Bacterial-host interactions in inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) is the collective name for a group of chronic relapsing infections of the intestinal tract. There is increasing evidence that the composition of the human microbiota plays a key role in the development of IBD. The control of symbiotic and pathogenic bacteria is tightly regulated by both the adaptive and innate immune responses. IBD is a multifactorial disorder and identification of the most relevant effectors associated with IBD can give us new insights for future therapy strategies. In this review we combine data concerning the innate and adaptive immune response towards intestinal microbes and discuss possible mechanisms by which microbes play a role in IBD disease pathogenesis.

Defining features of the human Microbiota composition

The biggest population of microbes can be found in the distal ileum and colon. Approximately 100 trillion organisms reside within the intestine and most of these organisms are bacteria[1] The mucosa is colonized by many different bacteria. The collective genomic content of this microbiota is called the microbiome. The microbiome contains approximately 200.000 to 300.000 different genes and is highly diverse within the human population.[2] Individual difference is characterized by specific bacteria and the ratio of bacteria belonging to different phyla. [3] The human body hosts multiple phyla of bacteria like Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, Cyanobacteria, and Fusobacteria. The most important phyla in the gut are the Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria. The presence of these four phyla of bacteria are shared in the entire human population but the ratio varies between individuals and body sites.[2]

Firmicutes are Gram-positive, strictly anaerobic bacteria [7] and comprise of 250 genera such as *lactobacillus, Streptococcus, Mycoplasma and Clostridium*. Within the phylum *Firmicutes* the genus *Clostridium* is strongly represented in the human microbiota. Bacilli like *lactobacillus, Streptocuccus* are commonly present but make up a smaller portion of the total amount of *Firmicutes* [3] *Firmicutes* are non spore forming rods and some use sugars and produce lactate, butyric acid and hydrogen,[8] however they do not produce succinate or propionate. [2] In gut of five human subjects analysed by Rajilic-Stojanovic et al., *Firmicutes* were by far the most abundant phylum accounting for 47.21% to 90.35% of the entire microbiota. (Figure 1) [3, 9,105]

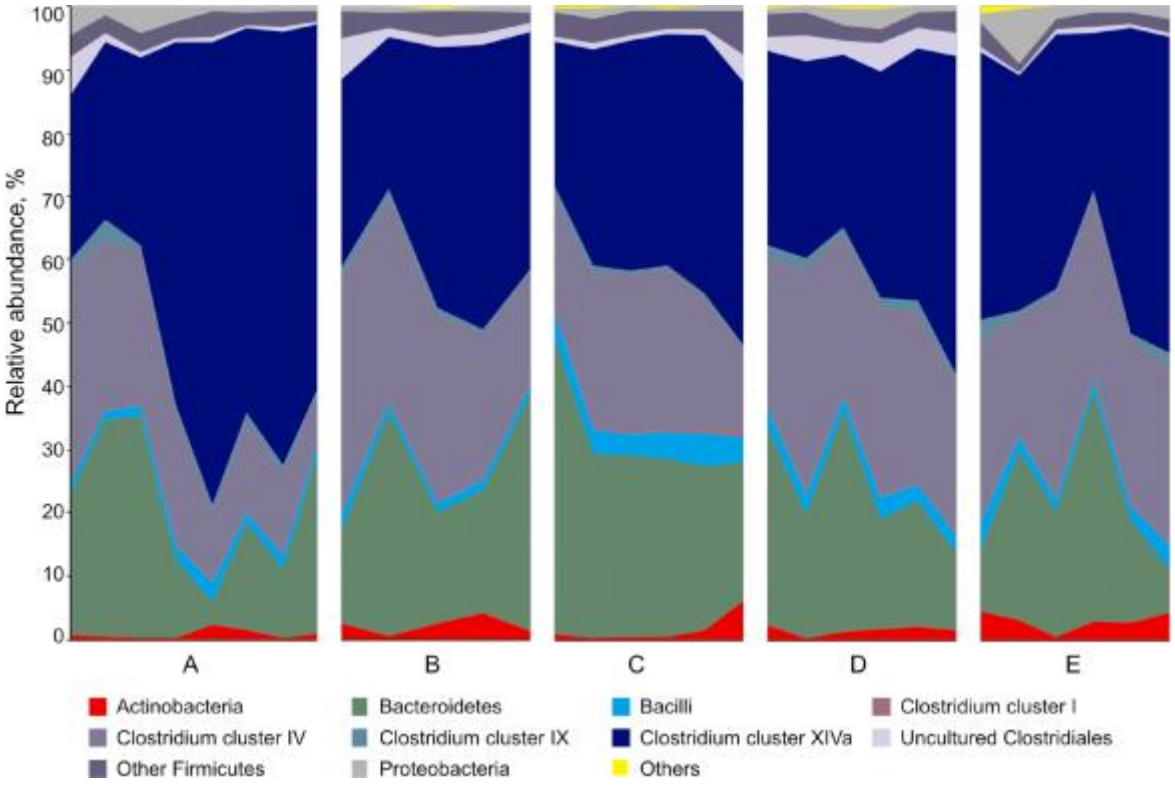

Figure 1: Rajilic-Stojanovic et al. Environ. Microbiol. 2012.
Relative abundance of the Level 1 phylogenetic groups over an 8-12-year period on five unrelated healthy people using Phylogenetic microarray's. [3]

Bacteroidetes are Gram-negative, bile-resistant, non-spore-forming strictly anaerobic bacteria[7] and comprises of more than 20 genera.[2] *Bacteroidetes* is a phylum of bacteria comprised of three classes: *Bacteroides, Flavobacteria*, and *Sphingobacteria* and many of them are present in the distal small intestinal and colonic microbiota.[10] Bacteroidetes are able to digest a wide variety of indigestible polysaccarides.[13] Genomic and proteomic analyses show that Bacteroidetes are versatile bacteria capable of utilising many different nutrient sources. Bacteroidetes also influence the rest of the microbiota. For example Bacteroidetes possess sugar utilization enzymes: this can promote the growth of other bacteria that have a selective advantage in presence of simple sugars.[10] Flexibility in nutrient source enables Bacteroidetes to be a more stable resident of the intestine.[3] Of the anaerobe bacteria in the human intestine approximately 25% belongs to the phylum *Bacteroidetes*. [11, 9] In the five subjects studied by Rajilic-Stojanovic etal., there portion amounts for 46.63% to 3.68%. (Figure 1)[3,105]

Actinobacteria are Gram-positive and unlike Firmicutes this phylum possesses a high guanine and cytosine content. *Actinobacteria* are stable residents of the microbiota but less numerous then *Bacteroidetes* and *Firmicutes*.[3] Interestingly in some studies Actinobacteria was the second most abundant phylum in the lower intestine accounting for 14.6% to 9.8%.[2]Whether the amount of Actinobacteria is dependent on the location within the intestine or that the methods of measuring bacterial abundance is done differently by the two research groups remains unclear.[14] However there seems to be concordance in the claim that Actinobacteria are stable residents within the gut microbiota.

To find an answer on the question how microbiota influences human health we must first define what a healthy human Microbiota is. Therefore multiple large scale projects were conducted to characterize the human microbiota in both the European [147] and American population. [148] Based on culture dependent studies the microbiota of healthy humans share multiple bacterial species. Researcher have identified several core species like Bacteroides uniformis, Roseburia intestinalis, and Faecalibacterium prausnitzii. [145]However when using techniques like pyrosequencing and phylogenetic microarrays the relative abundance of some of these core species was below 0.5%. [146] Since the presents of even these commonly shared species varies it's difficult to make a general statement about the species composition of the human microbiota. To make a distinction between different individuals researchers use measurements of bacterial diversity rather than specific species .[124]

Besides variety in bacteria diversity, the microbiota also varies in the ratio of different phyla. [123] Every phylum has its specific biological properties and knowing de distribution of the different phyla should represents the functional profile. To do these comparisons bacteria have to be grouped according to their taxonomy. However if a specific species is very abundant the phylum it belongs to might be overestimated. [145] A more direct measurement for functional profiles is shotgun metagenomics. With this technique different functions can be measured for example the ability to synthesis ATP or purine metabolism. Comparing these enzyme functional profiles show a far more similar distribution between human individuals compared to genus level phylotypes.[148] The presence of this core repertoire of functions was confirmed among different mammalian gut microbiomes. [149] The lack of real core species in the microbiota could be explained by the hypothesis that functional niches can be occupied by different species.

When comparing a Korean cohort to a US cohort the microbiota was significantly different.[139] Both diversity in bacterial species and ratios of phyla can be influenced by external factors. One of the most obvious external factors that influence microbiota composition is diet. For example Individuals consuming a western diet have a high number of Faecalibacterium prausnitzii and Eubacterium rectale.[6] Another example of the influence of diet on microbiota composition can be observed in a group of Japanese subjects. Japanese people have a diet which contains different types of raw seaweeds and when compared to the gut metagenome of North American subjects there microbiome contained genes specific for marine bacteria. This shows that the type of food consumed is associated with genes from environmental bacteria. [99]

By using shotgun metagenomics the influence of diet on functional niches was examined in 33 mammalian species. Researchers found a difference in microbioom between herbivores and carnivores. Compared to carnivores, herbivores show a strong expression of enzymes involved in the biosynthesis of different amino acids, especially the pathways involved in the glutamate biosynthesis was significantly increased. Moreover many breakdown enzymes of amino acids including glutamate are significantly lower in herbivores.[149] When comparing a human western diet (protein rich) to diets that contain mainly corn and cassava the fecal microbiomes are similar to the microbioom of carnivores versus herbivores seen in animal species.[6] The relationship between diet and changes in microbiota is thought to be important in human development. The genus Bifidobacteria is associated with the ability to

utilize milk oligosaccharide and decreases during the first year of life due to switch to more solid foods. Interestingly children receiving breast milk have an abundance of Bifidobacteria compared to children on formula-milk. [141] The change in food and age also influences the ratio of the different phyla. Babies have a lower representation of Bacteroidetes and Firmicutes than adults.[105] Both phyla contain multiple genus of bacteria responsible for the biosynthesis of essential nutrients like vitamins and folate.[142] This change might be responsible for the nutritional needs of babies compared to children in later stages of development. Diet changes the microbiota and possibly affects human development.[105] Other factors like geographic location, social structure, exposure to animals and health status are also possible factors in the difference in microbiome. However correcting for the effect of diet in these groups has been a challenge.[4]

The hypothesis that there is a genetic component that changes the microbiome is based on the observation that the microbiome between family members is more similar compared to non-family members. [4] To assess if genetic diversity has an influence on the microbiome, comparative twin studies were conducted. Both subjects were raised under similar conditions and with similar diets. The results show no more similarities between monozygotic (MZ) twins compared to dizygotic (DZ) twins proving that kinship does not affect the microbiota.[140, 105]Another possible explanation of the similarity between family members is the exchange of microbiome during birth. Yatsunenko et al. proved by comparing the microbiome of subjects with their biological parents that this is not the case. The microbiome of a group of children were no more similar to the mother compared to the microbiome of the biological father. [124]

To study if social structure influences the composition of the microbiome a cohort study on elderly people was conducted. [4]The theory is that when people get exposed to more people there microbiome is more diverse. To test this theory they compared so called community dwellers (elderly people that still come in contact with other people) to people in long-stay care. They found that the community dwellers have a more diverse microbiota and are less frail compared to people in long-stay care. All subjects in this study had a relatively similar age and diet and the auteurs claim that an less diverse microbiota due to lack in social contact result in a decreased in health status[4] . However the association could also be seen the other way around. People in long stay care are there because of frail health and as a result have a less diverse microbiome. The association between social contact and microbiome seems logic, however in this study health status cannot be ignored as a possible confounder. [125]

Microbiota affects host immunity

To investigate the effect of the microbiota on host immunity different association studies were conducted in the past decade. In a population of 116 Swedisch infants with a high risk of developing allergies, cultures of the intestinal microbiota were examined and compared to earlier intestinal colonisation studies.[100] They found a significant change in microbiota of infants that were allergic compared to non-allergic infants. They claims that the changed in colonization pattern increases the risk of allergies in this cohort. [100] In another human study they looked at the first discharge of newborns. This discharge is not sterile but contains intrauterine bacteria. Mothers that have a history of eczema get children with a microbiota that is less diverse than mothers without a history of eczema. [126] The association between

the development of IgE-associated eczema and a less diverse microbiome was confirmed in a pyrosequencing study of 1 month old infants. This lack of diversity was most prominent in the phylum Bacteroidetes.[127]

However it is still very difficult to study microbiota- immune association in the human population due to the diversity of microbiota. Therefore researchers use other microbiota models which are easier to standardize. The effect of the microbiota on immune status is extensively studied in animal models. Some of these experiments date back 40 years and were conducted using animals kept under axenic (germ-free) conditions. Comparing these axenic mice with mice kept under normal conditions gave researchers the first clues that environmental microbiota effects host immunity. In 1973, Glaister et al., showed that the number of the epithelial lymphocytes (plasma cells) increases after exposure to a conventional environment in axenic mice. [101] Using the same model researchers also found that 5-Hydroxytryptamine/histamine levels in the wall of the small intestine is lower in axenic compared to normal housed animals . [102] Appling immunohistochemical methods it was shown that axenic mice have decreased MHC II expression on epithelial cells of the small intestine [103] and a decrease in expression of activation markers on intestinal macrophages.[104] Overall, these studies indicate that the lack of a microbiota in mice has severe consequence for the hosts ability to induce an adequate immune response.

Cebra and colleagues did multiple studies using a germfree mouse model to evaluate the effects of microbes on the host immunity. Germfree mice were colonised with segmented filamentous bacteria (SFB) and multiple immunological parameters were measured during 190 days of colonization.[17,18] SFB are commonly found bacteria in many species and colonization with these bacteria increases IgA production. More intriguing was the observation that IgA production by B cells was only for a small part specific for SF bacteria. That the total IgA level goes up but not the specific IgA level pleads that these 'normal' bacteria boost the nonspecific mucosal immunity. [17, 18, 7] In addition to this observation both the percentages of Th17 and Treg cells are reduced in germfree mice and when transplanted with SFB this reduction is restored.[128] The presence of a microbiota affects host immunity and changes can lead to pathologies like allergies. Allergies are associated with changes in the the Th17 response. However their effect on the Treg population also shows the immune suppressive function of the microbiota.

The regulation of PRRs is important in intestinal tolerance

The distinction between symbiotic and pathogenic bacteria is one of the biggest challenges for the host. Microbiota influences the immune response in many different ways. There are specific triggers for the activation of the immune response. These triggers are responsible for the dysregulation of the tolerogenic cascades and influence signalling cascades that promote a pro-inflammatory response. Both the adaptive and innate immune responses are affected by interactions with microbial recognition patterns.[7] Bacteria present different Pathogen-associated molecular patterns (PAMPs) that trigger the immune system. PAMPs comprise of lipopolysacharide (LPS), peptidoglycan (PGN), flagellin and non-host nucleic acids. The recognition of these PAMPs goes though the pathogen recognition receptors (PRRs).[19] Three classes of PRRs were identified: Toll-like receptors (TLRs), Nucleotide-binding oligomerization domain-containing protein (NOD-like) receptors (NLRs), and the retinoid acid-inducible gene-1 receptors (RLRs) [20, 21]

It was shown that the presence of microbes influences the expression of TLRs. Germ-free mice exhibit less TLRs on there intestinal epithelial cells (IECs).[111] IECs are essential in the gut since they form the physical barrier between the lumen and the immune system [111] TLRs are transmembrane receptors with an extramembranous and a cytoplasmic domain. The extramembrane domain is responsible for the protein recognition within the endosome or extracellular surface. The cytoplasmic domain is involved in the signal transduction..[30] Activation of NF-kB by TLRs with the exception of TLR3 and the alternate TLR4-TRIF pathway is only possible through the involvement of the Myeloid differentiation primary response gene-88 (MyD88). TLRs can also signal trough IRF resulting in a type I IFN response.[21] Toll-like receptors are vital in the activation of the innate immune response. (Figure 2)

TLRs are activated by PAMPs from both Gram-positive bacteria and Gram-negative bacteria. The response of TLR on the different PAMPs depends on the type of TLR. [112] (Figure 2) Muzio at al., shows by Northernblot analyses of different sets of human cells types that TLRs are variable between cell types.[31] This expression of TLR is dynamic and is influenced be external factors. Pro inflammatory signals like TNF-α, IL-1β are known to up regulate the expression of some TLRs and signals like IL-10 can block certain TLR. [3,12] Taken together we can state that the expression of TLRs is different per cell type and is influences by the microbiota and external stimuli.

NLRs are a special class PRR and have a primary role in the hosts defence. NLRs are mostly expressed by lymphocytes and antigen-presenting cells (APC's), however they are also expressed by non-immune cells like epithelial and mesothelial cells. NLRs are intracellular receptors and interact with its C-terminal leucine-rich repeat to peptidoglycans (PGN) which are present on the cell wall of both Gram-positive and Gram-negative bacteria. [22 23] TLRs share multiple signalling pathways with NLR's such as MAPK and NF-kB however there activation does not depend on PGNs.[22 23] The activation of NLR triggers different cascades depending on domain organisation of the NLR.[20] It can either result in activation of the pathway though RICK/RIP2, MAPK or the formation of the caspase-1-activated inflammasome. [24, 20] NOD receptors are well studied receptors among the NLR-family which consists of 22 proteins. NOD activates NK-kB, RICK/RIP2 and mitogen-activated protein kinase dependant pathways. [25]

RLRs are cytosolic helicases and the best studied RLRs are retinoic acid inducible gene protein I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5). [32, 20] RLRs are intracellular receptors and primarily recognize nucleic acids belonging to viruses and the C-terminal regulatory domain provides the ability to discriminate between host and viral nucleic acids.[33] As a response to uncapped 5'triphosphate NF-κB and IRFs pathways are activated inducing type I interferon's. RIG1 and MDA5 have copies of the CARD that bind the adaptor proteins necessary for the NF-kB and IRF regulated immune response.[32, 21]

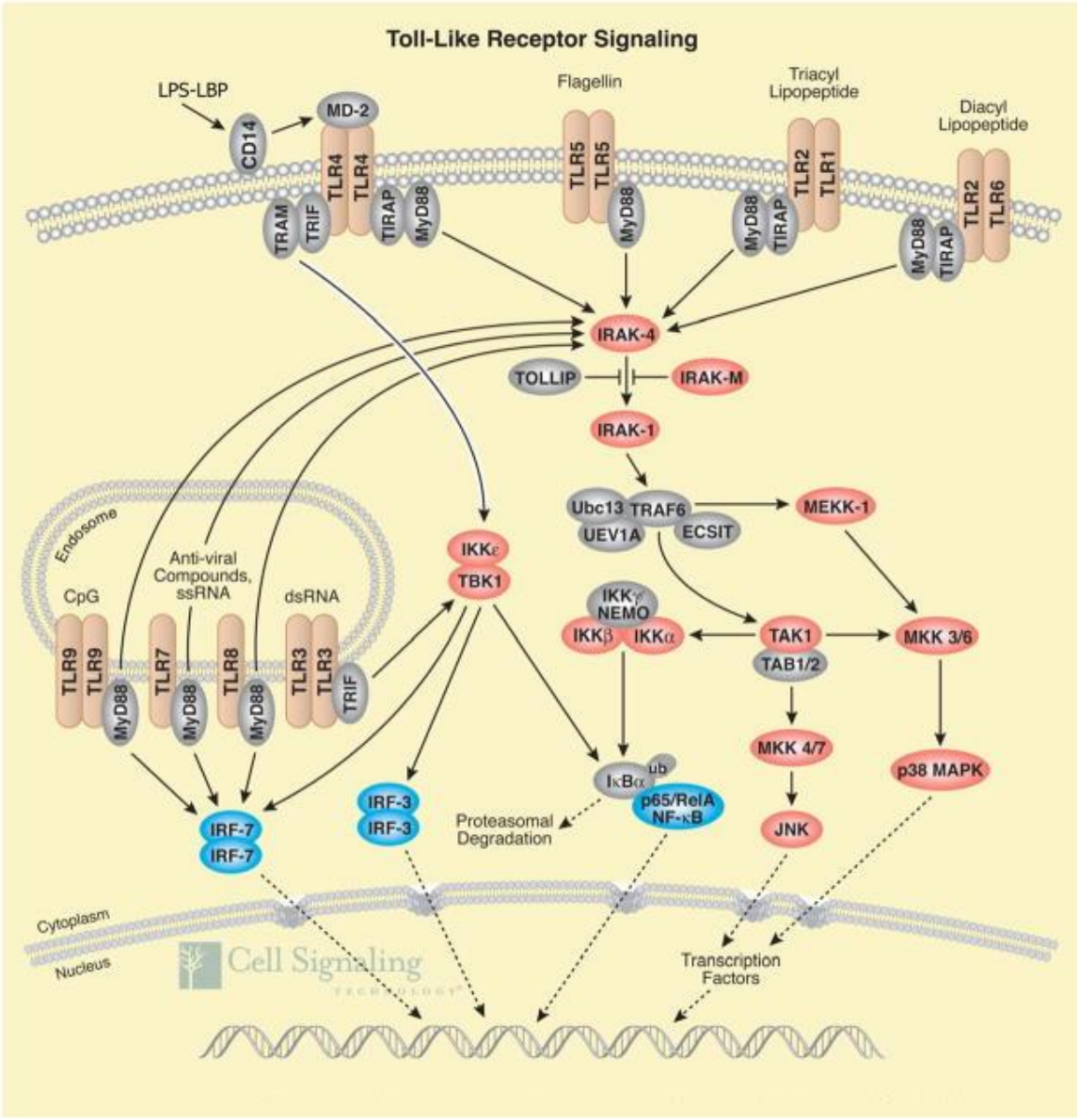

Figure 2: Buchholz, B. M. & Bauer et al. Neurogastroenterol 2010.
TLRs share multiple signaling pathways and act on different PAMPs within the endosome or extracellular surface. TLR stimulation eventually leads to transcriptional activity inducing the type I IFN or MAPK or NF-kB mediated immune response. [112]

The regulation of PRRs is important in intestinal tolerance. Their location determine the type of immune response. For example some types of PRR's (TLR5) are only expressed on the basolateral site of the epithelial cells and not the apical site.[34] Other PRRs (TLR9) are expressed on both sites but activate different pathways depending on the enviorment.[35] The cell type is also relevant, intestinal monocytes do not secrete cytokines in response to PRRs activation in contrast to peripheral monocytes that activate the NF-kB pathway.[36] Studying PRR is relevant in understanding the interactions between the host immune system and the microbiota. [20]

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is the collective name for a group of chronic relapsing infections of the intestinal tract. IBD can be subdivided into several classes, most IBD patient

suffer from either Crohn's disease (CD) or ulcerative colitis (UC). The combined prevalence of Crohn disease and ulcerative colitis is 200 to 300 per 100,000 in the United States.[47] CD mainly affects the Ileum and colon but can be found throughout the intestinal tract. UC is confined to the lower part of the intestines namely the colon and rectum. The main pathological difference between CD an UC is that in 80% of the CD patients deeper layers of the gut wall are affected and ulcerative colitis tents to be more superficial. A minority of the IBD patients suffer from intestinal diseases like Collageneuze colitis, Lymfocytaire colitis, Ischemische colitis, Diversiecolitis, Ziekte van Behçet.[48] In the western world the incidence of autoimmune diseases such as inflammatory bowel disease is increasing. Change in microbiota composition is a possible explanation for this rise in IBD incidence. [101]

The microbiota has several functions within the host. Firstly it provides the host with the capacity to hydrolise plant sugars, detoxifying xenobiotics and sythesis vitamins. Secondly it functions as a physical barrier by making the epithelium less permeable. Thirdly it provides a competitive barrier for pathogenic microbes. Lastly it also stimulates the immunological barrier by priming the innate and the adaptive immune system for action. [12] Since the microbiota affects so many processes in the gut it is thought that it plays an key role in gut pathologies like IBD.

Genetic susceptibility and IBD

There are numerous different IBD susceptibility genes. Different IBD susceptibility genes have been identified using genome-wide association studies, most of the susceptibility genes are involved in immunological processes. [65, 66] On the basis of these human genetic studies different transgenic and knock-out mouse strains were generated to study the effect of identified genes on intestinal inflammation.[67] Multiple innate genes like PRRs but also adaptive immune genes, autophagy genes, tolerance genes and metabolic genes are associated with susceptibility of IBD. [65, 66]

<u>Nod1 and Nod2</u>

Experiments using Nod knockout mice suggest a link between bacterial interactions and IBD.[20, 71, 72] This observation can be explained by two processes. Firstly, a mutation in Nod 2 results in a deficiency in barrier function and decreases the attraction of immune cells to clear bacteria.[70] This loss in barrier function could be a mechanism that perpetuates IBD and the invasion of bacteria could lead to increased inflammation.[65, 73] Secondly, a primary dysfunction of the innate immunity leads to excessive activation of pro-inflammatory signals causing IBD.[20] Which of the two mechanisms is important in IBD is still under discussion. In other words is the susceptibility for IBD due to changes in microbiota or is it the biota changed because of the disrupted immune response. By evaluating the effect of immune genes on the protection of the integrity of the gut mucosa we can explore if immune deficiencies cause or perpetuate IBD.

NOD polymorphisms are significantly associated with early onset IBD [24] Missense mutations (R702W and G9008R) and one frameshift mutation (L1007fss) in NOD2 gene suggests that a loss in gene function raises the susceptibility to CD. These mutations disrupt the normal innate response causing an compensational adaptive response. This adaptive response might be to strong thereby causing IBD. An excessive immune response caused by NOD receptors can be facilitated by their effect on the MAPK and NF-kB pathways. Hyper activation of both

these pathways increase inflammatory proteins and are associated with IBD [68,20,69]Using Western blot analysis Waetzig et al., showed that MAPK's (p38-alpha, JNKs and ERK1/ERK2) were significantly more activated in patients with inflammatory bowel disease. [6820] Furthermore Hyper activation of p65 (a subunit of the NF-kappa-B transcription factor) induced a IBD like phenotype in mice. [20,69] Further proof for the involvement of the MAPKs and NF-kB pathways is the association between the transcriptional activity of the NOD domains and IBD. Activation of MAPKs and NF-kB is regulated by the same upstream regulatory proteins, Hsu et al., shows that the adaptor protein Caspase Recruitment Domain 9 (CARD9) part of the NOD receptor is a discriminating factor in the activation of either the MAPK or NF-kB pathway.[26] In a Mucosal genome-wide methylation scan CARD9 methylation was associated with IBD. The association between methylation of the CARD9 and IBD status points to the importance of the MAPK pathway. CARD9 seems to activate the MAPK pathway and less so the NF-kB pathway. [115] NOD 1 and 2 triggers different cascades depending on their domain organisation. Deregulation by for instance methylation of the CARD domain could disrupt gut homeostasis by hyper activation of the MAPK pathway.[26,29,116]

To asses if Nod mutations lead to a primary dysfunction of the innate immunity the activation of proinflammatory signals were assessed by looking at inflammatory parameters in knockout mice. Nod1 and Nod2 knockout mice on a C578L/6 background were administered with Dextran sulphate sodium and compared to single knockouts. The level of Regenerating islet-derived protein 3 gamma (RegIII-γ) was the same in Nod1-/-;Nod2-/- vs Nod1-/+;Nod2-/+ under the condition that they both had a comparable microbiota. (RegIII-γ is the mice homolog for HIP/PAP c-type lectin that has a direct activity against Gram-positive bacteria) This observation suggests that the primary immune response against bacteria in the mucosa is not responsible for the IBD phenotype.[72]

Is it the increased permeability that causes inflammation or does increased inflammation contributes to increased permeability? A predisposition to IBD as a result of breaches in the intestinal barrier seems likely. However in mice with disrupted tight junction function, intestinal permeability went up but mice did not develop spontaneous colitis.[76] Histological analysis of the intestines from Nod1-/-;Nod2 -/- knockout mice show no granulocyte infiltration. The lack in Nod does not result in spontaneous inflammation, nevertheless 5 % of these knockout mice do not have the ability to maintain bacteria within mucosal compartment.[72] If we compare this with the situation in the human population we see the same trend. Only a small percentage of people with the Nod2 mutation develop symptoms characteristic for IBD patients. [70] Loss of barrier function is therefore unlikely the primary cause of IBD but rather a mechanism that perpetuates the IBD phenotype. Combining the results of these experiments we could hypothesize that there must be a threshold above which the barrier function becomes essential in maintaining intestinal integrity.

The barrier function is more relevant in the perpetuating IBD rather than the primary immune response. In a study performed by *Buhner et al.*, IBD patients possessing a mutation in the Nod2 gene have an increased permeability of the gut. [70] In biopsies taken from IBD patients it was shown that the there is a disruption of the actin cytoarchitecture in the epithelial cells and paracellular area. [63] Nod1-/-;Nod2 -/- knockout mice have a barrier dysfunction and showed colonic paracellular permeability and decreased expression in E-

cadherin.[72] The ability of Nod2 in limiting bacterial invasion is attributed to the effect it has on Paneth cells. Nod2 receptors can activate NF-kB dependent production of alfa-defensins, specifically alfa-defensin 5 and 6.[74] Both the loss in E-cadhedrin and alfa-defensins cripple the barrier function and make the layers underneath intestinal epithelium accessible for bacteria. NOD dysfunction affects the barrier function leading to inflammation of the intestine. This observation is supported by the results from an experiment using a zebrafish embryo *Salmonella* infection model. In this experiment they showed that a lack in Nod reduces the ability to control systemic infection.[75] A lack in PPR signalling give opportunist bacteria the change to cause infection in the gut.

In humans the role of a healthy gut function in protecting against opportunist Bacterial infections becomes apparent when it is changed. Human patients receiving cytotoxic chemotherapy develop systemic bacterial infections. Several parts of the protective mucosal barrier are damaged by chemotherapy giving pathogens a higher chance of infecting the host. [129] The normal gut flora can also be changed by using antibiotics. Analyses of human fecal samples by culturing them for specific exogenous bacteria showed an expansion of these bacteria as a result to the use of antibiotics [130] Fluoroquinolone is an antibiotic that reduces the presence of Proteobacteria in the gut by a 10 fold. Patients that receive Fluoroquinolone before Hematopoietic Stem Cell Transplantation have a lower risk for Gram-negative bacteremia. Non-treated patients with an higher presence of Proteobacteria have an 5-fold increased risk of developing gram-negative bacteremia. [131] The conclusion from this observation is that when the diversity and stability of the microbiota is compromised the result is that the dominant bacteria can cause an infection. So when gut integrity is compromised be it either by chemotherapy or genetic factors a balanced microbiota can protect against bacterial infections.

In mice the administration of antibiotics prior to pathogen introduction can also increase the susceptibility to infection. The introduction of specific bacteria within an axenic mouse show an increased susceptibility to pathologic bacterial infections like *Shigella flexneri*,[107] *Bacillus anthracis*[108] and *Listeria*[109] Even in lower animal species the lack of an healthy microbiota is associated with an increase in susceptibility to infection. For example microbe-free aseptic mosquitoes have an increased susceptibility to Plasmodium and this effect is normalised after they were fed with bacteria.[110] The surface of the mucosa is colonised by many different populations of bacteria. By changing the microbiota in a susceptible host some type bacteria have a higher chance in causing infections which in the healthy microbiota would not cause problems.

Less self-tolerance of CD4 T cells is associated IBD

Intestinal integrity is highly important for the immune response against bacteria in the gut. Normally the microbiota is limited to the intestinal lumen and the mucosal immune compartment. Immune responses is tightly regulated in the gut and prevent exaggerated adaptive immunity to commensal bacteria.[117] Escape of commensal bacteria by NOD dysfunction or other innate deficiencies is not enough to cause IBD and depends on the type of immune response it induces.[118] How the immune system processes the antigens on these fugitive bacteria determines the adaptive response.

If a T cell encounters a specific antigen in the absence of an infection it becomes tolerant. TCR interactions without the presence of co stimulatory signals like CD28 or ICOS or a strong activation of negative co-receptors for example CTLA4, PD1 and BTLA cause the expression of tolerance genes. The most studied example of a tolerance gene is E3 ubiquitin ligase, this gene inhibits TLR signaling and recruits transcriptional suppressors of cytokine genes.[40] This tolerance prevents a misdirected immune response that might lead to IBD. Hyperresponsivenes is one of the hallmarks for the development of IBD.[79] *Duchmann et all.,* showed in an experiment using Lamina propria monocuclear cells and periferal monocuclear cells from IBD patients that the activation markers on both CD4 and CD8 T cells are up regulated when co cultured with indigenous bacteria. [80] In healthy controls this effect did not occur, demonstrating that there is less self-tolerance in CD4 and CD8 T cell populations in IBD patients.

Patients carrying a mutation in the Aatg16L1 allele (autophagy-related gene) have a higher risk developing CD and show abnormal Paneth cell morphology.[81] In a study in mice it was shown that the gene Atg16L1 only causes Crohn like symptoms in the presence of a specific virus.[77] Interestingly the same researchers also found a link between CD and the Nod2 receptor. Atg16L1 is a gene involved in the antibacterial response by the innate immune system, more specifically in the incorporation of the bacteria in the autophagosome. The Nod2 receptor is intracellular and therefore depends on autophagy. Using Fluorescence microscopy, researchers found that mutant Nod2 failed to recruit atg6L1 to the plasma membrane thereby failing to induce bacterial autophagy. (The atg16L1 mutant they used for these cell experiments had the same frameshift mutation that is found in the human population) With other in vivo and in vitro experiments they also showed that this mechanism is independent on the adaptor RIP2 and NF-kB pathway.[78] The NOD2 and atg genes associated with CD are both important in the autophagy-dependent antibacterial pathway. Both the genes act on the same axis and protect in the development of CD.

Autophagy related genes (NOD2, IRGM and atg16L1) seem to be of important in determining the fate of T cell tolerance. Whether or not we can extrapolate the findings on CD for a general conclusion about IBD remains unclear. There are approximately 100 confirmed loci associated with IBD and most of them are involved in the innate immunity. The genes that are specific for ulcerative colitis (HNF-α, CDH1, LAMB1) are all involved in the barrier function of the epithelial the genes associated with CD in autophagy-dependent antibacterial pathways. This seems to be in concordance with the fact that CD is a disease caused by a defect in the deeper layers of the gut wall and ulcerative colitis more superficial.[66, 83] (Figure 3) It also means that because IBD is not one disorder the future of IBD treatment will possibly be in personalised treatment.

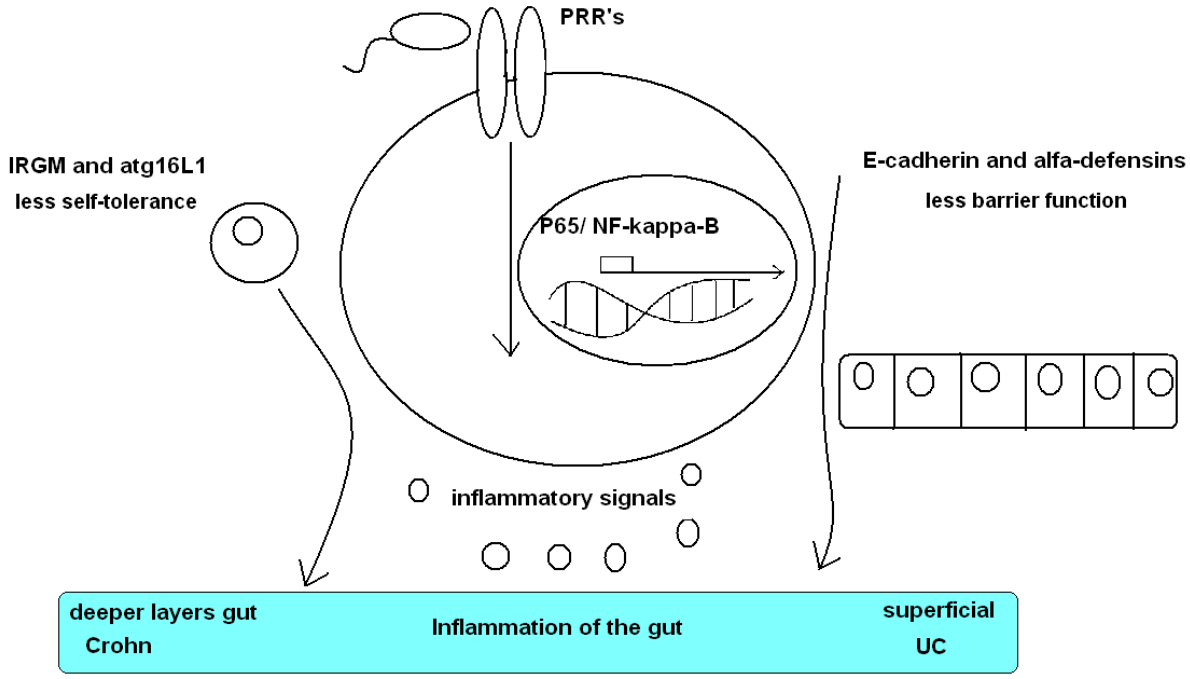

Figure 3: Multiple antibacterial pathways are associated with the development of IBD. When affected these different susceptibility genes dictate the clinical manifestation of IBD. Genes involved in the autophagy-dependent antibacterial pathways are correlated with Crohn's and genes involved in gut barrier function more with UC. Genes on the PRR-Nf-kappa-B/IFN axis effect both clinical manifestations of IBD. [66, 83]

IL10

IL-10 is one of the genes that is associated with susceptibility to IBD. When taking a more detailed look at this data we can conclude that IL-10 and IL10β deficiencies are associated with UC rather than CD. [65, 66] In concordance with this observation, patients that have a homozygous mutation of the IL-10R develop deep ulceration of the intestinal mucosa. [157] Multiple knockout studies in mice suggest an regulative function of IL-10R in the intestinal track however IL-10 molecule therapies seem to vary in their effectiveness against IBD.[163] This difference in effect of IL-10 on IBD status can be attributed to different causes like systemic side effects, dividing patients in the right subgroup or the way it is administered. Using genetically modified bacteria expressing the human IL-10 researcher were able to deliver IL-10 directly in the mucosa. In a phase 1 trial transplanting these bacteria into IBD patients a decrease in severity was observed.[162]

IL-10 deficiencies show that IL-10 has an anti-inflammatory effect. [163] The antigens presented by commensal bacteria do not cause a strong immune response within the gut thanks to the expression of chemokines and cytokines like IL-25 and IL-10. [155,162] These cytokines and chemokines are excreted by epithelial and myloid derived cells and orchestrate the immune response within the gut.[155] NOD2 signaling leads to secretion of IL-10 effecting intestinal homeostasis [164]Futermore IL-10 is known to inhibit the TLR signaling in IEC's thereby limiting Th1/ Th17 response.[160]

IL-10R is expressed by multiple cells such as T cells and monocytes/ macrophages. Monocytes have IL-10R and these cells in IBD patients are less responsive to IL-10.[158]

Monocytes are also known to produce less IL-10 in IBD patients. [158] Macrophages are activated by pro inflammatory signals and this pathway is inhibited by IL-10. Lowering macrophages activation leads to secretion of cytokines skewing the Treg response(Foxp3+).[88,89] There are various subsets of Tregs, it is thought that inducible Tregs are most relevant for the gut immunity.[111] This assumption is basses on the reduced expression of IL-10 in the GALT of germfree mice.[115] In Patients with IBD the presence of Tregs increases in the intestine. However the accumulation of intestinal Tregs does not correlate with IBD severity. [156] In concordance with this observation patients with IPEX syndrome (These patients have a defects in the transcription factor FOXP3) also develop a colitis like phenotype.[159] Therefore the change in Treg (CD4+ CD25+) response due to deficient IL-10 signaling cannot fully explain the IBD phenotype.

Rather than expansion it is the FOXP3 expression in Tregs that regulate there immunosuppressive capabilities. Either a mutation in IL-10R or FOXP3 makes Tregs loose there inhibitory effect on effector T cells. Using a T cell transfer model researcher found that it is the secretion of IL-10 by monocytes (CD11c+CD11b+F4/80+) in the gut that regulate this FOXP3 expression in Tregs thereby preventing colitis.[161] In summary IL-10 limits PRR signaling in IEC's and myeloid cells in the case of chronic stimulation by commensal bacteria. IL-10 also bridges the regulation between microbial sensing and the effector T cells.

IL23R

In multiple genome wide association studies there is a significant association between CD and the IL23R gene.[150,151] Multiple noncoding variants of the IL23R gene are associated with CD and even a rare variant was found that seems to protect against CD.[151] This so called R381Q variant is the result of a single nucleotide polymorphism (Arg381Gln). This polymorphism is located in the binding domain with JAK2 kinase. SNP's in the JAK2 are also associated with IBD .[82] Since Intracellular phosphorylation of JAK2 is known to be responsible for STAT3 dependant up regulation of IL17 this IL-23- IL17 axis might be involved in IBD.[15] In another population study not the entire control group has this variant and consistently the CD group is not completely deprived from this R381Q variant.[152] Therefore this IL23R isoform does not fully explains the function of IL23R in CD. Instead of the IL-23 effect on Il-17 production, IL-23 is also involved in the control and expansion of Th17 cells.[154]

Th17 cells contribute to the host defence but are also infamous contributors to auto-inflammatory functions. The increase in IL-23 skews towards an Th17 phenotype that could potentially cause an inflammatory response.[84, 85] In concordance with this observation neutralisation of IL-23 rescues the IBD phenotype in mice models.[86,84] Using Il23-/- mice it was shown that Il-23 drives intestinal inflammation but not systemic inflammation. [88] When Th17 response was measured using intracellular flow cytometry no Th17 skewing was observed in the blood.[87] To specify on the Tcell responsible for the IL-23 inflammatory response in IBD we must look at which T cell subsets that are present in the gut. CD4 T cell subsets which are affected in the lamina propria of axenic mice are intraepithelial lymphocytes (IELs) and γδ T cells. [111, 114] IL-23 is known to promote a T cell subset called ILC

(Thy1+ Sca1+ RORγt+), expressing both IL-17 and IFNγ. This is an interesting observation since ILCs are thought to be important in the guts pathogen sensing. The T cells found in the intestines depend on stimulation by adenosine 5' –triphosphate a trigger that activated inflammatory pathways through RORγt.[33] Innate lymphoid cells (ILCs) are both RORγt positive and IL-23 sensitive [88] forming a possible candidate for being the subtype effected by Il-23 regulated inflammation in IBD.

The question how the Microbiota influences this IL23-IL17 axis in CD4+ RORγt+ T cells is still unanswered. To find the answer to this question we must again look at how commensal Bacteria are detected in the gut. The intestinal epithelial cells (IECs) are the first cells to come in contact with the commensal bacteria in the gut lumen. Detection by TLRs trigger the release of cytokines and chemokine's that can negatively regulate the frequency of Th17 cells.[154] When GF mice were compared with normal mice IL25 mRNA expression was reduced in the intestine. [155] IL-25 is produced by IEC's and can negatively regulate the frequency of Th17cells by inhibiting the production of IL-23. By isolating different sets of intestinal cells and testing them for IL-25 responsiveness CD11b+ cells were identified as the link between IEC and CD4+ cells. IL-25 treatment on CD11b+ cells inhibits their ability to produce IL-23.[155] All together these observations lead us to conclude that commensal bacteria limit the Th17 response in the gut by inhibiting IL-23 Through IL-25 production. The cells involved in this regulation are IEL's, CD11b+ macrophages and CD4+ RORγt cells (Figure 4)

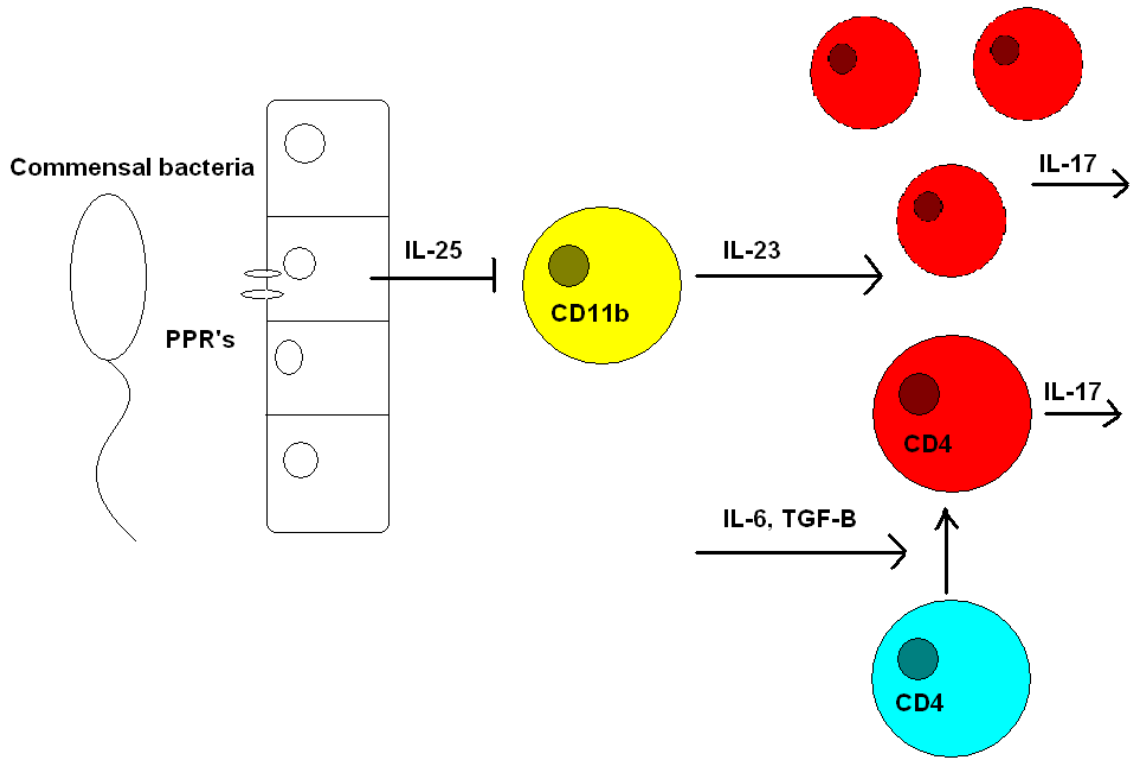

Figure 4: Summary of the effect of commensal bacteria on the IL-23-IL17 axis. Commensal Bacteria signal through TLR's on IEC's causing the expression of chemokine's and cytokines like IL-25. Expression of IL-25 inhibits the expression of IL-23 by CD11b positive cells. The lack of IL-23 limits the expansion of Th17 cells in the gut[155150,151]

Altered microbial community in IBD patients

We have already discussed that genetic defect alone are not enough to cause the IBD phenotype. [72] The human microbiota is thought to plays a key role in the development of IBD; the shift in composition drives the chronic inflammation in these patients.To prove this claim researchers have compared biopsies from IBD patients (6 CD and 6 UC patients) with healthy controls (n = 5). [49] By in dept sequencing they found that there is less microbial diversity in IBD patients compared to controls. More specifically, patients with IBD have a microbiota that is less diverse among members of the *Firmicutes* and *Bacteroidetes* than controls. The total number of *Firmicute* was reduced and there was an increase in the number of Bacteroidetes.[9] A larger scale analysis by Culture-independent rRNA sequence analysis showed a similar shift in composition, however they show a decrease in the number of *Bacteroidetes*. This inconsistent result is possibly due to inter-individual variation. Another important observation is that there is a strong increase in the proportion of *Proteobacteria* in the IBD microbiota. (Figure 5) [49]In inflamed biopsies of CD patients there is a significant increase in a specific family of bacteria called *Entrobacteriaceae* compared to non-inflamed biopts.[9] This family of bacteria belongs to the phylum *Proteobacteria* and includes *Campylobacter concisus Escherichia, Shigella* and *enterohepatic Helicobacter* which are all associated with the pathogenesis of IBD [56]

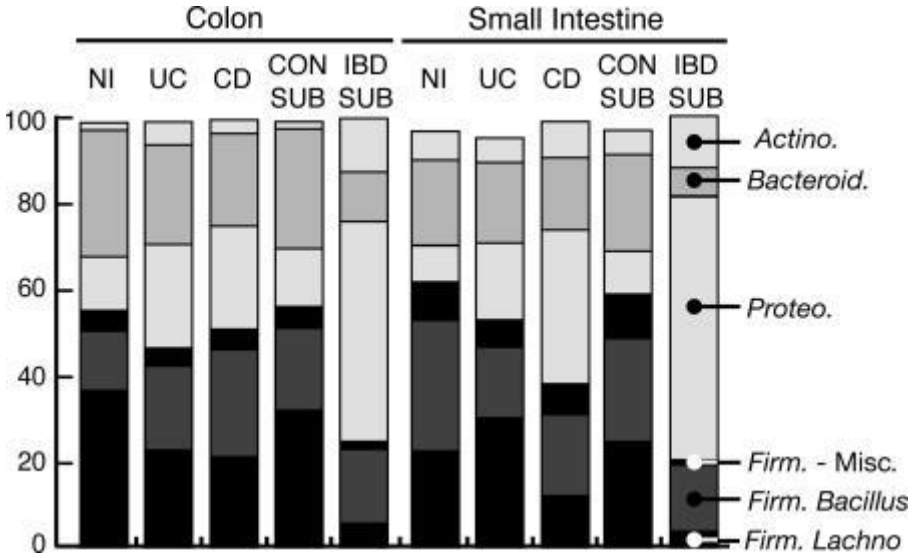

Figure 5: Frank et al. Proc. Natl 2007.

Culture-independent rRNA sequence analysis comparing tissue from CD, UC patients to non-IBD controls. Graph shows a representation of different phylum-levels depicted in percentages. Bars do not reach 100% because rare phyla were not included. IBD subsets represent no inflamed (NI), ulcerative colitis (UC) Crohn's disease (CD) and Control subset (CON SUB)[49]

Escherichia/Shigella are closely related (ID: 561) and are significantly more present in the microbiota of IBD patients compared to healthy controls.[54]. Escherichia and Shigella belong to the proteobacteria and are associated with pathological conditions in IBD mice models. A microbiotic shift towards more *proteobacteria* is possibly caused by the compromised gut integrity and not the other way around. A Plausible explanation for the presence of high number of *Escherichia/Shigella* in the intestines of IBD patients is that the tissue-destruction

due to inflammation provides the environment which favors bacteria with an enhanced fitness. There is also the possibility that the difference in energy source between the different bacteria is of importance.

By using an analytic tool called spare multivariatable linear modelling researchers were able to correct for multiple confounders within an exceptionable big database containing the microbiota information of a big group of IBD patients. One of the findings was that there is an increase in the glutathione transporter gene, N-acetylgalactosamiphosphotransferanse (NAG transport) and the metabolism in the intestine shifts towards a more cystiene rich environment. [54] This increase in glutathione and cysteine has a strong effect on the mucosal barrier. This barrier strongly relies on mucin which is full of glycosylated sugars and cystiene. Because of the higher expression of mucin in IBD, bacteria like *Escherichia coli* are allowed to thrive since they can use mucin as their primary energy source. This assumption is based on multiple experiments in which they knock out the MUC gene or block its expression, as a consequence of this they see a decrease in bacteria related to *Escherichia coli.* [58, 59] However mice with a deleted MUC2 gene develop inflammation of the colon.[60] The protective role of mucins is a double edged sword on the one hand forming a protective barrier on the other it is a medium for pathogenic bacteria to grow on. [61]

Besides a competitive advantage due to the enhanced mucin levels *Escherichia/Shigella* has another trick up its sleeve. *Escherichia* and related bacteria can use ethanolamine as a nitrogen source. [62] This ability gives them a major advantage when coping with oxidative stress. By measuring nitric oxide (NO) and markers of oxidative injury (carbonylation and nitrotyrosination) the group of Keshavarzian et al., [63] showed that oxidative injury correlated with the phenotype severity in IBD. [64] This indicates that *Escherichia* has an advantage over other bacteria like those of the Firmicutes phylum when there is an increase in ROS. Altered community composition of the microbiota and the presence of proinflamatory microbes (*Escherichia/Shigella*) may promote the increase in pro inflammatory Tcell populations perpetuating the IBD phenotype. Together with host susceptibility (genetic immune-specific mutations) and the lack in commensal bacteria (*Lactobacillus plantarum, Lachnospiraceae*) and pathobionts (*Bacteroides. Fragilis*) it creates an environment susceptible for developing IBD (Figure 6)

How can microbes alter susceptibility towards IBD?

In the human intestine it is important to differentiate between pathogenic and mutualistic bacteria. Autoimmunity arises when there is less self-tolerance in CD4 and CD8 T cell populations. [40] The modulation of CD4 cells depends on cytokine milieu and results in their specific homing and phenotype (Th1, Th2, Treg or Th17). Disregulation of the intestinal lamina propria results in an increase in Th1 and Th17 population and a decrease in Tregs. [41] Disturbance of the T cell homeostasis results in less tolerance against bacterial epitopes . [43] The presence of a microbiota modulated the presentation to the immune system thereby affecting the adaptive response.

The generation and clonal expansion of specific T and B cells play a vital role in the adaptive response against pathogenic bacteria.[119] The process of T and B cell tolerance is dependable on both central and peripheral control. The central control of T cells is mediated in the thymus. In peripheral control intrinsic (Treg and tolerogenic DCs) and extrinsic (apoptosis,

phenotype skewing transcriptional and epigenetic) mechanisms are involved. Research shows that the commensal microbiota programmes the process of T cell differentiation. Intestinal adaptive immune cells require a microbiota for their development and function. For the adaptive immune response against microbes the balance between regulatory T cell and inflammatory lymphocytes such as Th1 and Th17 T is essential. [39]

The presence of Firmicutes and Bacteroidetes are beneficial within the hosts gut and both the bacteria and human experience increased fitness as a result of this relationship.[12] When Bacteroidetes however escape the intestinal luminal environment they can cause pathology, including bacteremia and abscesses. [10] For example Bacteroides thaetaomicron a symbiotic bacteriodete present in the gut can become pathogenic when the host is deficient in either IL-10 or TGF-β. [132] Location and gut integrity are key in preventing bacterial infection. There are however bacteria that are more likely pathogens like transient bacteria. They are foreign and can be a problem within the gut because they can induce a strong immune response. These bacteria did not co-evolve with the host and therefore did not develop a mutualistic relationship with the host. This forceful colonization can triggers a persistent immunological consequence even after this transient bacteria is cleared. [133]

Firmicutes
Lactobacillus plantarum belongs to the phylum of *Firmicutes* . This bacterium secretes a peptide which has a characteristic abundance in serine and threonine within its sequence. In vitro studies show that this protein (STp) causes DC to produce IL-10. IBD Patients lack this peptide in the gut hinting to the possibility that the presence of *Lactobacillus plantarum* is beneficial for IBD patients. [50] *Lachnospiraceae* another *Firmicutes* was significantly decreased in IBD patients again pointing to the commensal interactions of *Firmicutes* with the host. When it comes to the Firmicutes it is difficult to make a generalisation whether or not they are mutualistic. The Firmicutes contain different clusters that are known to produce toxins and are mainly pathological. Within the family of Clostridia, cluster I is pathological because of their toxin producing nature. Cluster IV and XIV however contain a lot of non-toxin forming bacteria like *Roseburia intestinalis* . [54]

The correlation between the presence of *Rosarburia and IBD* was shown in an experiment using the microbiota of intestinal biopsies and stool samples from 231 IBD patients. 16S gene pyrosequencing and shotgum metagenomics was performed to characterize the microbiota of IBD patients. After adjusting for multiple confounders they found two types of bacteria namely *Roseburia* and *Phascolarctobacterium* that were significantly reduced in both UC and CD. [54] *Roseburia intestinalis* (ID: 166486), *Eubacterium* (ID: 1730) and *Phascolarctobacterium* (ID: 33025) all belonging to the pylum of Firmicutes [8, 96, 97] The levels of these bacteria were correlated with the number of Tregs present in the gut of the host. Besides the higher number of Tregs the levels of IL-10 was also higher showing that cluster IV and XIV Clostridia of the *Firmicutes*increase the hosts regulatory T cell response.[128]

Bacteroidetes
The microbiota composition is regulated by diet. In African children the microbiota was dominated by *Bacteroidetes* 73% compared to 27% *Bacteroidetes* in European children.[6] Most relevant difference in diet was the high fibre intake in the African group. This difference in percentage of *Bacteroidetes* is associated with less autoimmunity in African

children. High fibre diets and the presence of *Bacteroidetes* are beneficial for IBD patients.[52, 53] *Bacteroidetes* like *B.fragilis* induce a Treg and IL-10 response in the mucosa and are beneficial for IBD patients.[51,119]Introduction of *B. fragilis* that lack expression of polysaccharide A is however not able to induce a Treg and IL-10 response. This mutated *Bacteroidete* tips the Tcell balance towards Th17. PSA present on many Bacteroides can signal through specific PRR's like TLR-2 underlining the importance of PRRs-Tcell interaction on differentiation in intestinal tolerance. However which other PPR's are responsible for the effect of PSA on Th17 cells is not completely clear. [134]

Microbiota influences inflammatory T cell subtypes shaping IBD status

Bacteria like *Bacteroides fragilis* are beneficial in a colitis mice model by increasing the Tregs response.[119] *Bacteroides. Fragilis* is a filamentous bacteria and is able to induce a Th17 response however it does not cause intestinal inflammation. Why is it that the Th17 induced by this bacteria does not cause inflammation? The answer might be in the fact that both Tregs and Th17 share common cytokine and transcriptional networks. TGF- β is essential to both Tcell subsets and there transcriptional regulators RORγt and FoxP3 are inhibited by the same effectors. Strangely *Bacteroides fragilis* is not beneficial for other inflammatory diseases like rheumatoid arthritis. This effect was unexpected since rheumatoid arthritis is associated with the increase in Th17 phenotype and decrease in Tregs.[120] *Bacteroides. Fragilis* has the potential to both induce an inflammatory response and to develop tolerance. Because *Bacteroides. Fragilis* is a common bacterium within the host gut, controlled inflammation is necessary. The alteration of the host immune system due to autoimmune-specific mutations within the genome combined with a decrease in tolerance against *Bacteroides. Fragilis*. could possibly effect IBD status [121]

It's also possible that the function of Th17 induced by Bacteroides. Fragilis are different from those found in rheumatoid arthritis experiments. The group of *Ono et al.* even claims to have found intestinal Th17 cells with a regulatory activity. Besides the recruitment and development of Th17cells the microbiota is also involved in the functions of these cells. When doing an adaptive transfer of Th17 cells from normal mice compared to colitis mice only the last one causes inflammation. Not all Th17 are the same in activity for example the presence of a microbiota in mice lowers the expression of IL-22 in cells that express RORγt+[135]. Another way the microbiota influances the function of lymphocytes is by chemokine expression. Th17 needs IL-1β and IL-6 to activate the IL-17 specific transcription factor RORγt. Increased Il-6 and IL-6R levels are correlated with IBD severity [165] and the presence of a healthy microbiota lowers IL-1β.[166] This shows the importance of the activity of these cells and not only skewing of the Tcell phenotype.[137]If it is true that the Th17 response induced by Bacteroides. Fragilis is functionally different from the classic idea of a Th17 response, this might explain why they induce a TH17 response but do not cause inflammation.

Introduction of Bacteriodetes like *B. fragilis* in mice protect the host against inflammation of the gut by expansion of both Th17 and suppressive Tregs. Mutualistic bacteria like *B. fragilis* provide immune protection by directing the development of all subsets of Tcells effecting the immune response in general. The difference between mutualistic and pathogenic bacteria is that the change in immune response is more subtle thereby keeping the immune system on standby. The composition of the microbiota shapes the balance between immune

regulatory T cells and pro inflammatory T cells. In a healthy host both subsets are available. When one of the two subsets is overrepresented the balance either shifts toward autoimmunity or immune deficiency.

Effect of microbial products on intestinal inflammation in IBD patientsBacteriodetes and some type of Firmicutes have evolved in a mutualistic fashion with the host providing essential nutrients that increase the hosts fitness . The intestines are full of Short chain fatty acids (SCFA) such as acetate, propionate and butyrate they are the product of the fermentation process of undigested polysaccharides by bacteria.[53] The different species produce beneficial by-products from these polysaccharides for the host. *Firmicutes* and Bacteriodes have several hundred proteins involved to metabolize dietary polysacharide.

A study conducted by the group of Den hond et al., show that patients with active IBD have a decreased colonic butyrate oxidation. This lack in oxidation suggests that the mucosa in these patients is not able to maintain the availability of butyrate.[93] Butyrate influences a number of cellular effects such as cell proliferation and differentiation, it is beneficial for the epithelial barrier and is a substrate for colonocytes.[94,71] The lack in intracellular butyrate in Coloncytes causes a down regulation of monocarboxylate transporter MCT1. Thibault et al. shows in both colonic tissues from patients with IBD and in intestinal epithelial cells (HT-29) that MCT1 mRNA expression is negatively correlated with IL-1b mRNA expression.[95] The results from these experiments combined leads us to conclude; that decreased butyrate oxidation has clinical consequences and the reduction of the intracellular availability of butyrate in colonocytes causes downregulation of MCT1, thereby increasing IL-1b expression. Influencing the metabolic rate of butyrate would be an interesting target for IBD treatment.[5] Promoting butyrate metabolism has a possible positive effect in treating IBD. Influencing the microbiota would be an indirect but elegant way to influence the butyrate metabolism. However it is difficult to study the influence of different colonies of bacteria within the gut because of the diversity of microbiota within the human population.

Van den Abbeele et al. developed a model in which they can create an in vitro microbial ecosystem to mimic this unique microbiota. The Inter-individual differences among human subjects were characterised by human intestinal tract Chip (HITChip) and the unique microbial patterns were simulated in vitro. With the model created by Van den Abbeele et al. they were able to show that the bioavailability of butyrate can be increased by colonizing the mucin layer with *Roseburia intestinalis* and *Eubacterium*.[55] *Roseburi* uses acetate and produces butyrate, *Phascolarctobacterium* is a propionate producer. [98] The biggest amount of butyrate in the human large intestine is produced by Firmicutes.[98] Another possible candidate to increase butyrate metabolism is by increasing the number of Faecalibacterium prausnitzii which make up 8% of the total microbiota.[5,6] Reduction of bacteria responsible for butyrate metabolisme seem to be associated with IBD. These bacteria thrive in the presence of indigestible fibres completing the SCFA circle (Figure 5).

Diet and other lifestyle changes can influence the microbiota and may represent an additional way to influence butyrate metabolism. Comparing the microbiota of African children to western children, Shigella and Escherichia were significantly underrepresented. [6] Limiting Shigella and Escherichia by changing your diet is possible however there could be a more elegant way of limiting these bacteria but sparing? the rest of the microbiota. This is

possible by targeting specific characteristics of this bacteria with a drug[15]. If *Escherichia/Shigella* deteriorates IBD status both a high fibre diet and targeted antibiotics are possible targets for treatment.[5]

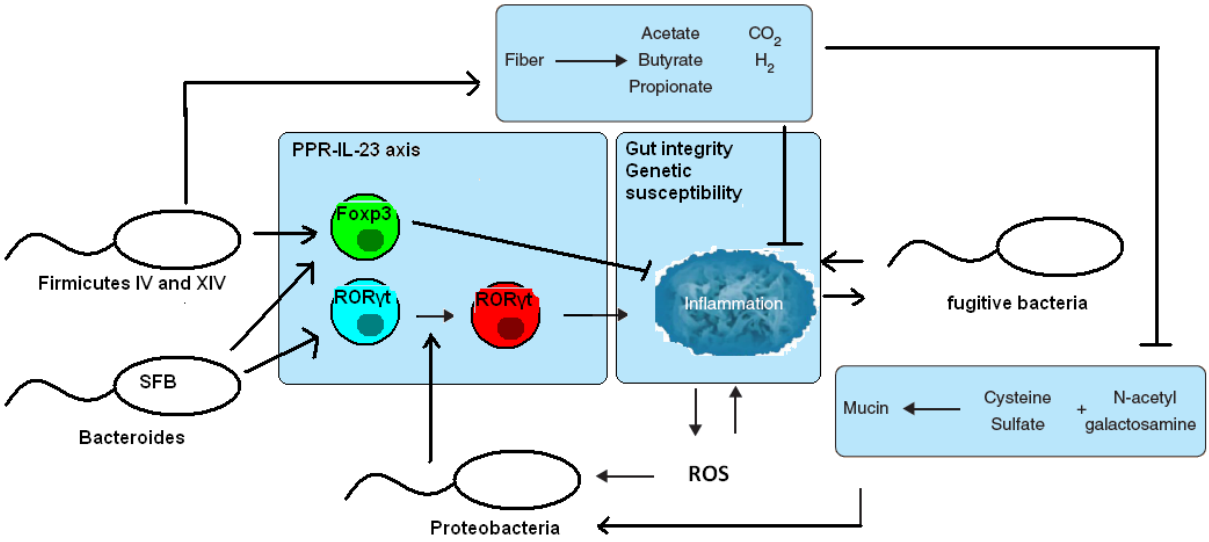

Figure 6: Adapted from Morgan et al. Genome Biology 2012
Summary microbiota balance in IBD: Bacteriodetes and some type of Firmicutes increase the bioavailability of butyrate and increase Tregs and IL-10 expression. Presence of these bacteria recruits Tcell subsets to the gut in a subtle manner increasing the barrier for pathogenic bacteria (green: Foxp3 Bleu:RORγt). Higher expression of mucin and ROS in IBD shifts the microbiota balance towards proteobacteria. Gut integrity and genetic susceptibility increase the chance of infection of the gut by fugitive bacteria perpetuating inflammation though expression of ROS and Th17 like T cell response. (Red: active RORγt IL-17 producing cells)

Conclusion

It has become increasingly clear that IBD is a highly integrated process. The microbiota of IBD patients is less diverse among members of the Firmicutes and Bacteroidetes pylum. In contrast to the Firmicutes and Bacteroidetes certain types of proteobacteria are overrepresented. By changing the microbiota the host becomes more susceptible for some type of infections which in the healthy microbiota would not cause problems. Both in protecting against pathogenic bacteria and developing autoimmunity colonisation with SFB's can be beneficial. Therefore therapeutic strategies to affect the microbiota may increase the quality of life for IBD patients. Lifestyle and diet influence the susceptibility for IBD by modifying the microbiota. A high fibre diet, transplantation with SFB's and an increase of intracellular butyrate in colonocytes could help IBD patients. Specific anti-proteobacterial targets like inhibiting acetyltransferase YhhK could also help rescue the IBD phenotype by decreasing proinflammatory bacteria.

Another important process in finding new IBD treatments is the regulation of IBD related pathways by innate triggers like PRRs. Regulatory compounds effecting PPRs or there downstream targets could effect both the innate and adaptive response to bacteria by affecting the cytokine production and antigen presentation. The importance of T cells on IBD is evident since the best treatment available at this point acts on the T cell axis. The monoclonal antibody for TNF-α infliximab and the human version Adalimumab are effective in treating IBD.[122] More research is necessary to asses the importance of PRR-pathways in IBD related T and B cell tolerance and subset-skewing to find other immune targets relevant for IBD.

Susceptibility for IBD can be summarized in four basic effectors. Firstly, susceptibility is increased by autoimmune specific mutations of the host. Secondly, diet and lifestyle influence the composition of the microbiota and thereby IBD status. Thirdly, the presence of commensal bacteria can positively affect the IBD phenotype by inducing tolerance. Lastly, the presence of proinflammatoy bacteria can perpetuate IBD. Which of these effectors is most relevant for IBD susceptibility remains unclear. IBD is a complex disorder containing multiple subtypes, the difference between those subtypes is not yet fully understood but is possibly due to the difference in microbiota and immune status of the host. This diversity will force de development of future IBD treatment towards personalised treatment depending on IBD susceptibility fingerprint.

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