. As a constituent of the cell wall PGN is constantly shed and subsequently recognized by TLR2 and intercellular Nod1 and Nod2 receptors expressed on IECs and APCs (Wolfert et al., 2007). Stimulation of TLR2 triggers association with MyD88, whereas for the activation of Nod receptors an effector molecule RICK is needed. Other molecules participate in the process as well, form intricate complexes that in the end lead to activation of NF-κB and MAPK signaling pathways (Strober et al., 2006). That results in production of pro-inflammatory cytokines, activation of adaptive immunity (priming of T cells) and recruitment of

neutrophils to the site of infection (Fritz et al., 2007). Since PGN can vary in its structure, the response to it differs in strength. Some pathogenic species, including *Staphylococcus aureus* use the ability to alter PGN structure to escape recognition by receptors and invade the host (Wolfert et al., 2007).

Another important polysaccharide belonging to PAMPs is **β-glucan**, which is a component of fungal cell wall. It is recognized by Dectin-1, a transmembrane receptor belonging to yet another family of PRRs, the C-type lectin receptors (CLRs). Dectin-1 is expressed on phagocytes (macrophages, DCs and neutrophils) and plays a crucial role in control of fungal infection by triggering an anti-microbial response (Taylor et al., 2007). Upon activation by β-glucan, Dectin-1 recruits and activates Syk (Spleen Tyrosine Kinase), leading to NF-κB pathway activation. Naturally, there are many other not so well explored microbial components, which as well play important roles in interaction with the host. Such are: exopolysaccharides (EPS) produced by species of *Lactobacillus*, teichoic and lipoteichoic acid which are components of gram-positive bacteria, or staphylococcal enterotoxins (SEA). They are however less well known, and their thorough discussion is beyond the capacity of this thesis.

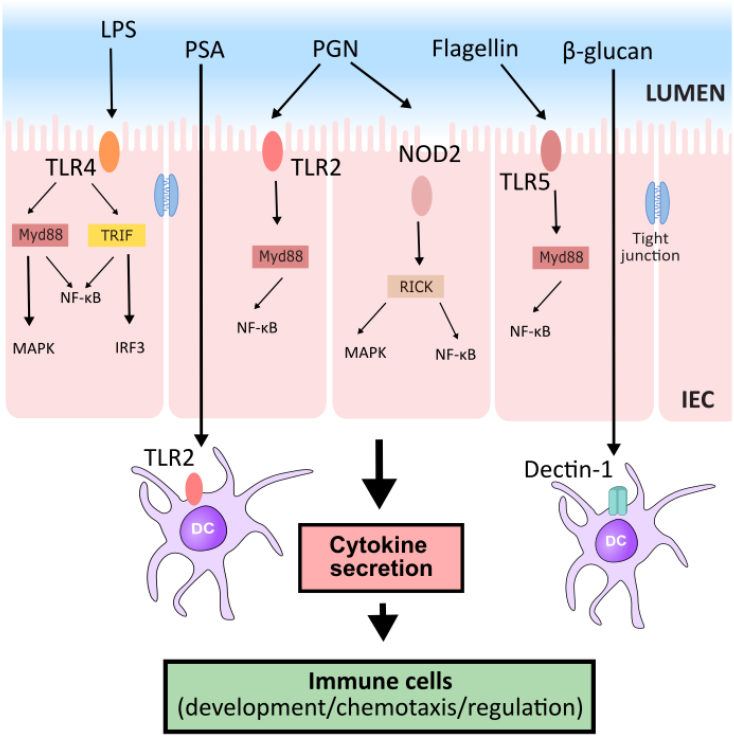

Figure 3: ***Schematic representation of intestinal epithelial cells (IECs) microbial ligands and associated receptors.*** *(Templates were kindly provided by my supervisor MUDr. Miloslav Kverka Ph.D).*

4.1.2 Metabolites

A significant and beneficial property of microbiota is its capacity to metabolize dietary compounds, like fiber or certain polysaccharides, that are indigestible for the host due to absence of necessary enzymes. Microbes use these compounds as a source of carbon and convert them into other substances, that the host can utilize. These metabolites, most significant of which are described in this subchapter, serve apart from nutrition for the host also as a source of energy for intestinal epithelial

cells and particularly as important immunomodulatory molecules. Regarding the immunomodulatory properties of gut microbiota metabolites, both tolerogenic and inflammatory effects were reported, and the immune state of the host plays a critical role in determining which of the mechanisms will be preferred. The schematic representation of these metabolites, along with accompanying receptors and signaling pathways is showed in Figure 4.

Short chain fatty acids (SCFAs), namely acetate, butyrate and propionate, are metabolic products of anaerobic microbial fermentation of non-digestible carbohydrates in the gut. The most abundant acetate, together with propionate are mainly produced by *Bacteroidetes*, while butyrate is a product of *Firmicutes* (Collins et al., 1994). These bacteria produce enzymes, that break down the fiber carbohydrates into sugars (glucose, xylulose) which are then transferred via phosphotransferase system into bacteria and fermented. Produced water-soluble SCFAs are then taken up by colonocytes by simple diffusion or soluble transporters in their apical membrane (Miyauchi et al., 2004). SCFAs are metabolized in the colonocytes, where they serve as a source of energy and regulate cell proliferation and differentiation as well as promote production of molecules important for the maintenance of barrier function like mucins (Finnie et al., 1995). SCFAs predominantly exert anti-inflammatory effects, influence both innate and adaptive immune responses as well as epithelial cells. Their effects are observed locally within the gut as well as systemically, underscoring their considerable importance.

Interestingly, SCFAs are often produced as a result of metabolic cascades, when metabolic activity of one species provides substrate for other bacteria and this is known as cross-feeding. For instance, lactate and succinate are often metabolized to butyrate and propionate by cross-feeding species (Belenguer et al., 2006). Another example is a butyrate producer *Eubacterium hallii*, that participates in vitamin B synthesis, which is then utilized by *Akkermansia* leading to production of propionate (Belzer et al., 2017). On the other hand, *E. hallii* generates butyrate upon utilizing lactate and acetate made by bifidobacteria (Duncan, Louis, & Flint, 2004).

SCFAs are sensed by G-protein coupled receptors (GPCRs), primarily GPR41 and GPR43 which are expressed on IECs and on immune cells (Brown et al., 2003). Stimulation of GPCRs with SCFAs has an anti-inflammatory effect as it leads to inhibition of NF-kB in these cells (Segain et al., 2000). This puts SCFAs in the perspective as possible therapeutic agents for some inflammatory diseases. To demonstrate, administration of butyrate by ulcerative colitis (UC) patients leads to downregulated expression of NF-κB in intestinal macrophages and has therefore anti-inflammatory effects (Lührs et al., 2002). Interestingly, some phagocytes can sense the gradient of the SCFAs. Activation of the receptor GPR43 in neutrophils enables their chemotaxis towards the source of SCFAs (Vinolo et al., 2011). MAPK is another signaling pathway that can be influenced by SCFAs. Its activation in IECs leads to production of cytokines and chemokines, thus preparing the cells for undelayed appropriate

reaction to infections (Kim et al., 2013). Further anti-inflammatory effect of SCFAs lies in inducing colonic Foxp3+ Treg population, that produces anti-inflammatory IL-10 and therefore dampens inflammatory response (Atarashi et al., 2013; Smith et al., 2013). In contrast, depending on the immunological setting, the SCFAs can also enhance effector T cells, particularly differentiation into Th1 and Th17 during infection (M. H. Kim et al., 2013).

Finally, SCFAs have the ability to regulate gene expression of the cells via inhibition of histone deacetylases (HDACs). HDACs are enzymes that remove acetyl groups from histone proteins. Under their influence, histones are more compact and transcriptionally inactive. When the HDACs are inhibited, histones become acetylated and form more relaxed euchromatin that is accessible for RNA polymerase and thus transcriptionally active. Through their HDAC inhibitory activity, SCFAs impact B-cells, promoting their differentiation into plasma cells and enhancing their production of both IgA and IgG. Administration of SCFAs by mice led to increased measures of IgA and IgG in both intestine and blood circulation, showing that SCFAs impact is both local and systemic (Kim et al., 2016). Particularly **butyrate** shows many capabilities in regulation and influencing the host cell processes. By inhibiting HDACs it epigenetically regulates the gene expression in cTreg (Waldecker et al., 2008) and suppresses inflammatory response to commensal microbiota in intestinal macrophages by rendering them hyporesponsive (Chang et al., 2014). Butyrate also promotes intestinal barrier integrity by influencing TJ. It upregulates transcription of TJ protein claudin (Wang et al., 2012) and induces AMPK (AMP-activated protein kinase) that confers to TJ proteins assembly (Peng et al., 2009). On top of that, butyrate also serves as a main source of energy for colonic IECs (Roediger, 1980).

Proteins and certain amino acids that escape utilization in the small intestine are other example of dietary materials metabolized by the gut microbiota in colon (Macfarlane et al., 1988). The proteins are first broken down into amino acids which then serve as substrates for various metabolic pathways within the microorganisms. **Indole and tryptophan metabolites** serve as great examples of significant secondary metabolites, from the immunological point of view, as they play important roles in modulating immune responses. They activate a ligand-activated transcription factor, the aryl hydrocarbon receptor (AhR) (Heath-Pagliuso et al., 1998). AhR is expressed on immune cells and its activation has impact on regulation of immune responses, for example by inducing and controlling the differentiation of Treg and Th17 populations (Quintana et al., 2008). AhR is a necessary transcriptional factor for the development of IL-22 producing innate lymphoid cells (ILC3) which protect integrity of intestinal mucosa by interleukin secretion (Lee et al., 2012). The activation of AhR also promotes differentiation of monocytes into DCs over macrophages (Goudot et al., 2017). These metabolites also interact with PXR (pregnane X receptor) and were shown to play an important role in TLR4 mediated control of barrier function (Venkatesh et al., 2014). For instance, activation of PXR in IECs leads to inhibition of NF-κB pathway and promotes intestinal barrier integrity (Sun et al., 2022).

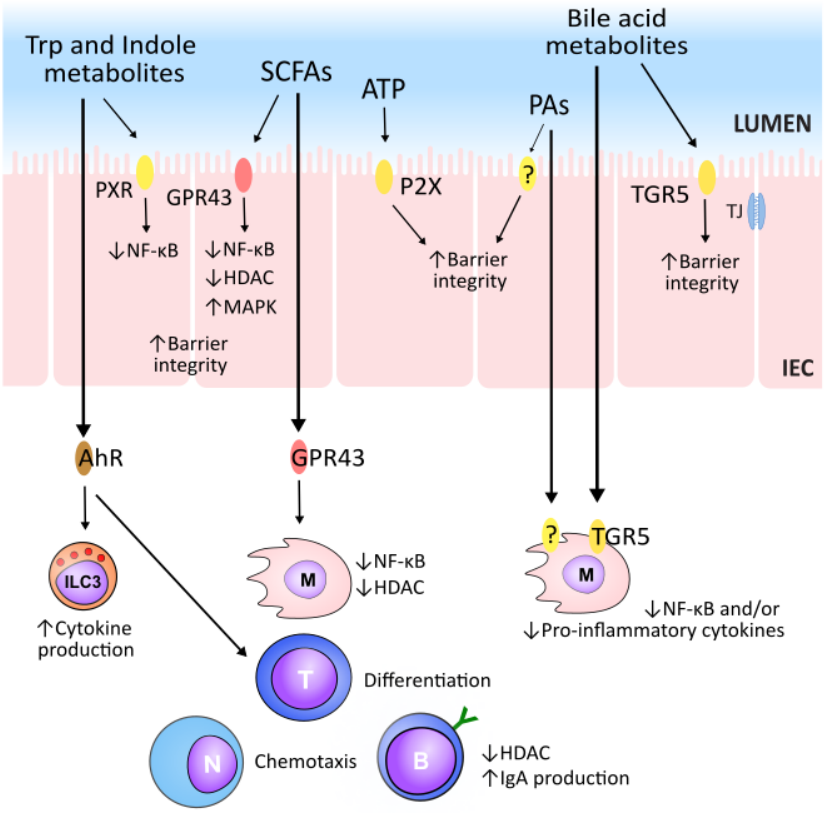

Figure 4: Schematic representation of microbial metabolites, sensing receptors and associated effects.
(Templates were kindly provided by my supervisor MUDr. Miloslav Kverka Ph.D).

Polyamines (PAs), including spermine, spermidine and putrescine are utilized from polyamine rich foods that contain amino acidic precursors, by gut microbes like *E. coli* and *Bacteroides*. Polyamines promote gut barrier integrity by enhancing adherent junction protein E-cadherin in IECs (Liu et al., 2009). They also take part in inhibiting expression of pro-inflammatory cytokines in monocytes and macrophages and therefore confer to intestinal homeostasis (Zhang et al., 1999). The precise molecular mechanisms behind the function of PAs, including receptors to sense them have not yet been discovered (Postler & Ghosh, 2017). Microbiota is furthermore capable of producing **bile acid metabolites**, like deoxycholic acid (DCA) and lithocholic acid (LCA), from bile acids, which interact with a GPCR receptor TGR5 among others. TGR5 is expressed on various immune and epithelial cells in the small and large intestine and the importance of DCA and LCA anti-inflammatory effects was shown on models deficient in the mentioned receptors, which ultimately led to worsened induced colitis (Biagioli et al., 2017; Renga et al., 2013). Signaling via TGR5 also inhibits production of pro-inflammatory cytokines, particularly TNF-α, by intestinal macrophages (Yoneno et al., 2013). These findings highlight the role of bile acid metabolites and their receptors in promoting immune tolerance.

The above-mentioned metabolites are produced by bacteria either from dietary components (SCFAs, indole derivatives and polyamines) or by modifying host metabolites (bile acid metabolites). However, certain metabolites, for instance **ATP**, can also be synthesized by bacteria *de novo*. Apart from its universal intracellular role as an energy source, ATP can also be secreted extracellularly and serve as a

signaling molecule. Extracellular ATP (eATP) is secreted not only by host cells, released mainly by immune cells and injured IECs, but also by the luminal microbes (Atarashi et al., 2008). It promotes the inter-species communication between the host and the microorganisms via interacting with purinergic receptors, such as P2X and P2Y expressed on immune and epithelial cells (Coutinho-Silva et al., 2005). Presence of eATP in gut lumen promotes Th17 cell differentiation (Atarashi et al., 2008).

4.2 Microbiota in IBD

Human inflammatory bowel diseases (IBD) are chronic immune-mediated inflammatory disorders that include two distinct conditions – Crohn's disease (CD) and ulcerative colitis (UC). They arise from a combination of genetic predispositions and environmental factors and are characterized with impaired barrier function, disrupted microbiota composition and chronic inflammation. While UC localization is restricted to the colon, CD can affect any part of the GIT and is typical in its patchy pattern and transmural occurrence of inflammation (Mulder et al., 2014). Common features accompanying IBD are bloody diarrhea and irregular crypt architecture in the case of UC and fistulas (pathological connections between two epithelial lined surfaces), skip lesions and granulomas (clusters of immune cells, also used as histological marker for CD) in CD. Abdominal pain and weight loss frequently accompany both diseases (Baumgart & Sandborn, 2007). IBD has been associated with intestinal dysbiosis (Postler & Ghosh, 2017). Microbes play a significant role in initiating, maintaining and determining the phenotype of IBD and several features connected to microbiota, like microbial sensing and translocation are altered in individuals with IBD. The involvement of microbiota in mediating inflammation in IBD is the topic of this last chapter.

4.2.1 Microbial sensing

Recognition of conserved microbial PAMPs via PRR receptors is necessary for proper functioning of the intestinal barrier, where the host cells are continuously exposed to microbial and dietary antigens. But to make matters more complicated, the IS must reliably distinguish between harmful and harmless species, so it does not trigger an excessive defense reaction against commensals but at the same time prevents infection by pathogens. The PRRs however do not inherently distinguish between commensal and pathogenic bacteria, as their components can often be similar for both types. Nevertheless, there are certain options how the immune cells can distinguish pathogens, stemming from the invasivity of the species and the structure of their components.

Regarding IECs, there are several mechanisms for recognition of pathogens. One is the polarized expression of TLRs as noted previously. Given the abundance of commensal bacteria in the gut lumen, the apical sides of IECs express fewer TLRs, leading to tolerance towards harmless species. Conversely, a greater number of receptors is expressed on the basolateral side of the IECs, and their activation indicates presence of a pathogen that infiltrated the epithelium or epithelial injury, which are both signals for launching an inflammatory response (Lebeer et al., 2010). Expression of TLRs is

upregulated in IBD patients compared to healthy controls. Interestingly, this is evident in both affected and non-inflamed tissue. While increased TLR4 expression is likely a consequence of impaired tolerance to LPS, increased TLR2 expression suggest activation of innate immunity cells by a not yet known bacterial component (Frolova et al., 2008). Another peculiar way the IECs react to distinct microbiota is by expressing the enzyme intestinal alkaline phosphatase which neutralizes (by dephosphorylation) the endotoxin lipid A present in LPS of gram-negative bacteria and reduces TLR4 signaling. As this enzyme is primarily expressed apically by IECs, if a pathogen breaches the barrier, its endotoxin is not neutralized and that prompts an inflammatory response (Bates et al., 2007). More generally, the components of pathogens and commensals that are ligands for TLRs, can also vary in structure, which allows for their effective recognition by the immune system (Lebeer et al., 2010).

Commensals also typically lack adhesive properties, whereas pathogens are able to penetrate the mucus layer in the intestine and come to direct contact with the IECs and therefore initiate the immune responses. Mutated pathogenic species that lack the ability to adhere to epithelium do not activate the IS as opposed to the adhesive strains (Atarashi et al., 2015). Adhesive ligands like fimbriae, confer to the virulence of pathogens. For instance, pathogenic strain of *E. coli* that expresses adhesive fimbriae activates TLR4 signaling pathway which induces local inflammation and attracts immune cells, while non-adhesive commensal *E. coli* strain does not (Fischer et al., 2006). In addition, commensals also actively confer to the maintenance of homeostasis, as they suppress epithelial responses by attenuating the NF-κB pathway (Neish et al., 2000).

Ideally, commensal bacteria restricted to the gut lumen are recognized as harmless and are therefore tolerated by the immune system. That is the case in healthy individuals. However, this essential detection and control of microbes is breached in individuals with IBD. In their case, there can be an aberrant immune response when the immune system mistakenly identifies harmless bacteria as pathogens. That triggers a chronic inflammatory response, which leads to the ongoing inflammation characteristic of IBD. Along with unfavorable environmental conditions, the genetic predispositions of the host take part in the onset of IBD. An excessive response to commensal bacteria is known to promote the development of IBD and host's genetic factors, especially defects in defense against microbes, play a significant role in the recognition and clearance of microbiota.

For instance, polymorphism in gene for the innate immunity PRR receptor Nod2 are associated with abrogated clearance of bacteria in IBD. Nod2 plays a critical role in the control of bacterial flora in the gut, via its detection and induction of bacteria-killing activity. It is expressed on APCs and IECs including Paneth cells and recognizes a microbial ligand muramyl dipeptide derived from peptidoglycan (Strober, Fuss, & Mannon, 2007). Increased exposure to bacteria, due to compromised bacterial killing results in increased stimulation of T cells and confers to dysbiosis. An increased

amount of commensal microorganisms is found in the gut of Nod2-deficient mice and their ability to resist pathogen species colonization is diminished (Petnicki-Ocwieja et al. 2009).

4.2.2 Microbial translocation

In healthy state, excessive bacterial translocation is effectively prevented by intestinal barrier function. In such case, the innate and adaptive immune system encounters the microbial antigens, and occasionally also whole viable microbes, in a strictly controlled manner as described above. The antigens/microbes are engulfed by APCs or eliminated by macrophages residing in the LP. On the contrary, uncontrolled translocation of microbes and their components, i. e. passage through the epithelium and infiltrating otherwise sterile blood and tissues is a common sign of IBD. This increased exposure to bacterial antigens overwhelms immune tolerance and leads to chronic inflammation.

Intestinal permeability and therefore bacterial translocation are significantly increased in IBD patients (Benjamin et al., 2008). The intestinal barrier function in IBD can be compromised due to many interrelated factors, ranging from genetic defects in immunocompetent mechanisms of the host to gut microbiota dysbiosis. Translocation in IBD is for instance associated with increased bacterial adherence which can be caused by numerous defects, such as deficiency in the production of defensins and mucus (Kocsis et al., 2008). NF-κB regulates the expression of antimicrobial defensins in IECs via chemokines and blocking of this pathway results in increased bacterial translocation (Nenci et al., 2007). The translocated microbes and their components serve as ligands for TLRs and stimulate immune responses that promote intestinal inflammation in IBD. Furthermore, TLR4 deficient mice show increased bacterial translocation, due to impaired neutrophil recruitment to LP followed by decreased clearance of the bacteria (Fukata et al., 2005).

Translocation of fragments of bacterial DNA into blood is a risk factor that can lead to relapse of CD, as the bacterial antigens stimulate systemic induction of pro-inflammatory cytokines that exacerbate the intestinal inflammation (Gutiérrez et al., 2016). Additionally, bacterial translocation may contribute to systemic inflammation and complications outside the gut, including autoimmune reactions and extra-intestinal manifestations associated with IBD. It is also a significant problem since it can cause sepsis after surgical treatment of IBD. *E. coli* has been identified as the most prevalent species part-taking in this phenomenon (O'Boyle et al., 1998). Microbiota itself can promote the translocation in susceptible conditions, as pathogens with the ability to translocate damage the barrier, and therefore allow other species, even commensals to translocate. On the other hand, microbial components also promote restitution of the epithelium after damage, which prevents bacterial translocation.

4.2.3 Associated species and potential treatment

Localization of IBD preferentially in intestinal segments with highest microbial concentrations implicates the role of microbes in pathogenesis of IBD. Moreover, IBD interestingly does not occur in

GF animals, in contrast to their colonized relatives (Sellon et al., 1998). The composition of microbiota and its function are altered in IBD and although neither CD nor UC are characterized by a uniform altered microbiota, some similarities that distinguish IBD patients from healthy individuals can be drawn. IBD patients contain abnormal microbiota characterized both by depletion in commensal species and higher numbers of potential pathogens.

Commensal bacteria, mainly *Bacteroidetes* and *Firmicutes* are decreased in number in IBD. That ultimately leads to decrease in protective commensal species and species that produce SCFAs and enhance mucus production, such as *Faecalibacterium prausnitzii* and therefore loss of anti-inflammatory effects (Frank et al., 2007). Conversely, *Enterobacteriaceae*, including among others *E. coli* are significantly increased. IBD is associated with elevated number of mucosa-associated adhesive species, for instance, *B. fragilis* mucosal biofilm is recognized as a prominent feature of IBD (Swidsinski et al., 2005). Furthermore, adherent strain of *E. coli* that is able of adhesion and infiltration of IECs is frequently found in patients with CD. It infiltrates and replicates in intestinal macrophages. That is partly responsible for formation of granulomas and an exaggerated production of pro-inflammatory cytokine TNF-α which supports the chronic inflammatory response in CD (Barnich & Darfeuille-Michaud, 2007).

Other bacteria, *Listeria* and *Streptococcus*, together with *E. coli* frequently invade the ulcers and fistulae in CD (Ying Liu et al., 1995). *Mycobacterium avium paratuberculosis* was suggested as a unifying species causing the IBD in genetically susceptible patients some forty years ago. However, following research has shown that while it plays a role in the disease development in some patients, it is not a causative effect in most cases of CD (Eckburg & Relman, 2007). Similarly, none of the so far examined bacterial species have been assigned as a sole cause of the IBD onset and so the most likely explanation for IBD occurrence remains the combination of genetic susceptibility and environmental factors, to which belongs the composition of intestinal microbiota.

The role of microbes in promoting the inflammatory responses and causing a relapse in IBD lead to treating IBD, especially in patients after surgical treatment of the same, with antibiotics. ATB can either selectively eliminate pathogenic species or globally decrease luminal concentration, which both relieve the seriousness of IBD. Additionally, administration of probiotics can help to restore beneficial species such as *Lactobacillus* and *Bifidobacterium*. Both administration of ATB and probiotics show improvement in IBD treatment and prevention of disease relapse (Sartor, 2004). Another promising approach firstly introduced for treating recurrent *C. difficile* infection and lately also for IBD treatment, is the fecal microbiota transplantation (FMT). This method involves transplantation of fecal matter from a healthy donor to patient and restoring healthy composition of intestinal microbiota (Nanki et al., 2018). Microbiota-targeted therapy of IBD is therefore a potent clinical approach that can be used together with traditional anti-inflammatory and immunosuppressive therapies of IBD.

5 Conclusion

The intricate interplay between the human gut microbiota and the host's immune system is a dynamic and bidirectional process. The complex microbial community, inhabiting the human gut yields many benefits for the host, facilitating nutrient metabolism and protecting against pathogen invasion through mechanisms like colonization resistance. Its composition is shaped by numerous factors, including diet, medications, genetic predispositions, and the capacity of the immune system. Conversely, the mucosal immune system actively interacts with the microbes, samples microbial antigens, distinguishes between commensals and pathogens, and directly shapes the composition of the intestinal microbiota. Immune cells in the gut mucosa play a vital role in maintaining homeostasis at the interface between the microorganisms and host tissues.

Central to the host-microbiota interactions are the immunomodulatory molecules produced by the gut microbes. They come in form of structural components such as lipopolysaccharides, flagellins and peptidoglycans, and metabolites, for instance short chain fatty acids. Recognition of these molecules by receptors expressed on immune and epithelial cells leads to activation of signaling pathways and subsequent production of cytokines and induction of immune cells. The molecules regulate the permeability of the intestinal barrier, stimulate immune responses, and contribute to the development of the immune system and play therefore a crucial role in maintaining the intestinal homeostasis.

Essentially, the interaction between the gut microbiota and the immune system represents a delicate equilibrium, where each entity continuously influences the other. If the homeostasis is breached it influences all the participants, as it leads to aberrant immune responses and disruption in the microbial composition, termed dysbiosis. Dysbiosis has been linked to numerous diseases, one particular being inflammatory bowel disease (IBD) which is on a global rise, yet its pathogenesis is still not fully understood. Microbial sensing is altered in IBD, translocation of live bacteria and their antigens is enhanced due to impaired barrier function and this all leads to an aberrant immune response and sustaining of chronic inflammation. IBD is the result of genetic predispositions combined with environmental factors, where microbiota plays a significant role. Treatment with antibiotics and probiotics as well as transplantation of fecal microbiota, are useful approaches in IBD therapy, but for it to work properly, we must be able to understand the intricate interactions between the intestinal microbes and the IS.

This thesis contributes to the understanding of interactions between the immune system and the colonizers that inhabit the body and highlights the importance of further research in this field. For me personally, working on this thesis brought a useful revision of the topics of mucosal immunity, while learning about microbiota and its interactions with the said immune system confirmed my interest in studying these mechanisms. In my future studies I would like to continue in investigating the influence of intestinal microflora in mediating immune responses, particularly its role in IBD.

6 References

Aas, J. A., Paster, B. J., Stokes, L. N., Olsen, I., & Dewhirst, F. E. (2005). Defining the normal bacterial flora of the oral cavity. *Journal of Clinical Microbiology*, *43*(11). https://doi.org/10.1128/JCM.43.11.5721-5732.2005

Albenberg, L., Esipova, T. V., Judge, C. P., Bittinger, K., Chen, J., Laughlin, A., … Wu, G. D. (2014). Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology*, *147*(5). https://doi.org/10.1053/j.gastro.2014.07.020

Arumugam, M., Raes, J., Pelletier, E., Paslier, D. Le, Yamada, T., Mende, D. R., … Zeller, G. (2011). Enterotypes of the human gut microbiome. *Nature*, *473*(7346). https://doi.org/10.1038/nature09944

Atarashi, K., Nishimura, J., Shima, T., Umesaki, Y., Yamamoto, M., Onoue, M., … Takeda, K. (2008). ATP drives lamina propria TH17 cell differentiation. *Nature*, *455*(7214). https://doi.org/10.1038/nature07240

Atarashi, K., Tanoue, T., Ando, M., Kamada, N., Nagano, Y., Narushima, S., … Honda, K. (2015). Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells. *Cell*, *163*(2). https://doi.org/10.1016/j.cell.2015.08.058

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., … Honda, K. (2013). Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*, *500*(7461). https://doi.org/10.1038/nature12331

Atuma, C., Strugala, V., Allen, A., & Holm, L. (2001). The adherent gastrointestinal mucus gel layer: Thickness and physical state in vivo. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *280*(5 43-5). https://doi.org/10.1152/ajpgi.2001.280.5.g922

Ayabe, T., Satchell, D. P., Wilson, C. L., Parks, W. C., Selsted, M. E., & Ouellette, A. J. (2000). Secretion of microbicidal α-defensins by intestinal Paneth cells in response to bacteria. *Nature Immunology*, *1*(2). https://doi.org/10.1038/77783

Bäckhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., … Finlay, B. B. (2012). Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. *Cell Host and Microbe*, Vol. 12. https://doi.org/10.1016/j.chom.2012.10.012

Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., … Jun, W. (2015). Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host and Microbe*, *17*(5). https://doi.org/10.1016/j.chom.2015.04.004

Barnich, N., & Darfeuille-Michaud, A. (2007). Adherent-invasive Escherichia coli and Crohn's disease. *Current Opinion in Gastroenterology*, Vol. 23. https://doi.org/10.1097/MOG.0b013e3280105a38

Bartosch, S., Fite, A., Macfarlane, G. T., & McMurdo, M. E. T. (2004). Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Applied and Environmental Microbiology*, *70*(6). https://doi.org/10.1128/AEM.70.6.3575-3581.2004

Bates, J., & Diehl, L. (2014). Dendritic cells in IBD pathogenesis: An area of therapeutic opportunity? *Journal of Pathology*, Vol. 232. https://doi.org/10.1002/path.4277

Bates, J. M., Akerlund, J., Mittge, E., & Guillemin, K. (2007). Intestinal Alkaline Phosphatase Detoxifies Lipopolysaccharide and Prevents Inflammation in Zebrafish in Response to the Gut Microbiota. *Cell Host and Microbe*, *2*(6). https://doi.org/10.1016/j.chom.2007.10.010

Baumgart, D. C., & Sandborn, W. J. (2007). Inflammatory bowel disease: clinical aspects and established and evolving therapies. *Lancet*, Vol. 369. https://doi.org/10.1016/S0140-6736(07)60751-X

Belenguer, A., Duncan, S. H., Calder, A. G., Holtrop, G., Louis, P., Lobley, G. E., & Flint, H. J. (2006). Two routes of metabolic cross-feeding between Bifidobacterium adolescentis and butyrate-producing anaerobes from the human gut. *Applied and Environmental Microbiology*, *72*(5). https://doi.org/10.1128/AEM.72.5.3593-3599.2006

Belzer, C., Chia, L. W., Aalvink, S., Chamlagain, B., Piironen, V., Knol, J., & de Vos, W. M. (2017). Microbial metabolic networks at the mucus layer lead to diet-independent butyrate and vitamin B12 production by intestinal symbionts. *MBio*, *8*(5). https://doi.org/10.1128/mBio.00770-17

Benjamin, J., Makharia, G. K., Ahuja, V., Kalaivani, M., & Joshi, Y. K. (2008). Intestinal permeability and its association with the patient and disease characteristics in Crohn's disease. *World Journal of Gastroenterology*, *14*(9). https://doi.org/10.3748/wjg.14.1399

Berlin, C., Berg, E. L., Briskin, M. J., Andrew, D. P., Kilshaw, P. J., Holzmann, B., … Butcher, E. C. (1993). α4β7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell*, *74*(1). https://doi.org/10.1016/0092-8674(93)90305-A

Biagioli, M., Carino, A., Cipriani, S., Francisci, D., Marchianò, S., Scarpelli, P., … Fiorucci, S. (2017). The Bile Acid Receptor GPBAR1 Regulates the M1/M2 Phenotype of Intestinal Macrophages and Activation of GPBAR1 Rescues Mice from Murine Colitis. *The Journal of Immunology*, *199*(2). https://doi.org/10.4049/jimmunol.1700183

Blekhman, R., Goodrich, J. K., Huang, K., Sun, Q., Bukowski, R., … Clark, A. G. (2015). Host genetic variation impacts microbiome composition across human body sites. *Genome Biology*, *16*(1). https://doi.org/10.1186/s13059-015-0759-1

Boix-Amorós, A., Collado, M. C., & Mira, A. (2016). Relationship between milk microbiota, bacterial load, macronutrients, and human cells during lactation. *Frontiers in Microbiology*, *7*(APR). https://doi.org/10.3389/fmicb.2016.00492

Brabec, T., Vobořil, M., Schierová, D., Valter, E., Šplíchalová, I., Dobeš, J., … Filipp, D. (2023). IL-17-driven induction of Paneth cell antimicrobial functions protects the host from microbiota dysbiosis and inflammation in the ileum. *Mucosal Immunology*, *16*(4). https://doi.org/10.1016/j.mucimm.2023.01.005

Brown, A. J., Goldsworthy, S. M., Barnes, A. A., Eilert, M. M., Tcheang, L., Daniels, D., … Dowell, S. J. (2003). The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *Journal of Biological Chemistry*, *278*(13). https://doi.org/10.1074/jbc.M211609200

Cario, E., Gerken, G., & Podolsky, D. K. (2004). Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C. *Gastroenterology*, *127*(1). https://doi.org/10.1053/j.gastro.2004.04.015

Chang, P. V., Hao, L., Offermanns, S., & Medzhitov, R. (2014). The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(6). https://doi.org/10.1073/pnas.1322269111

Clayburgh, D. R., Shen, L., & Turner, J. R. (2004). A porous defense: The leaky epithelial barrier in intestinal disease. *Laboratory Investigation*, Vol. 84. https://doi.org/10.1038/labinvest.3700050

Collado, M. C., Rautava, S., Aakko, J., Isolauri, E., & Salminen, S. (2016). Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Scientific Reports*, *6*. https://doi.org/10.1038/srep23129

Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., Fernandez-Garayzabal, J., Garcia, P., … Farrow, J. A. E. (1994). The phylogeny of the genus Clostridium: Proposal of five new genera and eleven new species combinations. *International Journal of Systematic Bacteriology*, *44*(4). https://doi.org/10.1099/00207713-44-4-812

Coombes, J. L., Siddiqui, K. R. R., Arancibia-Cárcamo, C. V., Hall, J., Sun, C. M., Belkaid, Y., & Powrie, F. (2007). A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-β -and retinoic acid-dependent mechanism. *Journal of Experimental Medicine*, *204*(8). https://doi.org/10.1084/jem.20070590

Coutinho-Silva, R., Stahl, L., Cheung, K. K., De Campos, N. E., De Oliveira Souza, C., Ojeius, D. M., & Burnstock, G. (2005). P2X and P2Y purinergic receptors on human intestinal epithelial carcinoma cells: Effects of extracellular nucleotides on apoptosis and cell proliferation. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *288*(5 51-5). https://doi.org/10.1152/ajpgi.00211.2004

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., … Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484). https://doi.org/10.1038/nature12820

Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C. R., Yu, W. H., … Wade, W. G. (2010). The human oral microbiome. *Journal of Bacteriology*, *192*(19). https://doi.org/10.1128/JB.00542-10

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(26). https://doi.org/10.1073/pnas.1002601107

Duncan, S. H., Louis, P., & Flint, H. J. (2004). Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Applied and Environmental Microbiology*, *70*(10). https://doi.org/10.1128/AEM.70.10.5810-5817.2004

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., … Relman, D. A. (2005). Microbiology: Diversity of the human intestinal microbial flora. *Science*, *308*(5728). https://doi.org/10.1126/science.1110591

Eckburg, P. B., & Relman, D. A. (2007). The role of microbes in Crohn's disease. *Clinical Infectious Diseases*, Vol. 44. https://doi.org/10.1086/510385

Edelblum, K. L., Sharon, G., Singh, G., Odenwald, M. A., Sailer, A., Cao, S., … Turner, J. R. (2017). The Microbiome Activates CD4 T-cell–mediated Immunity to Compensate for Increased Intestinal Permeability. *CMGH*, *4*(2). https://doi.org/10.1016/j.jcmgh.2017.06.001

Evans, D. F., Pye, G., Bramley, R., Clark, A. G., Dyson, T. J., & Hardcastle, J. D. (1988). Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut*, *29*(8). https://doi.org/10.1136/gut.29.8.1035

Faith, J. J., McNulty, N. P., Rey, F. E., & Gordon, J. I. (2011). Predicting a human gut microbiota's response to diet in gnotobiotic mice. *Science*, *333*(6038). https://doi.org/10.1126/science.1206025

Ferrer, M., Ruiz, A., Lanza, F., Haange, S. B., Oberbach, A., Till, H., … Suarez, A. (2013). Microbiota from the distal guts of lean and obese adolescents exhibit partial functional redundancy besides clear differences in community structure. *Environmental Microbiology*, *15*(1). https://doi.org/10.1111/j.1462-2920.2012.02845.x

Finnie, I. A., Dwarakanath, A. D., Taylor, B. A., & Rhodes, J. M. (1995). Colonic mucin synthesis is increased by sodium butyrate. *Gut*, *36*(1). https://doi.org/10.1136/gut.36.1.93

Fischer, H., Yamamoto, M., Akira, S., Beutler, B., & Svanborg, C. (2006). Mechanism of pathogen-specific TLR4 activation in the mucosa: Fimbriae, recognition receptors and adaptor protein selection. *European Journal of Immunology*, *36*(2). https://doi.org/10.1002/eji.200535149

Fordtran, J. S. (2006). Colitis Due to Clostridium Difficile Toxins: Underdiagnosed, Highly Virulent, and Nosocomial . *Baylor University Medical Center Proceedings*, *19*(1). https://doi.org/10.1080/08998280.2006.11928114

Frank, D. N., St. Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N., & Pace, N. R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(34). https://doi.org/10.1073/pnas.0706625104

Friedman, E. S., Bittinger, K., Esipova, T. V., Hou, L., Chau, L., Jiang, J., … Wu, G. D. (2018). Microbes vs. chemistry in the origin of the anaerobic gut lumen. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(16). https://doi.org/10.1073/pnas.1718635115

Fritz, J. H., Le Bourhis, L., Sellge, G., Magalhaes, J. G., Fsihi, H., Kufer, T. A., … Philpott, D. J. (2007). Nod1-Mediated Innate Immune Recognition of Peptidoglycan Contributes to the Onset of Adaptive Immunity. *Immunity*, *26*(4). https://doi.org/10.1016/j.immuni.2007.03.009

Fritz, J. V., Desai, M. S., Shah, P., Schneider, J. G., & Wilmes, P. (2013). From meta-omics to causality: Experimental models for human microbiome research. *Microbiome*, Vol. 1. https://doi.org/10.1186/2049-2618-1-14

Frolova, L., Drastich, P., Rossmann, P., Klimesova, K., & Tlaskalova-Hogenova, H. (2008). Expression of Toll-like receptor 2 (TLR2), TLR4, and CD14 in biopsy samples of patients with inflammatory bowel diseases: Upregulated expression of TLR2 in terminal ileum of patients with ulcerative colitis. *Journal of Histochemistry and Cytochemistry*, *56*(3). https://doi.org/10.1369/jhc.7A7303.2007

Fukata, M., Michelsen, K. S., Eri, R., Thomas, L. S., Hu, B., Lukasek, K., … Abreu, M. T. (2005). Toll-like receptor-4 is required for intestinal response to epithelial injury and limiting bacterial translocation in a murine model of acute colitis. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *288*(5 51-5). https://doi.org/10.1152/ajpgi.00328.2004

Gonzalez, A., Stombaugh, J., Lozupone, C., Turnbaugh, P. J., Gordon, J. I., & Knight, R. (2011). The mind-body-microbial continuum. *Dialogues in Clinical Neuroscience*, *13*(1). https://doi.org/10.31887/dcns.2011.13.1/agonzalez

Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., … Ley, R. E. (2014). Human genetics shape the gut microbiome. *Cell*, *159*(4). https://doi.org/10.1016/j.cell.2014.09.053

Goudot, C., Coillard, A., Villani, A. C., Gueguen, P., Cros, A., Sarkizova, S., … Segura, E. (2017). Aryl Hydrocarbon Receptor Controls Monocyte Differentiation into Dendritic Cells versus Macrophages. *Immunity*, *47*(3). https://doi.org/10.1016/j.immuni.2017.08.016

Grönlund, M. M., Lehtonen, O. P., Eerola, E., & Kero, P. (1999). Fecal microflora in healthy infants born by different methods of delivery: Permanent changes in intestinal flora after cesarean delivery. *Journal of Pediatric Gastroenterology and Nutrition*, *28*(1). https://doi.org/10.1097/00005176-199901000-00007

Gutiérrez, A., Zapater, P., Juanola, O., Sempere, L., García, M., Laveda, R., … Francés, R. (2016). Gut bacterial DNA translocation is an independent risk factor of flare at short term in patients with crohn's disease. *American Journal of Gastroenterology*, *111*(4). https://doi.org/10.1038/ajg.2016.8

Hailman, E., Lichenstein, H. S., Wurfel, M. M., Miller, D. S., Johnson, D. A., Kelley, M., … Wright, S. D. (1994). Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. *Journal of Experimental Medicine*, *179*(1). https://doi.org/10.1084/jem.179.1.269

Hanson, L. (1961). Comparative immunological studies of the immune globulins of human milk and of blood serum. *International Archives of Allergy and Immunology*, *18*(5). https://doi.org/10.1159/000229177

Heath-Pagliuso, S., Rogers, W. J., Tullis, K., Seidel, S. D., Cenijn, P. H., Brouwer, A., & Denison, M. S. (1998). Activation of the Ah receptor by tryptophan and tryptophan metabolites. *Biochemistry*, *37*(33). https://doi.org/10.1021/bi980087p

Henderson, G., Cox, F., Kittelmann, S., Miri, V. H., Zethof, M., Noel, S. J., … Janssen, P. H. (2013). Effect of DNA extraction methods and sampling techniques on the apparent structure of cow and sheep rumen microbial communities. *PloS One*, *8*(9). https://doi.org/10.1371/journal.pone.0074787

Hoshino, K., Takeuchi, O., Kawai, T., Sanjo, H., Ogawa, T., Takeda, Y., … Akira, S. (1999). Cutting Edge: Toll-Like Receptor 4 (TLR4)-Deficient Mice Are Hyporesponsive to Lipopolysaccharide: Evidence for TLR4 as the Lps Gene Product . *The Journal of Immunology*, *162*(7). https://doi.org/10.4049/jimmunol.162.7.3749

Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H., Chinwalla, A. T., … White, O. (2012a). Structure, function and diversity of the healthy human microbiome. *Nature*, *486*(7402). https://doi.org/10.1038/nature11234

Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H., … White, O. (2012b). The Human Microbiome Project HMP Consortium Structure function and diversity of the healthy human microbiome. *Nature*, *486*(7402).

Ivanov, I. I., Atarashi, K., Manel, N., Brodie, E. L., Shima, T., Karaoz, U., … Littman, D. R. (2009). Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell*, *139*(3). https://doi.org/10.1016/j.cell.2009.09.033

Iwashita, J., Sato, Y., Sugaya, H., Takahashi, N., Sasaki, H., & Abe, T. (2003). mRNA of MUC2 is stimulated by IL-4, IL-13 or TNF-α through a mitogen-activated protein kinase pathway in human colon cancer cells. *Immunology and Cell Biology*, *81*(4). https://doi.org/10.1046/j.1440-1711.2003.t01-1-01163.x

Jeffery, I. B., Lynch, D. B., & O'Toole, P. W. (2016). Composition and temporal stability of the gut microbiota in older persons. *ISME Journal*, *10*(1). https://doi.org/10.1038/ismej.2015.88

Jiménez, E., Fernández, L., Marín, M. L., Martín, R., Odriozola, J. M., Nueno-Palop, C., … Rodríguez, J. M. (2005). Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Current Microbiology*, *51*(4). https://doi.org/10.1007/s00284-005-0020-3

Johansson, M. E. V., Phillipson, M., Petersson, J., Velcich, A., Holm, L., & Hansson, G. C. (2008). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(39). https://doi.org/10.1073/pnas.0803124105

Jones, B. D., Ghori, N., & Falkow, S. (1994). Salmonella typhimurium initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. *Journal of Experimental Medicine*, *180*(1). https://doi.org/10.1084/jem.180.1.15

Kawai, T., & Akira, S. (2011). Toll-like Receptors and Their Crosstalk with Other Innate Receptors in Infection and Immunity. *Immunity*, Vol. 34. https://doi.org/10.1016/j.immuni.2011.05.006

Kim, M. H., Kang, S. G., Park, J. H., Yanagisawa, M., & Kim, C. H. (2013). Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology*, *145*(2). https://doi.org/10.1053/j.gastro.2013.04.056

Kim, M., Qie, Y., Park, J., & Kim, C. H. (2016). Gut Microbial Metabolites Fuel Host Antibody Responses. *Cell Host and Microbe*, *20*(2). https://doi.org/10.1016/j.chom.2016.07.001

Klaasen, H. L. B. M., Van der Heijden, P. J., Stok, W., Poelma, F. G. J., Koopman, J. P., Van den Brink, M. E., … Beynen, A. C. (1993). Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. *Infection and Immunity*, *61*(1). https://doi.org/10.1128/iai.61.1.303-306.1993

Knights, D., Ward, T. L., McKinlay, C. E., Miller, H., Gonzalez, A., McDonald, D., & Knight, R. (2014). Rethinking enterotypes. *Cell Host and Microbe*, Vol. 16. https://doi.org/10.1016/j.chom.2014.09.013

Kocsis, Á. K., Lakatos, P. L., Somogyvári, F., Fuszek, P., Papp, J., Fischer, S., … Mándi, Y. (2008). Association of beta-defensin 1 single nucleotide polymorphisms with Crohn's disease. *Scandinavian Journal of Gastroenterology*, *43*(3). https://doi.org/10.1080/00365520701682615

Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., … Ley, R. E. (2010). Microbes and Health Sackler Colloquium: Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America*.

Kumar, P., Monin, L., Castillo, P., Elsegeiny, W., Horne, W., Eddens, T., … Kolls, J. K. (2016). Intestinal Interleukin-17 Receptor Signaling Mediates Reciprocal Control of the Gut Microbiota and Autoimmune Inflammation. *Immunity*, *44*(3). https://doi.org/10.1016/j.immuni.2016.02.007

Kverka, M., & Tlaskalova-Hogenova, H. (2013). Two faces of microbiota in inflammatory and autoimmune diseases: Triggers and drugs. *APMIS*, Vol. 121. https://doi.org/10.1111/apm.12007

Kverka, M., & Tlaskalová-Hogenová, H. (2017). Intestinal Microbiota: Facts and Fiction. *Digestive Diseases*, Vol. 35. https://doi.org/10.1159/000449095

Ladinsky, M. S., Araujo, L. P., Zhang, X., Veltri, J., Galan-Diez, M., Soualhi, S., … Ivanov, I. I. (2019). Endocytosis of commensal antigens by intestinal epithelial cells regulates mucosal T cell homeostasis. *Science*, *363*(6431). https://doi.org/10.1126/science.aat4042

Larsen, N., Vogensen, F. K., Van Den Berg, F. W. J., Nielsen, D. S., Andreasen, A. S., Pedersen, B. K., … Jakobsen, M. (2010). Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS ONE*, *5*(2). https://doi.org/10.1371/journal.pone.0009085

Lebeer, S., Vanderleyden, J., & De Keersmaecker, S. C. J. (2010). Host interactions of probiotic bacterial surface molecules: Comparison with commensals and pathogens. *Nature Reviews Microbiology*, Vol. 8. https://doi.org/10.1038/nrmicro2297

Lécuyer, E., Rakotobe, S., Lengliné-Garnier, H., Lebreton, C., Picard, M., Juste, C., … Gaboriau-Routhiau, V. (2014). Segmented filamentous bacterium uses secondary and tertiary lymphoid tissues to induce gut IgA and specific T helper 17 cell responses. *Immunity*, *40*(4). https://doi.org/10.1016/j.immuni.2014.03.009

Lee, J. S., Cella, M., McDonald, K. G., Garlanda, C., Kennedy, G. D., Nukaya, M., … Colonna, M. (2012). AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. *Nature Immunology*, *13*(2). https://doi.org/10.1038/ni.2187

Lee, J. S., Tato, C. M., Joyce-Shaikh, B., Chen, Y., … Cua, D. J. (2015). Interleukin-23-Independent IL-17 Production Regulates Intestinal Epithelial Permeability. *Immunity*, *43*(4). https://doi.org/10.1016/j.immuni.2015.09.003

Ley, R. E., Peterson, D. A., & Gordon, J. I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, Vol. 124. https://doi.org/10.1016/j.cell.2006.02.017

Li, J. D., Feng, W., Gallup, M., Kim, J. H., Gum, J., Kim, Y., & Basbaum, C. (1998). Activation of NF-κB via a Src-dependent Ras-MAPK-pp90rsk pathway is required for Pseudomonas aeruginosa-induced mucin overproduction in epithelial cells. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(10). https://doi.org/10.1073/pnas.95.10.5718

Li, X., Li, C., Zhang, W., Wang, Y., Qian, P., & Huang, H. (2023). Inflammation and aging: signaling pathways and intervention therapies. *Signal Transduction and Targeted Therapy*, Vol. 8. https://doi.org/10.1038/s41392-023-01502-8

Lien, E., Sellati, T. J., Yoshimura, A., Flo, T. H., Rawadi, G., Finberg, R. W., … Golenbock, D. T. (1999). Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *Journal of Biological Chemistry*, *274*(47). https://doi.org/10.1074/jbc.274.47.33419

Linden, S. K., Sutton, P., Karlsson, N. G., Korolik, V., & McGuckin, M. A. (2008). Mucins in the mucosal barrier to infection. *Mucosal Immunology*, Vol. 1. https://doi.org/10.1038/mi.2008.5

Liu, L., Guo, X., Rao, J. N., Zou, T., Xiao, L., Yu, T., … Wang, J. Y. (2009). Polyamines regulate E-cadherin transcription through c-Myc modulating intestinal epithelial barrier function. *American Journal of Physiology - Cell Physiology*, *296*(4). https://doi.org/10.1152/ajpcell.00620.2008

Liu, Ying, Van Kruiningen, H. J., West, A. B., Cartun, R. W., Cortot, A., & Colombel, J. F. (1995). Immunocytochemical evidence of Listeria, Escherichia coil, and Streptococcus antigens in Crohn's disease. *Gastroenterology*, *108*(5). https://doi.org/10.1016/0016-5085(95)90687-8

Liu, Yuan, & Chen, Y. G. (2020). Intestinal epithelial plasticity and regeneration via cell dedifferentiation. *Cell Regeneration*, Vol. 9. https://doi.org/10.1186/s13619-020-00053-5

Lopera-Maya, E. A., Kurilshikov, A., van der Graaf, A., Hu, S., Andreu-Sánchez, S., Chen, L., … Sanna, S. (2022). Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch Microbiome Project. *Nature Genetics*, *54*(2). https://doi.org/10.1038/s41588-021-00992-y

Lührs, H., Gerke, T., Müller, J. G., Melcher, R., Schauber, J., Boxberger, F., … Menzel, T. (2002). Butyrate inhibits NF-κB activation in lamina propria macrophages of patients with ulcerative colitis. *Scandinavian Journal of Gastroenterology*, *37*(4). https://doi.org/10.1080/003655202317316105

Macfarlane, G. T., Allison, C., Gibson, S. A. W., & Cummings, J. H. (1988). Contribution of the microflora to proteolysis in the human large intestine. *Journal of Applied Bacteriology*, *64*(1). https://doi.org/10.1111/j.1365-2672.1988.tb02427.x

Macpherson, A. J., & Smith, K. (2006). Mesenteric lymph nodes at the center of immune anatomy. *Journal of Experimental Medicine*, Vol. 203. https://doi.org/10.1084/jem.20060227

Martín, R., Langa, S., Reviriego, C., Jiménez, E., Marín, M. L., Xaus, J., … Rodríguez, J. M. (2003). Human milk is a source of lactic acid bacteria for the infant gut. *Journal of Pediatrics*, *143*(6). https://doi.org/10.1016/j.jpeds.2003.09.028

Mayer, L., & Shlien, R. (1987). Evidence for function of Ia molecules on gut epithelial cells in man. *Journal of Experimental Medicine*, *166*(5). https://doi.org/10.1084/jem.166.5.1471

Mazmanian, S. K., Cui, H. L., Tzianabos, A. O., & Kasper, D. L. (2005). An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell*, *122*(1). https://doi.org/10.1016/j.cell.2005.05.007

Mazmanian, S. K., Round, J. L., & Kasper, D. L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*, *453*(7195). https://doi.org/10.1038/nature07008

Mazzini, E., Massimiliano, L., Penna, G., & Rescigno, M. (2014). Oral Tolerance Can Be Established via Gap Junction Transfer of Fed Antigens from CX3CR1+ Macrophages to CD103+ Dendritic Cells. *Immunity*, *40*(2). https://doi.org/10.1016/j.immuni.2013.12.012

McDole, J. R., Wheeler, L. W., McDonald, K. G., Knoop, K. A., … Miller, M. J. (2012). Goblet cells deliver luminal antigen to CD103 + dendritic cells in the small intestine. *Nature*, *483*(7389). https://doi.org/10.1038/nature10863

Medzhitov, R., Preston-Hurlburt, P., & Janeway, C. A. (1997). A human homologue of the Drosophila toll protein signals activation of adaptive immunity. *Nature*, *388*(6640). https://doi.org/10.1038/41131

Miyauchi, S., Gopal, E., Fei, Y. J., & Ganapathy, V. (2004). Functional Identification of SLC5A8, a Tumor Suppressor Down-regulated in Colon Cancer, as a Na+-coupled Transporter for Short-chain Fatty Acids. *Journal of Biological Chemistry*, *279*(14). https://doi.org/10.1074/jbc.C400059200

Mora, J. R., Iwata, M., Eksteen, B., Song, S. Y., Junt, T., Senman, B., … Von Andrian, U. H. (2006). Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science*, *314*(5802). https://doi.org/10.1126/science.1132742

Mowat, A. M., & Agace, W. W. (2014). Regional specialization within the intestinal immune system. *Nature Reviews Immunology*, Vol. 14. https://doi.org/10.1038/nri3738

Mowat, A. M. I. (2003). Anatomical basis of tolerance and immunity to intestinal antigens. *Nature Reviews Immunology*, Vol. 3. https://doi.org/10.1038/nri1057

Mulder, D. J., Noble, A. J., Justinich, C. J., & Duffin, J. M. (2014). A tale of two diseases: The history of inflammatory bowel disease. *Journal of Crohn's and Colitis*, Vol. 8. https://doi.org/10.1016/j.crohns.2013.09.009

Nadjsombati, M. S., McGinty, J. W., Lyons-Cohen, M. R., Jaffe, J. B., DiPeso, L., Schneider, C., … von Moltke, J. (2018). Detection of Succinate by Intestinal Tuft Cells Triggers a Type 2 Innate Immune Circuit. *Immunity*, *49*(1). https://doi.org/10.1016/j.immuni.2018.06.016

Nagpal, R., Mainali, R., Ahmadi, S., Wang, S., Singh, R., Kavanagh, K., … Yadav, H. (2018). Gut microbiome and aging: Physiological and mechanistic insights. *Nutrition and Healthy Aging*, Vol. 4. https://doi.org/10.3233/NHA-170030

Nakamura, Y., Mimuro, H., Kunisawa, J., Furusawa, Y., Takahashi, D., Fujimura, Y., … Hase, K. (2020). Microfold cell-dependent antigen transport alleviates infectious colitis by inducing antigen-specific cellular immunity. *Mucosal Immunology*, *13*(4). https://doi.org/10.1038/s41385-020-0263-0

Nanki, K., Mizuno, S., Matsuoka, K., Ono, K., Sugimoto, S., Kiyohara, H., … Kanai, T. (2018). Fecal microbiota transplantation for recurrent Clostridium difficile infection in a patient with ulcerative colitis. *Intestinal Research*, *16*(1). https://doi.org/10.5217/ir.2018.16.1.142

Nauta, A. J., Amor, K. Ben, Knol, J., Garssen, J., & Van Der Beek, E. M. (2013). Relevance of pre- and postnatal nutrition to development and interplay between the microbiota and metabolic and immune systems. *American Journal of Clinical Nutrition*, *98*(2). https://doi.org/10.3945/ajcn.112.039644

Neish, A. S., Gewirtz, A. T., Zeng, H., Young, A. N., Karmali, V., … Madara, J. L. (2000). Prokaryotic regulation of epithelial responses by inhibition of IκB-α ubiquitination. *Science*, *289*(5484). https://doi.org/10.1126/science.289.5484.1560

Nenci, A., Becker, C., Wullaert, A., Gareus, R., Van Loo, G., Danese, S., … Pasparakis, M. (2007). Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature*, *446*(7135). https://doi.org/10.1038/nature05698

Neutra, M. R., & Kraehenbuhl, J. P. (1992). Transepithelial transport and mucosal defence I: the role of M cells. *Trends in Cell Biology*, Vol. 2. https://doi.org/10.1016/0962-8924(92)90099-9

O'Boyle, C. J., MacFie, J., Mitchell, C. J., Johnstone, D., Sagar, P. M., & Sedman, P. C. (1998). Microbiology of bacterial translocation in humans. *Gut*, *42*(1). https://doi.org/10.1136/gut.42.1.29

Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J. Z., … Osawa, R. (2016). Age-related changes in gut microbiota composition from newborn to centenarian: A cross-sectional study. *BMC Microbiology*, *16*(1). https://doi.org/10.1186/s12866-016-0708-5

O'Hara, A. M., & Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO Reports*, Vol. 7. https://doi.org/10.1038/sj.embor.7400731

Oliveira, R. A., Cabral, V., Torcato, I., & Xavier, K. B. (2023). Deciphering the quorum-sensing lexicon of the gut microbiota. *Cell Host and Microbe*, Vol. 31. https://doi.org/10.1016/j.chom.2023.03.015

Palm, N. W., De Zoete, M. R., Cullen, T. W., Barry, N. A., Hao, L., … Flavell, R. A. (2014). Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell*, *158*(5). https://doi.org/10.1016/j.cell.2014.08.006

Pannaraj, P. S., Li, F., Cerini, C., Bender, J. M., Yang, S., Rollie, A., … Aldrovandi, G. M. (2017). Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. *JAMA Pediatrics*, *171*(7). https://doi.org/10.1001/jamapediatrics.2017.0378

Panzer, J. J., Romero, R., Greenberg, J. M., Winters, A. D., Galaz, J., Gomez-Lopez, N., & Theis, K. R. (2023). Is there a placental microbiota? A critical review and re-analysis of published placental microbiota datasets. *BMC Microbiology*, *23*(1). https://doi.org/10.1186/s12866-023-02764-6

Penders, J., Thijs, C., Stelma, F. F., Snijders, B., Kummeling, I., … Stobberingh, E. E. (2006). Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics*, *118*(2). https://doi.org/10.1542/peds.2005-2824

Peng, L., Li, Z. R., Green, R. S., Holzman, I. R., & Lin, J. (2009). Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *Journal of Nutrition*, *139*(9). https://doi.org/10.3945/jn.109.104638

Peterson, L. W., & Artis, D. (2014). Intestinal epithelial cells: Regulators of barrier function and immune homeostasis. *Nature Reviews Immunology*, Vol. 14. https://doi.org/10.1038/nri3608

Petnicki-Ocwieja, T., Hrncir, T., Liu, Y. J., Biswas, A., Hudcovic, T., Tlaskalova-Hogenova, H., & Kobayashi, K. S. (2009). Nod2 is required for the regulation of commensal microbiota in the intestine. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(37). https://doi.org/10.1073/pnas.0907722106

Poltorak, A., He, X., Liu, M. Y., Van Huffel, C., Du, X., … Beutler, B. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in Tlr4 gene. *Science*, *282*(5396). https://doi.org/10.1126/science.282.5396.2085

Postler, T. S., & Ghosh, S. (2017). Understanding the Holobiont: How Microbial Metabolites Affect Human Health and Shape the Immune System. *Cell Metabolism*, Vol. 26. https://doi.org/10.1016/j.cmet.2017.05.008

Prakash, T., Oshima, K., Morita, H., Fukuda, S., Imaoka, A., Kumar, N., … Hattori, M. (2011). Complete genome sequences of rat and mouse segmented filamentous bacteria, a potent inducer of Th17 cell differentiation. *Cell Host and Microbe*, *10*(3). https://doi.org/10.1016/j.chom.2011.08.007

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., … Zoetendal, E. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285). https://doi.org/10.1038/nature08821

Quintana, F. J., Basso, A. S., Iglesias, A. H., Korn, T., Farez, M. F., Bettelli, E., … Weiner, H. L. (2008). Control of Treg and TH17 cell differentiation by the aryl hydrocarbon receptor. *Nature*, *453*(7191). https://doi.org/10.1038/nature06880

Raetz, C. R. H., & Whitfield, C. (2002). Lipopolysaccharide endotoxins. *Annual Review of Biochemistry*, Vol. 71. https://doi.org/10.1146/annurev.biochem.71.110601.135414

Rahhal, R. M., Vanden Bush, T. J., Apicella, M. A., & Bishop, G. A. (2007). Differential effects of Francisella tularensis lipopolysaccharide on B lymphocytes . *Journal of Leukocyte Biology*, *82*(4). https://doi.org/10.1189/jlb.1206765

Renga, B., Mencarelli, A., Cipriani, S., D'Amore, C., Carino, A., Bruno, A., … Fiorucci, S. (2013). The Bile Acid Sensor FXR Is Required for Immune-Regulatory Activities of TLR-9 in Intestinal Inflammation. *PLoS ONE*, *8*(1). https://doi.org/10.1371/journal.pone.0054472

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., … Ricciardi-Castagnoli, P. (2001). Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature Immunology*, *2*(4). https://doi.org/10.1038/86373

Rios, D., Wood, M. B., Li, J., Chassaing, B., Gewirtz, A. T., & Williams, I. R. (2016). Antigen sampling by intestinal M cells is the principal pathway initiating mucosal IgA production to commensal enteric bacteria. *Mucosal Immunology*, *9*(4). https://doi.org/10.1038/mi.2015.121

Roediger, W. E. W. (1980). Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut*, *21*(9). https://doi.org/10.1136/gut.21.9.793

Rothschild, D., Weissbrod, O., Barkan, E., Kurilshikov, A., Korem, T., Zeevi, D.,… Segal, E. (2018). Environment dominates over host genetics in shaping human gut microbiota. *Nature*, *555*(7695). https://doi.org/10.1038/nature25973

Round, J. L., & Mazmanian, S. K. (2010). Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(27). https://doi.org/10.1073/pnas.0909122107

Ryu, J. K., Kim, S. J., Rah, S. H., Kang, J. I., Jung, H. E., Lee, D., … Kim, H. M. (2017). Reconstruction of LPS Transfer Cascade Reveals Structural Determinants within LBP, CD14, and TLR4-MD2 for Efficient LPS Recognition and Transfer. *Immunity*, *46*(1). https://doi.org/10.1016/j.immuni.2016.11.007

Salter, S. J., Cox, M. J., Turek, E. M., Calus, S. T., Cookson, W. O., Moffatt, M. F., … Walker, A. W. (2014). Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology*, *12*(1). https://doi.org/10.1186/s12915-014-0087-z

Sartor, R. B. (2004). Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: Antibiotics, probiotics, and prebiotics. *Gastroenterology*, *126*(6). https://doi.org/10.1053/j.gastro.2004.03.024

Segain, J. P., Raingeard de la Blétière, D., Bourreille, A., Leray, V., Gervois, N., Rosales, C., … Galmiche, J. P. (2000). Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. *Gut*, *47*(3).

Sellon, R. K., Tonkonogy, S., Schultz, M., Dieleman, L. A., Grenther, W., Balish, E., … Sartor, R. B. (1998). Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infection and Immunity*, *66*(11). https://doi.org/10.1128/iai.66.11.5224-5231.1998

Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biology*, *14*(8). https://doi.org/10.1371/journal.pbio.1002533

Shale, M., Schiering, C., & Powrie, F. (2013). CD4+ T-cell subsets in intestinal inflammation. *Immunological Reviews*, *252*(1). https://doi.org/10.1111/imr.12039

Shimada, Y., Kinoshita, M., Harada, K., Mizutani, M., Masahata, K., Kayama, H., & Takeda, K. (2013). Commensal bacteria-dependent indole production enhances epithelial barrier function in the colon. *PLoS ONE*, *8*(11). https://doi.org/10.1371/journal.pone.0080604

Shimazu, R., Akashi, S., Ogata, H., Nagai, Y., Fukudome, K., Miyake, K., & Kimoto, M. (1999). MD-2, a molecule that confers lipopolysaccharide responsiveness on toll- like receptor 4. *Journal of Experimental Medicine*, *189*(11). https://doi.org/10.1084/jem.189.11.1777

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., … Garrett, W. S. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic T reg cell homeostasis. *Science*, *341*(6145). https://doi.org/10.1126/science.1241165

Stepankova, R., Powrie, F., Kofronova, O., Kozakova, H., Hudcovic, T., Hrncir, T., … Tlaskalova-Hogenova, H. (2007). Segmented filamentous bacteria in a defined bacterial cocktail induce intestinal inflammation in SCID mice reconstituted with CD45RBhigh CD4+ T cells. *Inflammatory Bowel Diseases*, *13*(10). https://doi.org/10.1002/ibd.20221

Stokes, C. R., Soothill, J. F., & Turner, M. W. (1975). Immune exclusion is a function of IgA. *Nature*, *255*(5511). https://doi.org/10.1038/255745a0

Strober, W., Fuss, I., & Mannon, P. (2007). The fundamental basis of inflammatory bowel disease. *Journal of Clinical Investigation*, *117*(3). https://doi.org/10.1172/JCI30587

Strober, W., Murray, P. J., Kitani, A., & Watanabe, T. (2006). Signalling pathways and molecular interactions of NOD1 and NOD2. *Nature Reviews Immunology*, Vol. 6. https://doi.org/10.1038/nri1747

Sun, L., Sun, Z., Wang, Q., Zhang, Y., & Jia, Z. (2022). Role of nuclear receptor PXR in immune cells and inflammatory diseases. *Frontiers in Immunology*, Vol. 13. https://doi.org/10.3389/fimmu.2022.969399

Sun, S., Zhu, X., Huang, X., Murff, H. J., Ness, R. M., Seidner, D. L., … Fodor, A. A. (2021). On the robustness of inference of association with the gut microbiota in stool, rectal swab and mucosal tissue samples. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-94205-5

Swidsinski, A., Weber, J., Loening-Baucke, V., Hale, L. P., & Lochs, H. (2005). Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *Journal of Clinical Microbiology*, *43*(7). https://doi.org/10.1128/JCM.43.7.3380-3389.2005

Taylor, P. R., Tsoni, S. V., Willment, J. A., Rosas, M., Findon, H., … Brown, G. D. (2007). Dectin-1 is required for β-glucan recognition and control of fungal infection. *Nature Immunology*, *8*(1). https://doi.org/10.1038/ni1408

Theriot, C. M., Koenigsknecht, M. J., Carlson, P. E., Hatton, G. E., Nelson, A. M., Li, B., … Young, V. B. (2014). Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. *Nature Communications*, *5*. https://doi.org/10.1038/ncomms4114

Tlaskalová-Hogenová, H., Tpánková, R., Kozáková, H., Hudcovic, T., Vannucci, L., Tuková, L., … Funda, D. P. (2011). The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: Contribution of germ-free and gnotobiotic animal models of human diseases. *Cellular and Molecular Immunology*, Vol. 8. https://doi.org/10.1038/cmi.2010.67

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., … Gordon, J. I. (2009). A core gut microbiome in obese and lean twins. *Nature*, *457*(7228). https://doi.org/10.1038/nature07540

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The Human Microbiome Project. *Nature*, Vol. 449. https://doi.org/10.1038/nature06244

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, *444*(7122). https://doi.org/10.1038/nature05414

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. I. (2009). The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. *Science Translational Medicine*, *1*(6). https://doi.org/10.1126/scitranslmed.3000322

Uematsu, S., Fujimoto, K., Jang, M. H., Yang, B. G., Jung, Y. J., Nishiyama, M., … Akira, S. (2008). Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. *Nature Immunology*, *9*(7). https://doi.org/10.1038/ni.1622

Uematsu, S., Jang, M. H., Chevrier, N., Guo, Z., Kumagai, Y., Yamamoto, M., … Akira, S. (2006). Detection of pathogenic intestinal bacteria by Toll-like receptor 5 on intestinal CD11c+ lamina propria cells. *Nature Immunology*, *7*(8). https://doi.org/10.1038/ni1362

Venkatesh, M., Mukherjee, S., Wang, H., Li, H., Sun, K., Benechet, A. P., … Mani, S. (2014). Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and toll-like receptor 4. *Immunity*, *41*(2). https://doi.org/10.1016/j.immuni.2014.06.014

Vinolo, M. A. R., Ferguson, G. J., Kulkarni, S., Damoulakis, G., Anderson, K., … Curi, R. (2011). SCFAs induce mouse neutrophil chemotaxis through the GPR43 receptor. *PLoS ONE*, *6*(6). https://doi.org/10.1371/journal.pone.0021205

Volkova, A., Ruggles, K., Schulfer, A., Gao, Z., Ginsberg, S. D., & Blaser, M. J. (2021). Effects of early-life penicillin exposure on the gut microbiome and frontal cortex and amygdala gene expression. *IScience*, *24*(7). https://doi.org/10.1016/j.isci.2021.102797

Waldecker, M., Kautenburger, T., Daumann, H., Busch, C., & Schrenk, D. (2008). Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. *Journal of Nutritional Biochemistry*, *19*(9). https://doi.org/10.1016/j.jnutbio.2007.08.002

Wang, H. B., Wang, P. Y., Wang, X., Wan, Y. L., & Liu, Y. C. (2012). Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein claudin-1 transcription. *Digestive Diseases and Sciences*, *57*(12). https://doi.org/10.1007/s10620-012-2259-4

Wang, J., Thingholm, L. B., Skiecevičie, J., Rausch, P., Kummen, M., Hov, J. R., … Franke, A. (2016). Genome-wide association analysis identifies variation in Vitamin D receptor and other host factors influencing the gut microbiota. *Nature Genetics*, *48*(11). https://doi.org/10.1038/ng.3695

Wang, Y., Yin, Y., Chen, X., Zhao, Y., Wu, Y., Li, Y., … Xiang, C. (2019). Induction of Intestinal Th17 Cells by Flagellins From Segmented Filamentous Bacteria. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.02750

Wicherska-pawłowska, K., Wróbel, T., & Rybka, J. (2021). Toll-like receptors (Tlrs), nod-like receptors (nlrs) and rig-i-like receptors (rlrs) in innate immunity. tlrs, nlrs and rlrs ligands as immunotherapeutic agents for hematopoietic diseases. *International Journal of Molecular Sciences*, Vol. 22. https://doi.org/10.3390/ijms222413397

Wirt, D. P., Adkins, L. T., Palkowetz, K. H., Schmalstieg, F. C., & Goldman, A. S. (1992). Activated and memory T lymphocytes in human milk. *Cytometry*, *13*(3). https://doi.org/10.1002/cyto.990130310

Wolfert, M. A., Roychowdhury, A., & Boons, G. J. (2007). Modification of the structure of peptidoglycan is a strategy to avoid detection by nucleotide-binding oligomerization domain protein 1. *Infection and Immunity*, *75*(2). https://doi.org/10.1128/IAI.01597-06

Worbs, T., Bode, U., Yan, S., Hoffmann, M. W., Hintzen, G., Bernhardt, G., … Pabst, O. (2006). Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *Journal of Experimental Medicine*, *203*(3). https://doi.org/10.1084/jem.20052016

Wu, M., Li, P., Li, J., An, Y., Wang, M., & Zhong, G. (2020). The differences between luminal microbiota and mucosal microbiota in mice. *Journal of Microbiology and Biotechnology*, *30*(2). https://doi.org/10.4014/jmb.1908.08037

Xiong, Y., Xu, G., Chen, M., & Ma, H. (2022). Intestinal Uptake and Tolerance to Food Antigens. *Frontiers in Immunology*, Vol. 13. https://doi.org/10.3389/fimmu.2022.906122

Yasuda, K., Oh, K., Ren, B., Tickle, T. L., Franzosa, E. A., Wachtman, L. M., … Morgan, X. C. (2015). Biogeography of the intestinal mucosal and lumenal microbiome in the rhesus macaque. *Cell Host and Microbe*, *17*(3). https://doi.org/10.1016/j.chom.2015.01.015

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, Vol. 486. https://doi.org/10.1038/nature11053

Yoneno, K., Hisamatsu, T., Shimamura, K., Kamada, N., Ichikawa, R., Kitazume, M. T., … Hibi, T. (2013). TGR5 signalling inhibits the production of pro-inflammatory cytokines by in vitro differentiated inflammatory and intestinal macrophages in Crohn's disease. *Immunology*, *139*(1). https://doi.org/10.1111/imm.12045

Zegarra-Ruiz, D. F., Kim, D. V., Norwood, K., Wu, W. J. H., Saldana-Morales, F. B., … Diehl, G. E. (2021). Thymic development of gut-microbiota-specific T cells. *Nature*, *594*(7863). https://doi.org/10.1038/s41586-021-03531-1

Zhang, M., Borovikova, L. V., Wang, H., Metz, C., & Tracey, K. J. (1999). Spermine inhibition of monocyte activation and inflammation. *Molecular Medicine*, *5*(9). https://doi.org/10.1007/bf03402072

Zhou, L., Yoshimura, Y., Huang, Y. Y., Suzuki, R., Yokoyama, M., Okabe, M., & Shimamura, M. (2000). Two independent pathways of maternal cell transmission to offspring: Through placenta during pregnancy and by breast-feeding after birth. *Immunology*, *101*(4). https://doi.org/10.1046/j.1365-2567.2000.00144.x

Zoetendal, E. G., Von Wright, A., Akkermans, A. D. L., & De Vos, W. M. (2002). Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces. *Applied and Environmental Microbiology*, *68*(7). https://doi.org/10.1128/AEM.68.7.3401-3407.2002