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CHARACTERIZATION OF TRANSFERABLE INTESTINAL DYSBIOSIS IN MURINE CROHN'S DISEASE LIKE ILEITIS

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“...Dass ich erkenne, was die Welt

Im Innersten zusammenhält...“

Johann Wolfgang von Goethe

Faust - der Tragödie erster Teil

ABSTRACT

"*Dysbiosis*" of microbial communities is assumed to play a crucial role in the development of diverse pathologies, including inflammatory bowel diseases (IBD). Dysbiosis is commonly defined by compositional alterations of symbiotic microbial ecosystems with detrimental consequences for the host. The characteristics of dysbiotic ecosystems and the interrelated mechanisms of microbe-host interaction are still to be designated. Additionally, functional evidence for a causal role of dysbiosis in development of pathology is not provided, yet. Dysbiotic shifts in the gut ecosystem are repeatedly assumed to be causal for development of IBD, which is characterized by an overreaction of the immune system towards intestinal commensals. Especially for ileitis, as found in Crohn´s disease (CD), few data are available for the role of microbial triggers in development of inflammation. Consequently, the aim of the present study was to test the causal role and characteristics of dysbiosis in a genetically modified animal model of spontaneous ileal pathology.

Similar to pathology in CD patients, $TNF^{deltaARE}$ mice develop a TNF-driven transmural inflammation with predominant ileal involvement. To show the relevance of intestinal communities in the development of ileitis, $TNF^{deltaARE}$ mice were treated with antibiotics, or housed under conventional (CONV), specific-pathogen-free (SPF) and germfree (GF) conditions. The physiognomy of the respective ecosystems was analysed by 16S-profiling, metabolomics and metaproteomics. Microbiota transplant experiments should demonstrate the transferability of pathology or protection to GF or CONV mice.

The crucial role of microbial triggers in the development of disease was demonstrated, as GF $TNF^{deltaARE}$ mice were free of intestinal inflammation. Furthermore, antibiotics attenuated ileitis by microbiota-alteration, independent of reduction in bacterial density. Colitis in CONV $TNF^{deltaARE}$ mice was not reduced, which hints towards dependency of inflammation severity and location on the intestinal ecosystem. In contrast, SPF housed $TNF^{deltaARE}$ mice were free of colitis and developed a high variance in ileitis-severity. This was associated with gradual loss of antimicrobial defence, as well as specific compositional and functional shifts in the intestinal microbiota. Transplantation of these different microbial communities from donor mice with the same genotype, though different phenotype, was performed. Remarkably, only pathology-associated dysbiotic, but not healthy microbiota transmitted CD-like ileitis into GF recipients. Hereby, loss of Paneth cell antimicrobials was observed subsequent to inflammation development.

Therefore, the present study provides clear experimental evidence for the causal role of gut bacterial dysbiosis in the development of chronic ileal pathology.

ZUSAMMENFASSUNG

Dysbiose scheint mit der Entwicklung von verschiedenen Erkrankungen, wie z.B. chronisch entzündliche Darmerkrankungen (CED) assoziiert zu sein. Definiert wurde Dysbiose bisher als veränderte Zusammensetzung eines symbiontischen Ökosystems, mit negativen Konsequenzen für den Wirt. Besseres Verständnis über Charakteristika dysbiotischer Systeme, sowie deren spezifischen Wechselwirkungen mit dem Wirt ist dringend notwendig. Zudem steht der Beweis eines ursächlichen Zusammenhangs bisher noch aus. CED sind durch Überreaktion des Immunsystems gegenüber kommensalen Mikroorganismen charakterisiert. Daher wird dysbiotischen Ökosystemen eine kausale Rolle in der Entwicklung von CED beigemessen. Jedoch gibt es bisher für Entzündungen des Dünndarms, wie sie bei Morbus Crohn (MC) vorliegen, nur wenig Erkenntnis. Zielsetzung war es daher, kausale Zusammenhänge sowie Merkmale von Dysbiose in der Entwicklung von Ileitis zu beschreiben.

Ähnlich dem Krankheitsbild in MC Patienten, entwickeln $TNF^{deltaARE}$ Mäuse eine transmurale Entzündung, welche überwiegend im terminalen Ileum lokalisiert ist. Um die Entzündungsentwicklung in Abhängigkeit der Mikrobiota darzustellen, wurden $TNF^{deltaARE}$ Mäuse (sowie ihre Wurfgeschwister) in konventionellen, spezifisch Pathogen freien (SPF) oder keimfreien Hygienebedingungen gehalten, oder mit verschiedenen Antibiotika behandelt. Das Profil der luminalen Mikrobiota, des Metaproteoms und der Metaboliten wurde ermittelt. Durch Mikrobiota-Transplantation sollte bewiesen werden, ob Entzündungsinduktion oder Protektion auf keimfreie oder bereits kolonisierte Tiere übertragen werden kann.

Mikrobielle Signale wurden klar als Entzündungsauslöser identifiziert, da keimfreie $TNF^{deltaARE}$ Mäuse frei von Darmentzündung waren. Zudem wurde gezeigt, dass Antibiotika die Schwere der Ileitis reduzieren, ohne dabei jedoch die bakterielle Dichte zu verringern. Antibiotikagabe führte nicht zu einer Linderung der Colitis, was darauf schließen lässt, dass die Lokalisation, als auch Schwere der Entzündung abhängig vom jeweiligen Ökosystem sind. Interessanterweise entwickeln $TNF^{deltaARE}$ Mäuse in SPF-Haltung keine Colitis und zeigten hohe Variabilität im Ileitis-Belastungsgrad. Dies ging mit Verschiebungen in der bakteriellen Zusammensetzung der Mikrobiota, als auch einem graduellem Verlust der antimikrobiellen Abwehr einher. Durch Übertragung dieser unterschiedlichen Ökosysteme aus Spendertieren mit gleichem Genotyp aber ungleicher Symptomatik, sollte die Ursächlichkeit von Dysbiose in der Entzündungsinduktion gezeigt werden. Bemerkenswerterweise induziert nur die dysbiotische Mikrobiota aus entzündeten Tieren Ileitis, mit anschließendem Verlust der Paneth-Zell-Funktionalität.

In der vorliegenden Studie konnte daher klar gezeigt werden, das bakterielle Dysbiose in kausalem Zusammenhang mit Ileitis-Entwicklung steht.

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1 INTRODUCTION

1.1 Microbe-host-interactions in health and disease

Microorganisms populate all environmentally exposed surfaces on the human and animal body. Hereby the location-specific microbial ecosystem develops in response to prevailing biological and physiochemical properties of the respective environment.[1, 2] The gastrointestinal tract harbours a high number of taxonomically diverse microorganisms. Due to very close proximity to the richest immune-structure of the host, the intestinal ecosystem is of crucial importance to prime microbe-host interaction in a homeostatic immune-situation. The totality of these organisms - including bacteria, viruses, archaea and eukaryotes (yeasts, protozoa) and their genes - represents the "intestinal microbiome". The most abundant microorganisms in the human and murine intestine are bacteria of the phyla *Bacteroidetes* and *Firmicutes;* followed by *Proteobacteria, Actinobacteria, Verrucomicrobia, Tenericutes* and *Fusobacteria.*[3, 4] At lower taxonomic levels, the diversity is very high, with approximately 150-200 dominant bacterial species per individual.[5] However, great inter-individual differences were observed in the overall microbial community.[6, 7] The recently described high intra-species variation at strain level puts even more emphasis on this high diversity.[8] Large-scale sequencing studies like the European MetaHIT project and the US Human Microbiome Project (HMP), confirmed this great inter-individual variability in the overall bacterial composition.[9] Results from the HMP also showed that despite compositional variance, great redundancy of metabolic pathways are found, which seem equally distributed along all intestinal samples. **This highlights that it is virtually impossible to clearly define a "healthy" microbiota solely based on evaluation of relative abundance of different gut inhabitants**.

1.1.1 Disruption of microbe-host-interaction in inflammatory bowel diseases

Due to the close proximity to the host´s largest immune structure, the intestinal microbiota gains centre stage for development of Crohn's disease (CD) and ulcerative colitis (UC), the two main forms of inflammatory bowel diseases (IBD). While inflammation in CD patients mainly occurs in the terminal ileum and is found in the proximal colon only occasionally, inflammation in UC is confined to the colon.[10, 11] The aetiology of IBD is not yet fully known, however, an over reactive mucosal immune system responding upon commensal stimuli is regarded as reason for pathology onset.[12, 13] This hypothesis was supported by the observation of delayed recurrence of ileitis after faecal stream diversion in CD patients.[14] Furthermore, genome-wide association studies identified genes involved in microbial signaling, underlining the role of gut microbes in IBD pathogenesis. At present, more than 200 genetic polymorphisms were identified, and there are more to come.[15-17] However, disease concordance in identical twin studies explains only up to 40% of CD risk, leading to the general

agreement that environmental factors (in their sum regarded as the "exposome") dominate IBD risk and development. Epidemiologic studies showed higher IBD prevalence in industrialized countries and adoption of higher incidence rates in the second generation of immigrants, hinting to the fact that adoption of lifestyle (most likely diet) may be a risk factor for later IBD development.[18-20] Genetic predispositions and inflammation were shown to be accompanied by altered composition and metabolic activity of intestinal microbial communities. **This alteration in microbial composition that was associated with pathology, was defined as "*dysbiosis*"**.[21-26] In coexistence with genetic susceptibility to IBD, the microbiota is assumed to be causative for disease development, ***"…but whether the changes are causal or secondary to inflammation remains uncertain*."**[27]

Also other pathologies like obesity, diabetes, cardiovascular disease and even depression or multiple sclerosis have been recently connected to intestinal dysbiosis.[24, 28-34] However, the proof of a clear causative relation is still missing. Furthermore, those pathologies are quite diverse, therefore common physiognomies of dysbiosic ecosystems are difficult to define.

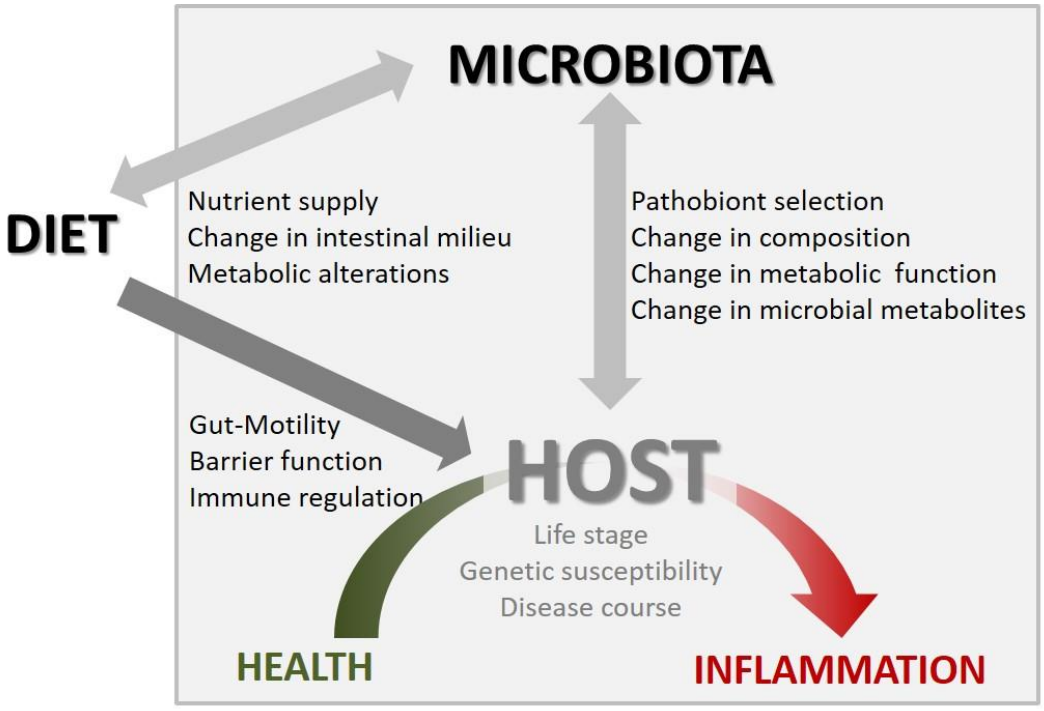


Figure 1: Direct and indirect interaction of diet, microbiota and host.
Diet influences the host and microbiota alike by provision of nutrients or creating a specific intestinal milieu. The host is, due to life stage and genetic susceptibility, more or less prone to microbial changes and inflammation development.

1.1.2 Characteristics of dysbiotic ecosystems

Under physiological conditions the microbiota shows both: plasticity and high resilience. Upon short-term perturbations (*e.g.* temporal change in dietary-pattern) the microbial composition adapts to alterations in the intestinal milieu, though soon resembles a pre-disturbance state.[7] The microbial ecosystem can also be changed without pathologic consequences for the host and stabilize within a new "alternative state".[35] Animal studies showed that the microbiota is capable to adapt to exposomal factors including diet, antibiotics or intrinsic factors like host genetics. Nevertheless, diet seems to overwrite the influence of genetic imprints.[36, 37]

Rapid resilience to perturbations is a key requirement for intestinal homeostasis in order to maintain a health-associated composition of the ecosystem ("*eubiosis*"). However, this resilience is lost in some pathologic conditions, like IBD. **It is important to note that dysbiosis displays several features, which will be categorized below in i) reduced bacterial diversity, ii) expansion of pathobionts, iii) changes in the microbial composition and iv) change in microbial functional capacity.** The appearance of these characteristics may occur solitarily, successively or simultaneously (**Figure 2**). Upon short term perturbations the microbiota may shift in diversity, composition and function and stabilize again in its previous state, or an alternative state due to high resilience. However, in IBD the microbiota shows low resilience and is subjected to changes finally leading to dysbiosis.

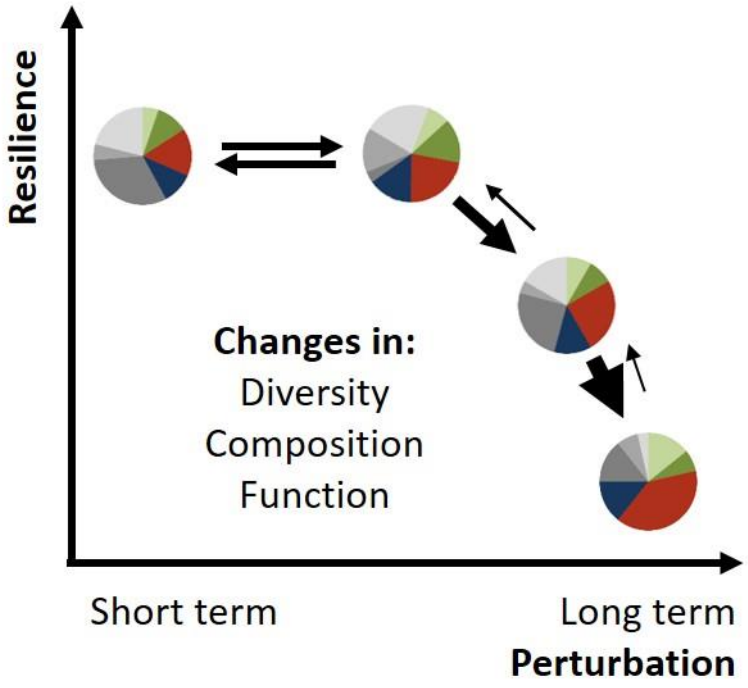


Figure 2: Long-term adaption of the gut ecosystem to perturbations.
Short-term dietary perturbations have no long lasting effect on the intestinal microbial diversity, composition and function. In the susceptible host, the intestinal resilience is reduced upon long-term perturbation and therefore also leading to further changes in the intestinal microbiota.

The widely discussed attribute of dysbiosis is **reduced bacterial diversity**. Based on numbers of bacterial species and their abundance found within one sample, the alpha diversity can be calculated. Many studies associated lowered bacterial diversity to disease, with the rationale of loss in metabolic redundancy.[32, 38-41] Furthermore, the ability to outcompete pathogens by a low-diverse microbiota is diminished. In patients that underwent frequent antibiotic treatments, the deteriorated intestinal diversity was shown to increase the risk of infection by opportunistic pathogens, such as *Clostridium difficile.*[42] In animal models, pathogens including *Salmonella, Citrobacter rodentium* or enterohaemorrhagic *E. coli* (EHEC) fail to colonize in the presence of a diverse, undisturbed microbiota, but elicit pathogenic traits if competing strains are missing.[43-46] The mechanisms of competitive exclusion may correspond to rivalry for nutrients or virulence-modulation of intruding strains.[47]

A further characteristic trait of dysbiosis is the **expansion of pathobionts**, *i.e.* single strains of the commensal microbiota that outgrow and cause detrimental effects in the host. The term pathobiont was defined by Chow and Mazmanian *et al.* as *"…symbiont that is able to promote pathology only when specific genetic or environmental conditions are altered in the host".*[48] While pathobionts are found only in low abundance in a healthy microbial setting, they overgrow in dysbiosis and cause disease in the susceptible (e.g. immune-compromised) host. Hereby, specificity in the combination of microbe and host-susceptibility is required. In animal studies *Bacteroides vulgatus* induced colitis in HLA/B27-ß2m rats, but not in interleukin (IL) 10 deficient (IL-$10^{-/-}$) mice and even prevented colitis in $IL2^{-/-}$ mice.[49, 50] Early studies regarded *Mycobacterium avium subspecies paratuberculosis* as the responsible pathobiont or even pathogen in IBD. However, this hypothesis could not be verified, as summarized by Packey and Sartor.[51] An increased abundance of *Enterobacteriaceae* is repeatedly observed in stool samples and mucosal specimens from IBD patients. Among these, the *Escherichia coli* strain LF82 is discussed to be a pathobiont.[23, 52, 53] The group around Darfeuille-Michaud was the first to describe adherent-invasive *E. coli* (AIEC), which selectively colonize the ileum of CD patients, suggesting that dysbiosis in IBD may also relate to strain-specific virulence factors.[54, 55]

Apart from single pathobionts, dysbiosis is in most cases regarded as **shifts in the overall microbial composition**, *i.e.* simultaneously increased or decreased abundance of certain commensals. Due to the new sequencing techniques and improved databases, great progress has been made in characterizing the intestinal microbiome also in larger cohorts. However, most samples were taken from patients who already underwent some kind of treatment, which exerts changes in the ecosystem and thereby impedes conclusive interpretation of findings.[56, 57] A study from Gevers *et al.* elegantly solved this issue by picturing the treatment naïve microbiome in children recently diagnosed for CD, before pharmaceutical or nutritional intervention.[26] They described an increase in *Enterobacteriaceae* preceding the onset of CD, an observation made by others as well.[34] However, one cannot exclude

that small inflammatory lesions can cause change in the microbiota, before the diagnosis of disease.[58] Thus, prospective cohorts would be of high value, but too demanding due to the low incidence rates.

Among the multitude of studies performed to detect IBD-associated bacterial taxa, little congruence is found between different cohorts. By combining the sequencing data from several studies, Walters *et al.* showed shifts in the bacterial composition at different taxonomic levels, which were at least partly consistent for several studies and cohorts.[53] These single taxa may serve as **indicator species with diagnostic value**, comparable to a biomarker. Walters *et al.* showed higher abundance of *Actinobacteria* and *Bacteroides* and a loss of *Prevotella* in CD patients compared to healthy controls. They also described at lower taxonomic level the loss of *Faecalibacterium prausnitzii*, the best known indicator species in IBD. This health-associated species was found in significantly lower levels in the inflamed intestine compared to healthy specimens and exerts positive immune-regulatory effects on the host. Therefore, loss of *F. prausnitzii* is speculated to be indicative for increased IBD risk.[59, 60] However, the use of only single indicator species as diagnostic tool may not be sufficient, as IBD-associated strains may be cohort or individual specific. Nevertheless, the novel approach of using overall drifts in the intestinal microbiota is a promising new tactic in diagnosis of IBD. Walters *et al* highlighted some universally valid disease-related shifts, which may be powerful enough to securely diagnose IBD, by combining the sequencing results from several studies.[53] By using stool microbiota, this non-invasive approach is even more advantageous.

Due to great progress in 16S ribosomal RNA sequencing methods, shifts in the prevailing bacterial members of the intestinal composition can be easily assessed and discussed (16S profiling). Hereby the gene amplicons of the V3/V4 region (approximately 450bp) of the 16S rRNA are assessed and identified via different databases. This finally allows the description of the bacterial profile in composition and diversity. However, most descriptions of intestinal dysbiotic communities fail to take fungal and viral contributions into consideration. Recently Chehoud *et al.* could show reduced fungal diversity in paediatric CD accompanied by increased *Candida* taxa.[61] Norman *et al.* showed marked differences in the intestinal virome in CD and UC patients compared to healthy controls.[62] The main difference was an increase in *Caudovirales* bacteriophages which was not secondary to bacterial dysbiosis. Interestingly, it is more likely to assume that viral dysbiosis contributes to pathology and changes in the bacterial ecosystem due to a “predator-prey” relationship.[63, 64] Nevertheless, studies of the role of other microbial taxa in IBD pathogenesis are awaited and will be essential to unravel dysbiotic patterns.

In addition to changes in composition and diversity, alterations in the **functional capacities** are characterizing a dysbiotic ecosystem. As shown by the human microbiome project, the healthy

intestinal ecosystem may be exceedingly different in composition, while its metabolic activity is highly similar.[9] An interesting study in IBD patients by Morgan *et al.* showed small perturbation of the intestinal composition, though quite distinct changes in microbial function.[65] In dysbiotic conditions, microbial pathways for oxidative stress tolerance, immune evasion, metabolite uptake and carbohydrate as well as amino acid biosynthesis were upregulated. An increase in carbohydrate-metabolism and especially changes in the capacity to utilize fucose, was reported in dysbiotic settings in CD patients and animal models of intestinal inflammation.[66, 67] Metaproteome analysis in a cohort of CD patients also proved a distinct signature, which was associated with CD.[68] By correlating these functional shifts to the bacterial ecosystem, *Bacteroides*-derived proteins related to survival in challenging environments (e.g. DnaKs and other chaperones) were found overrepresented. Along with changes in the intestinal microbial function, the profile of produced metabolites varies.[69] This leads to the assumption that dysbiosis may be more precisely characterized by changes in the microbial function rather than composition.

Furthermore, the definition of dysbiosis should not be a mere one-sided, microbial consideration, but should undoubtedly take the host into account. Palm *et al.* showed that IBD patients display an **altered immune recognition of a dysbiotic microbiota.** This was correlating with increased and more divergent Immunoglobulin A (IgA) coating.[70] By using an animal model of chemically induced colitis, they transferred disease-susceptibility and thereby proved the causal role of this IgA-coated ecosystem.

1.1.3 What causes dysbiosis?

The first factor influencing the acquisition of microbes is the mode of delivery. Also early nutrition (breast- or formula-feeding) or hygienic environment (lifestyle and geographic location) in which a child is raised determines the composition of microorganisms. In later life, the sum of environmental triggers, the so-called **exposome**, including e.g. diet, stress, hygienic milieu (contact to disinfectants or animals), drugs and geography (e.g. air pollution) contribute to shape the intestinal ecosystem.[3, 41, 71-79] The daily diet has an enormous impact on the intestinal ecosystem. Especially diet high in fat seems to shift the microbiota towards a more dysbiotic pattern, which is associated with increased risk of intestinal inflammation. Animal, as well as human studies showed the rapid adaption to changed dietary patterns. By feeding mice an exclusively animal- or plant-based diet for a short period of time, David *et al.* effectively showed the plasticity, as well as the stability of the intestinal ecosystem upon short-term perturbations.[80] However, also other studies showed that clear shifts in the intestinal ecosystem diversity and composition are not induced by short term changes in dietary habits or application of single nutrients, but require long term pressure on the ecosystem.[7, 81]

Beside exposomal factors, also **intrinsic factors** *i.e.* the respective genetic structure of the host, shape the intestinal microbiota. Several genes associated with altered immune function, microbial recognition or antimicrobial defence were shown to influence the intestinal ecosystem. In a meta-analysis Knights *et al.* highlighted the significant association between the NOD2 risk allele and increased abundance of *Enterobacteriaceae* in IBD patients.[82] In another study, Rausch *et al.* showed an association between the intestinal microbiota and the fucosyltransferase 2 (*FUT2*) secretor gene - a physiological trait that regulates gastrointestinal mucosal expression of blood group A and B antigens. Approximately 20% of humans lack the *FUT2* gene, which was shown to be a risk factor for CD development.[83] Within the group of CD patients, the microbiota from *FUT2* carriers differed from non-secretors in composition, diversity and metabolic functionality.[84, 85]

This observation of dysbiosis before onset of pathologies is exceptionally interesting, as recent studies also suggest that **inflammatory conditions** directly influence the intestinal community. This shift may occur due to insufficient bacterial adaptation to the changed milieu found during inflammatory processes. During inflammation, the secretion of antimicrobial peptides (AMPs) is upregulated. A recent study reported that the colonization fitness of commensals depends on the capability to adjust to increased AMP levels.[86] Therefore, some community members will be displaced during inflammation. While pathogens or pathobionts cannot survive these increased AMP levels, commensals are resistant. In this manner, stability and resilience during infections is mediated. The potential of AMPs to shape the intestinal ecosystem and their importance in IBD has been shown by others before.[87] The inflammatory reactions may also induce stress or upregulation of virulence-associated genes in the microorganism, as shown in monoassociation-studies with the gut commensals *Enterococcus faecalis* OG1RF or *E. coli* NC101.[88, 89] *E. coli* NC101 cells isolated from inflamed IL-10$^{-/-}$ mice displayed upregulated bacterial-stress response genes (e.g. heat shock proteins) compared to isolates from WT mice.[89, 90] However, inflammation may also promote growth-advantages and virulence of pathogens. For *Salmonella typhimurium* (*S. typhimurium*) it was shown that the host produces tetrathionate under inflammatory circumstances. This subsequently promotes the ethanolamine-utilization of *S. typhimurium* and thereby its colonization fitness.[91, 92] Also nitrate, which is produced by the host during inflammation, is assumed to promote growth of *Enterobacteriaceae.* Consequently, the often discussed increase of *Enterobacteriaceae* in IBD may also be regarded as secondary event to inflammatory processes rather than a causative association.[58]

Apart from host-derived factors, also microbe-derived mechanisms affect other commensals. The transfer of single strains or bacterial consortia to germ-free (GF) mice enables us to study mechanisms of **microbe-microbe interaction**. Commensals inhibit pathogen invasion by e.g. competition for nutrients, quorum-sensing-mediated colonization repression, as well as by expression of virulence

factors.[47, 93-95] Metabolic-interaction of commensals and pathobionts are also shaping the intestinal ecosystem, as some organisms depend on the conversion of dietary components or induction of host-derived nutrients by other members of the microbiota (food chain).[96, 97] Also quorum-sensing-mediated repression of colonization is regarded as an important mechanism of shaping the intestinal ecosystem, and recent findings from Thompson *et al.* demonstrate the role of autoinducer-2 in re-shaping antibiotic induced dysbiosis in the gut after antibiotic perturbation.[94, 98]

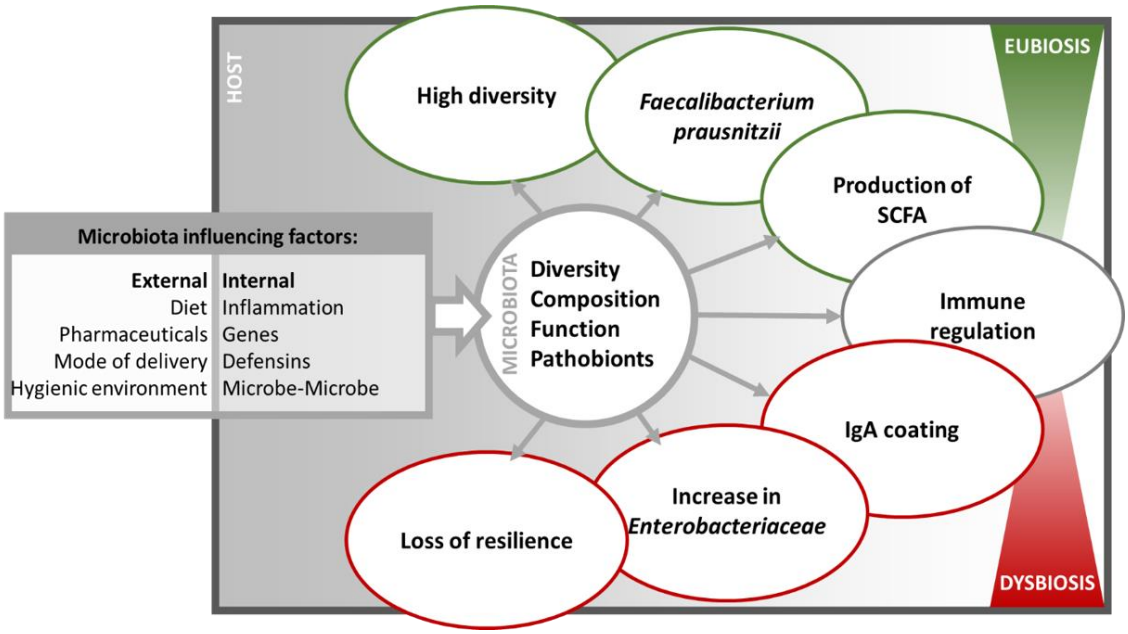


Figure 3: Influencing factors and characteristics of dysbiosis.
The intestinal microbiota is influenced in diversity, composition, function and prevalence of pathobionts by internal and external factors. Consequently, the microbiota varies in immune relevant characteristics and may thereby shift from eubiotic to a dysbiotic state.

1.1.4 Role of the microbiota in animal models of intestinal inflammation

Most dysbiosis-influencing factors cannot be studied under highly controlled and standardized conditions in humans. However, it is still of high interest to show, whether the correlation between dysbiotic microbial shifts and IBD are of true causative, or only associative character. For this, large prospective cohort-screenings would be necessary, which are hardly feasible due to low incidence rates of IBD (e.g. European annual CD incidence is 12.7 per 100,000 person-years).[99] Therefore, animal models of intestinal inflammation are gaining more and more importance, due to the fact that confounders like diet, genetic background and hygienic environment can be continuously controlled. **Genetically-driven animal models** developing inflammation without any chemical inductor (e.g. DSS or TNBS) are especially appropriate to study the interaction of the intestinal microbiota and pathology, as the chemical compound *per se* would influence the ecosystem. Ever since the availability of

germfree and gnotobiotic animal models, great progress has been made to portray microbe-host-interaction. Hereby many IBD mouse models were shown to be free of pathology in GF conditions and thereby clearly prove the causality of microbial triggers in IBD development.[100]

The best-studied model of colitis is the IL-10$^{-/-}$ mouse, which lacks the productions of the anti-inflammatory cytokine IL-10. In contrast to SPF and CONV housing, IL-10$^{-/-}$ mice do not develop inflammation in GF conditions. Interestingly, the time point of microbial annexation seems to be of importance in this context. IL-10$^{-/-}$ mice colonized at adult age developed more severe inflammation compared to mice colonized in weaning age.[101] A more recent model of UC is the Rag2$^{-/-}$ x Tbx21$^{-/-}$ (TRUC) mouse model. Those mice develop UC-like colitis due to the absence of an adaptive immune system. TRUC mice were free of colitis in GF conditions as well.[102] Another model, which is protected from inflammation under GF conditions, is the T cell receptor alpha knockout model. These mice are characterized by defective immunity as well, as αß T cells are absent.[103] Also severe combined immunodeficiency (SCID) mice, having an impaired B and T cell function, are free of colitis in GF housing conditions.[104]

However, all the above mentioned models display a colitis phenotype. To study mechanisms relevant in CD, which is characterized by ileal involvement, only few animal models are available. The SAMP1/YitFc mouse model is one of the rare models displaying a CD-like inflammation, with still uncertain mechanism of pathology induction.[105, 106] Nevertheless, a recent study suggests that inflammation may be mediated by loss of Chemokine (C-C motif) ligand 21 (CCL21) signalling and dendritic cell migration from the ileal lamina propria to mesenteric lymph nodes.[107] When housed in GF conditions, SAMP1/YitFc mice do develop ileitis (though with milder severity) at high age.[105, 108] A new interesting model is the SKG-model of spondylarthritis. These mice have a mutation in the ZAP-70 receptor and show CD-like ileal co-morbidity. However this ileal phenotype has to be induced by intraperitoneal injection with high doses (3mg) of curdlan (1,3-beta-glucan, the major component of bacterial and fungal cell walls).[109] Interestingly, no ileitis was observed in GF or LPS-injected GF mice. This emphasizes that ileitis-induction by curdlan needed the presence of intestinal microbial triggers.[110]

Another model of CD-like ileitis is the TNF^{deltaARE} mouse. These mice have increased TNF-levels due to a deletion of the adenosine-uracil-rich element (ARE) in the TNF transcript [111]. The pathology in TNF^{deltaARE} mice with their transmural ileitis and the Th-1 driven immune response has a very good resemblance to the inflammation in CD patients. In line with this, TNF-antibodies, which are a treatment option in CD patients, were shown to hamper inflammation in TNF^{deltaARE} mice.[112] Interestingly, TNF^{deltaARE} mice also develop extra-intestinal pathology in form of arthritis in the joints. In contrast to SAMP1/YitFc mice, the inflammation in TNF^{deltaARE} mice is not characterized by the

formation of “cobblestone” structures – a thickening of the gut wall with protruding lesions, which is also found in some patients with progressive IBD.[113] These divergent inflammatory phenotypes again highlight the necessity and basic requirement of different mouse models, to study the mechanisms and complexity of inflammation in IBD.

Recently, new mouse models were generated that display more hypothesis-driven mechanisms of intestinal inflammation. Especially, epithelial cell-specific knock-out models, *i.e.* autophagy-related protein 16L1 (ATG16L1$^{-/-}$) and X-box binding protein 1 (XBP-1$^{-/-}$) mice, allowed a more mechanistic understanding of CD-like disease phenotypes. These models also need an initial microbial trigger, as e.g. XBP-1$^{-/-}$ mice are protected from ileitis in GF housing but develop pathology in a Paneth cell dependent manner in SPF housing.[114, 115] Mice with a deletion of caspase 8 (a protease involved in apoptosis regulation) in the intestinal epithelial cells (Casp8^{deltaIEC} mice) develop inflammation in the terminal ileum due to increased necroptosis.[116] Notably, antibiotic-treated Casp8^{deltaIEC} mice were rescued from ileitis, indicating an important role for the intestinal microbiota in the development of the disease.[117]

The development of spontaneous animal models that mirror human genetic risk factors for IBD or other pathologies may be an important step toward unravelling mechanisms of pathology. **The pathology free status of animal models in GF housing is exploited as a valuable tool to investigate the development of dysbiosis, its influencing factors and mechanisms of microbe-host-interaction.**

1.1.5 Dysbiosis and microbe-host-interaction in gnotobiotic animal models

Specific mechanisms of disease induction can be studied by colonizing GF mice with single bacterial strains. Therefore, many annexation-studies have been performed for models of intestinal inflammation and other pathologies including obesity, diabetes and multiple sclerosis, as recently summarized.[100] However, by colonizing immunocompromised GF animals with selected single strains, several obstacles are met: not all microbes colonize equally and the induction of inflammation depicts bacteria as well as model specific traits.

In the IL-10$^{-/-}$ model, several monoassociations with candidate pathobionts (*Helicobacter hepaticus* and *E. coli*) failed to trigger colitis, while other bacteria (*Enterococcus faecalis* or *Bilophila wadsworthia* were effective in inducing pathology.[118-120] These findings suggest that an association with only one bacterial strain may not be sufficient for immune maturation, which is required for pathology. Atarashi *et al.* could show that one strain of *Clostridium* was not able to induce regulatory immune functions in former GF animals, as this relies on concerted actions of different strains.[121, 122] **It is tempting to speculate that not all members of the microbiota are equally sufficient to induce**

pathology, but rather require the interaction with other commensals. An example of this hypothesis encompasses the monoassociation of IL-10$^{-/-}$ mice with *Helicobacter hepaticus* or *Lactobacillus reuteri.* As single strains they did not induce inflammation, while the combination of the two bacteria led to colitis development.[119] Therefore, current studies tend to use complex microbial settings or defined microbial consortia to ensure sufficient immune maturation in GF animals. Garrett *et al.* showed that colitis in TRUC mice correlates with increased abundance in *Klebsiella pneumonia* and *Proteus mirabilis.* However, the combined colonization of TRUC or Rag2$^{-/-}$ recipient mice with both of these two bacterial strains did not induce inflammation. Only a combined colonization of *Klebsiella pneumonia*, *Proteus mirabilis* and a complex microbiota induced inflammation in recipient Rag2$^{-/-}$ mice.[102] However, it has to be mentioned, that WT cage-mates were also inflamed, suggesting a rather pathogenic than dysbiotic trait of the microbiota. Interestingly, Powell *et al.* identified *Helicobacter typhlonius* as a key driver of pathogenesis in TRUC mice.[123] In the SCID mouse model, Stepankova *et al.* showed inflammation initiation by segmented filamentous bacteria (SFB) only in combination with complex microbiota.[104] SFBs are known to be potent inducers of the host´s immune response as summarized elsewhere.[104, 124] This also points out the fact that monocolonizations may pose elegant ways to prove causality, though the multifaceted mechanisms of disease initiation may need more complex interactions. **However, these studies clearly hint toward a causal role of dysbiosis in intestinal pathology.**

In animal models of intestinal inflammation the causal relationship of a dysbiotic ecosystem in inducing inflammation was corroborated by direct microbiota transfer, or by co-housing of animals. The transfer of a complex microbiota from inflamed TRUC-mice induced colitis in gnotobiotic TRUC-mice. However, when the microbiota from antibiotic-treated mice in remission was transferred, the recipients developed only attenuated inflammation.[125] Also NOD2 mice develop genotype-induced dysbiosis. Interestingly, by co-housing NOD2 mice with WTs, also the WT-cage mates developed increased DSS susceptibility.[126] In these animal models with different mechanisms of pathology induction, a single microorganism is not capable to induce tissue inflammation, but needs the concerted action of a complex microbiota. These observations are further supported by the fact that no single pathobiont has been found that is consistently present in different IBD cohorts. Within these different studies, the microbiota-donors differed in genetic predisposition or therapeutic intervention. **However, the transfer of a donor-microbiota of animals with same genotype, though different phenotypes, would be of special interest to trace down dysbiotic patterns.** In the last decades, compelling evidence emerged pointing out the pivotal role of the intestinal ecosystem in defining the host immune homeostasis (e.g. by inducing pro- or anti-inflammatory responses).[127] Whether dysbiosis plays a causal role or is just an associated characteristic of pathology remains to be unravelled. However, understanding the mechanisms of microbial detection and defence, as well as factors that may induce

alteration in the microbial community are of fundamental importance to understand associations and causalities between gut microbes and immunity.

1.2 Mechanisms of microbe-host-interaction

The intestinal immune system is in close contact to trillions of microorganisms. Therefore, it continuously monitors for the presence of pathogens while it is simultaneously alert and tolerant to commensals. **To uphold this homeostasis, several lines of defence are keeping the intestinal inhabitants at bay**.

To strengthen the first line physical barrier, the host secretes several factors to keep a spatial segregation between the epithelial layer and the vast amount of microorganisms in the intestinal lumen. Goblet cells synthesize mucins that form a 150 μm-thick protective mucus cover in the colon. While the mucus layer is continuous in the colon, it shows a discontinuous pattern in the ileum.[128] The initiation of mucus-production needs a microbial stimulus and just recently it was shown that it takes up to seven weeks for colonized GF mice, to build up a mucus structure which resembles physical properties of conventionally housed mice.[129] Though the mucus layer is mainly produced to keep microbes at distance, it is an important site for microbe-host-interaction. The microbiota is capable to degrade certain structures in the mucus for energy production, though thereby alters it´s physical properties.[128, 130, 131]

Another luminal first-line defence mechanism is the secretion of IgA, which is secreted (sIgA) in its dimeric form at mucosal sites. SIgA promotes the clearance of antigens and pathogens from the gut lumen as it blocks their access to epithelial receptors and entraps them in the mucus-layer. Furthermore, sIgA selectively presents bacterial antigens to DCs and facilitates their removal.[132] GF animals express only low levels of sIgA, which again shows a microbe-host-interaction driven defence strategy.[133] Also, IgA-diversity displays plasticity and reacts rapidly to shifts in the intestinal community.[134] Recently it was shown that IgA-coating of luminal bacteria is a defence mechanism which the host engages to mark and distinguish disease-associated bacteria from other commensals.[70]

The first cell-layer-barrier is formed by intestinal epithelial cells (IEC). IECs form single-cell layer on the surface of the intestinal mucosa, which is securely sealed by different tight-junction proteins. Enterocytes are besides enteroendocrine cells, M-cells, goblet cells and Paneth cells, the most abundant type of IECs. However, they form not only the physical segregation into the apical and basolateral site, or absorb nutrients. Enterocytes play a pivotal role in the communication of microbial presence to immune cells. Thereby they influence the intestinal immune response and

homeostasis.[135] They sense the presence of bacteria by toll like receptors (TLRs) and the consecutive response (secretion of cytoprotective factors like interleukin (IL-6), tumour necrosis factor (TNF)-alpha, keratinocyte chemokine-1 (KC-1), and heat shock proteins) was shown to protect from epithelial injury.[136] Beside the classical enterocytes, also goblet cells and Paneth cells are crucial in microbe sensing, interaction and defence. Beside mucus secretion, goblet cells were shown to be capable of uptake and delivery of luminal antigens to dendritic cells (DC) in the lamina propria, similar to M-cells.[137] A cell type, which plays an important role in bacterial defence, though is solely found in the small intestine, are Paneth cells. In CD patients the function of Paneth cells was found to be impaired, which was especially associated to the ileal phenotype of pathology.[138] **Paneth cells are located at the crypt base of the small intestine in close proximity to the epithelial stem cell compartment and are considered as key players in innate defence, as well as microbial community regulation**.[139, 140] The spatial proximity to intestinal stem cells indicates their central role in the regulation of small intestinal epithelial renewal. Paneth cells sustain proliferation and epithelial restitution, by the secretion of epidermal growth factor (EGF), WNt3 and Notch ligands.[141] Furthermore, Paneth cells produce a wide array of antimicrobial peptides (AMPs) to enforce the spatial segregation of microbes and the IEC-layer. The cationic residues of defensins and cathelicidins are attracted to negatively charged bacterial lipid-rich membranes. Consequently, they create pores in the bacterial cell membrane and induce cell lysis. In contrast, C-type lectins selectively target Gram-positive bacteria by exclusively binding peptidoglycan.[87, 141-143]. Interestingly, the host´s AMP production and microbiota show reciprocal interaction. The expression and secretion of some AMPs is dependent on signals of microbial presence. In GF mice, beta-defensin 2, regenerating islet-derived genes 3 gamma (REG3γ), and the RNAse angiogenin 4, are expressed at very low levels. Upon colonization, however, they are rapidly upregulated.[141] On the other hand, Salzman *et al* could show that the selective secretion of different AMPs was even potent to modulate the composition of the intestinal microbiota.[87]

In close proximity to IECs, intestinal mononuclear phagocytes (iMP) –DCs and macrophages (MΦ) – are regarded as key players in the intestinal immune response, as they link innate and adaptive immunity. Intestinal DCs express several PRRs and secrete cytokines and chemokines upon stimulation. Consequently, they migrate to MLNs to promote adaptive immune responses, as well as oral tolerance.[144] Gastrointestinal MΦs maintain gut homeostasis as they are characterized by low production of pro-inflammatory mediators, but an intact phagocytic ability.[145] In CD patients however, intestinal MΦ express high levels of co-stimulatory molecules, show increased activation of NF-κB signalling and oxidative burst activity. Furthermore, they have a high expression of TLR2 and TLR4, as well as an augmented secretion of cytokines, including TNF and IL-23.[146-151]. Intestinal DCs also induce regulatory adaptive immunity by factors such as TGF-beta, IL-10 and retinoic acid, thereby

promoting forkhead box P3 (Foxp3)+ regulatory T cells (Treg).[152] Commensals can also directly influence Foxp3+ Treg cell induction, through TLR2-interaction on T lymphocytes.[153] Lathrop *et al* also showed, that Foxp3+ iTreg cells in the lamina propria express a unique subset of T cell receptors (TCRs) that recognised epitopes derived from the commensal microbiota. By manipulating the intestinal ecosystem via antibiotics, the TCR repertoire of colonic Foxp3+ Treg cells was altered.[154-156] Also bacteria-derived short chain fatty acids (SCFA), particularly butyrate, can directly induce the differentiation of intestinal Foxp3+ iTreg.[156-158] Hereby an interesting hypothesis is raised by Kabat *et al*.: Commensals also express pathogen associated molecular patterns (PAMPs), however the intestinal immune system is capable to distinguish pathogens from commensals. Therefore, **microbial metabolites like SCFA may therefore serve as "*surrogate for symbiosis*", enforcing a tolerant immune responses and intestinal homeostasis in the host.**[159] T cells also express PRRs that allow them to directly sense and respond to microbial patterns, as recently shown for γδ-T cells, which are enriched in the intraepithelial lymphocyte population (IEL). TLR2-signalling on γδ-T cells, potentiated Th17 differentiation and induction of effector cells.[160] In CD patients, exacerbated Th1- and Th17-mediated immune responses are observed, along with detrimentally high levels of TNFα, IFNγ, IL-17 and IL-22.[161-164]

Another, recently discovered lineage of immune cells that postures a microbial influenced route of immune homeostasis are innate lymphoid cells (ILC). These innate leukocytes display lymphoid morphology but lack rearranged antigen receptors and are also phenotypically distinct from myeloid lineages.[165] ILCs are enriched in mucosal tissues and were differentiated into three subgroups based on phenotypic and functional characteristics: ILC1 (T-bet+), ILC2 (GATA-binding protein 3 positive), and ILC3 (retinoic acid receptor-related orphan receptor-γt; RORγt). ILC3 play an important role in immune homeostasis. Upon microbial stimulation, they are the major innate source of IL-22 in the lamina propria, and therefore limit systemic dissemination of commensals.[166, 167] Subsequently, IL-22 signals through IL-22R on IECs, to activate signal transducer and activator of transcription 3 (STAT3) and induce AMPs, including Reg3γ and Reg3β, that protect against enteric pathogens.[168-170] ILCs are discussed to have an important role in CD development, as several studies showed increased ILC numbers in the lamina propria of CD patients, though their specific role in IBD still has to be unravelled.[171-173]

As IBD development is a complex interaction of innate and adaptive immune cells towards commensal stimuli, the therapeutical modulation of the immune response and its microbial triggers is multifaceted and difficult. **Due to the central role of the gut microbiota in pathology development, targeted modulation of the intestinal microbiota and the microbe-host interaction, may state an interesting new treatment approach.**

1.3 Therapeutic intervention in IBD

To induce and maintain remission in IBD, several modes of treatment are available. In adults 5-aminosalicylate, corticosteroids, immunomodulators and biological agents are the first line of treatment. Biologic agents, such as anti-TNF-therapy neutralize proinflammatory signals, such as TNF and thereby interrupt the inflammatory cascade.[174] Nowadays anti-TNF-antibodies are often combined with Methotrexate or Azathioprine to yield improved clinical outcome.[175] Though, pharmacological intervention is always associated to mild or severe side effects, therefore the option of dietary intervention is gaining more and more interest. Especially in paediatric patients, nutritional intervention (exclusive enteral nutrition) shows high efficacy.[176-178] Diet does not only exert a direct influence on intestinal immune and epithelial cell functions, but also shapes the intestinal microbial community by generating a milieu that favours the growth and metabolic activity of specific bacteria.

1.3.1 Shifting the balance: effect of antibiotics on microbe-host–interaction

Shifts in the intestinal ecosystem were shown to be associated to IBD as discussed above. Therefore, modulating the intestinal microbiota by antibiotics states another form of therapy in mild to moderate disease. The hereby most frequently used antibiotics are metronidazole and ciprofloxacin.[178] Several meta-analyses determined the placebo-superior effect of antibiotics and also refer to site-specificities of antibiotics.[179-181]

Beside their therapeutic implementation, antibiotics pose an important tool to gain knowledge about mutualistic relationships of commensals and their respective host.[182] One of the best known examples of antibiotic induced disturbances with pathologic consequence, is the bloom opportunistic pathogenic *Clostridium difficile.*[42] Also in murine models of *Salmonella* infection, an antibiotic-induced disturbance in the intestinal microbiota is a prerequisite for successful pathogen colonization.[183] The intention of antibiotic intervention is the targeted elimination of a single pathogen. Though, **the intestinal ecosystem is a network of co-dependent species, which are connected by food-chain or metabolite utilization interactions**.[96, 97, 184] Therefore, apart from direct reduction of antibiotic-sensitive bacteria, also metabolically related organisms will decline. The consequence is massive perturbation of the intestinal microbial composition. However, the therapeutical elimination of bacterial groups and therefore bacterial ligands or metabolites (e.g. SCFAs), induce alternated immune responses.[156, 185, 186]

To study mechanism of microbe-host interaction, GF animals can be colonized with single bacteria or clearly defined bacterial consortia in gnotobiotic setups. However, the incomplete immune maturation or mucus formation in GF animals states a relative disadvantage of gnotobiotic investigations.

Therefore, experimental microbiota-modulation in mice reared in colonized housing, may give a more realistic picture of the microbe-host-mutualism. Several antibiotic-intervention studies have been performed in animal models of intestinal inflammation, to describe the putative role of microbes in IBD development. Hereby frequently used antibiotics were metronidazole, vancomycin, norfloxacin and neomycin.[187] Those antibiotics differ in their spectrum of activity as well as intestinal absorption rates and mechanism of action as shown in **Table 1**. In different mouse models of colitis, the protective effect of antibiotics was shown to be time and site specific, an observation made in humans as well.[188, 189].

Table 1: Properties of most used antibiotics in IBD studies
Most frequently used antibiotics in animal models. Antibiotics used in the present study are marked by*.

Antibiotic	Category	Mechanism of action	Spectrum of antibacterial activity	Intestinal absorption
Ampicillin*	Beta-Lactam	Inhibition of cell wall formation	Broad	Poor
Ciprofloxacin	Fluoroquinolone	Inhibition of topoisomerase	Broad	Medium (>70%)
Imipenem	Beta-Lactam	Inhibition of cell wall formation	Broad	Poor
Metronidazole*	Nitroimidazole	Deactivation of enzymes	Narrow; mainly anaerobic	High (>90%)
Neomycin*	Aminoglycoside	Inhibition of protein synthesis	Broad; mainly gram negative	Poor
Norfloxacin*	Fluoroquinolone	Inhibition of DNA replication	Broad	Poor
Vancomycin*	Glycopeptide	Inhibition of cell wall synthesis	Narrow; mainly gram positive	Poor

Besides shifting the intestinal ecosystem, antibiotics were also shown to exert great influence on bacteria derived metabolites. Bile acids (BA) are synthesized in the liver as primary BA (cholic acid (CA), chenodeoxycholic acid (CDCA) and additionally in rodents α- and β-muricholic acid (MCA)). Before primary BAs are secreted into the bile and small intestine, they can be conjugated with glycine or taurine. In the intestine, bacteria convert primary BAs by 7alpha- dehydroxylation into the secondary BAs deoxycholic acid (DCA) and lithocholic acid (LCA) and in rodents to ω-MCA. Secondary BAs can reach even millimolar concentrations in the intestine and their composition varies widely among individuals. By microbial deconjugation, dehydrogenation, dihydroxylation or epimerization, the variety of BAs is further increased. BAs are ligands of the farnesoid X receptor (FXR) and the G-protein coupled BA receptor TGR5.[190, 191] Both FXR and TGR5 control several elements of glucose, lipid and energy metabolism. Consequently, BAs are considered as modulators of (postprandial) metabolism.[192] In FXR knockout mice, the importance of FXR signalling in epithelial barrier integrity could be shown.[193] Therefore, **bacterial derived secondary BA are assumed to be central for intestinal integrity and IBD prevention**.[194] Antibiotics were shown to exert great and specific shifts in the composition of the BA pool. However, clear correlations to certain members of the microbiota could not yet been drawn.[195, 196]

Antibiotics therefore still raise a variety of questions regarding their possibility to alter microbe-host mutualism. Especially in a host predisposed to pathology. The definition of “health-associated-communities” is not yet possible. Nevertheless, characterization of different ecosystems is of high importance, as the donation of allogenic ecosystems poses potential for clinical implementation. Therefore further studies are required to fully exploit the possibilities lying within microbiota orientated prevention of IBD development.

2 AIM

The aim of the present work was to prove the causal role of dysbiosis in the development of intestinal inflammation in a mouse model of CD-like ileitis. Many studies assume an association between dysbiotic intestinal communities and IBD. However, evidence is missing to show whether this association is a consequence or truly causative requirement for pathology. Moreover, compositional patterns and functional traits of IBD-related dysbiosis are still to be unravelled. Knowledge about ileitis-relevant consortia will be fundamental to identify microorganism with clinical significance as indicators for increased risk.

The essential role of microorganisms in development of pathology was proven in models of colitis by germfree housing, or administration of antibiotics. In contrast, in models of CD-like ileitis, this correlation was not shown, yet. First evidence hinting towards a causal association was shown for simplified bacterial consortia only. Prove of this causal relation with a complex, dysbiotic ecosystem is still lacking. Consequently, knowledge about functional characteristics of inflammation-related ecosystems and their metabolites is limited.

In the present work, the genetically susceptible $TNF^{deltaARE}$ mouse was used as model of CD-like ileitis. This mouse model gives good resemblance to human CD, shown by TNF-driven, transmural inflammation in the terminal ileum and occasionally proximal colon. By housing $TNF^{deltaARE}$ mice in GF conditions, the necessity of bacterial triggers for development of pathology was evaluated. To describe the role of intestinal microbial composition on disease characteristics, the intestinal microbial structure was changed by housing $TNF^{deltaARE}$ mice either in SPF or CONV hygienic conditions. Via antibiotic therapy, a pharmaceutical and more drastic alteration of the phylogenetic structure was achieved. In a setting of antibiotic induced attenuated inflammation, characterization of the microbe-metabolite-mutualism was possible. Hereby, the protective therapeutic potential of shifting the intestinal ecosystem, as well as dynamic of relapse were studied. Transferability of protective microbial effects via microbiota-transplant experiments was tested. Therefore, the microbiota from donor $TNF^{deltaARE}$ mice was transplanted to recipient mice from GF or CONV housing. By engrafting dysbiotic microbiota from donors with same genotypic susceptibility, though variance in ileitis-severity, final prove of causality should be provided.

3 MATERIAL AND METHODS

3.1 Ethics statement

Animal use was approved by the local institution in charge (Regierung von Oberbayern, approval no. 55.2-1-54-2531-75-10 and 55.2-1-54-2531-99-13). All animals were housed in mouse facilities at the Technische Universität München (School of Life Sciences Weihenstephan).

3.2 Housing conditions

Heterozygous $TNF^{deltaARE}$ and WT littermates (C57BL/6N) were kept in CONV, SPF or GF conditions (12 h light/dark cycles at 24-26°C) until the maximum age of 18 weeks.

Table 2: Members of the mouse gut microbiota that are unique to housing conditions.
Only microorganisms that were exclusively found either in SPF or CONV $TNF^{deltaARE}$ mice are displayed. Analysis was performed using high-throughput 16S rRNA gene sequencing, FELASA-guided analysis or cultivation (yeast-specific Sabourand-agar). Sequencing was done as described in the supplementary methods (taxa with a sequence abundance >0.5% are shown). For FELASA analysis, results of three different analysis time points were considered (previous, during and after performing the experiments). Samples from sentinel mice were taken from intestinal content, fur swap, organs and rectum swap. Analysis was performed by an independent, certified lab according to FELASA recommendations from 2014. *microorganisms generally regarded as pathogenic to rodents.[197]

Housing	Taxon	relative abundance (% ± SD)	Lineage	Method of analysis
SPF	*unknown_Clostridiales*	2.4 ± 1.2	Bacteria	sequencing
	unknown_Desulfovibrionales	0.5 ± 0.3	Bacteria	sequencing
CONV	*Helicobacteraceae**	9.6 ± 3.4	Bacteria	sequencing
	Bacteroidaceae	5.5 ± 2.9	Bacteria	sequencing
	Desulfovibrionaceae	5.1 ± 1.6	Bacteria	sequencing
	Verrucomicrobiaceae	2.8 ± 1.6	Bacteria	sequencing
	Prevotellaceae	2.0 ± 1.0	Bacteria	sequencing
	Deferribacteraceae	1.5 ± 0.9	Bacteria	sequencing
	Defluviitaleaceae	1.0 ± 0.7	Bacteria	sequencing
	Sutterellaceae	0.9 ± 0.4	Bacteria	sequencing
	Bifidobacteriaceae	0.8 ± 0.9	Bacteria	sequencing
	Erysipelotrichaceae	0.8 ± 0.7	Bacteria	sequencing
	Clostridiaceae	0.7 ± 1.1	Bacteria	sequencing
	Peptostreptococcaceae	0.6 ± 0.9	Bacteria	sequencing
	*Chlamydiaceae**	0.6 ± 0.6	Bacteria	sequencing
	*Murine norovirus**	positive	Virus	FELASA
	*Helicobacter spp.**	positive	Bacteria	FELASA
	*Pasteurella spp.**	positive	Bacteria	FELASA
	*Syphacia spp.**	positive	Animalia	FELASA
	*Trichomonas spp.**	positive	Animalia	FELASA
	Candida tropicalis	positive	Fungi	cultivation
	Kazachstania heterogenica	positive	Fungi	cultivation

All mice were fed a standard diet (autoclaved Ssniff, Soest, Germany, R/M-H for SPF and CONV, or M-Z V1124-300 for GF-animals) *ad libitum* and were sacrificed by CO_2. $TNF^{deltaARE}$ mice were made

germfree by hysterectomy (Institute for Laboratory Animal Science; Hannover). Sterility was checked by cultivation of faeces in LB or WCA broth (OXOID) every 10-14 days and at sampling, and microscopic observation of Gram-stained faecal smears. A mould-trap was used to indicate the presence of mould. No contaminations were observed during any of the experiments.

3.3 Antibiotic treatment

CONV-TNF^{deltaARE} and -WT mice received antibiotics for 28 days from either 4-8 or 8-12 weeks of age. Antibiotics were freshly prepared twice a week and administered *ad libitum* via drinking water in light-protected bottles. Mice were sacrificed 0, 2, 4 and 6 weeks after cessation of antibiotic therapy. The antibiotic combinations used were: VM: 0.25 g/l vancomycin and 1.0 g/l metronidazole (Sigma-Aldrich and Fluka); Amp: 1.0g/l ampicillin (Sigma-Aldrich); Nor: 0.25 g/l Norfloxacine (Sigma-Aldrich); MIX: 0.25 g/l vancomycin and 1.0 g/l metronidazole; 0.25 g/l neomycin and 0.25 g/l norfloxacin (Sigma-Aldrich; Fluka).

3.4 Transfer of caecal microbiota

Caecal content from donor mice was collected and immediately suspended (1:10, w/v) in filter-sterilized PBS/glycerol (20%), snap frozen and stored at -80 °C. Aliquots were centrifuged (300g/ 3 min/ 4°C) to pellet debris. Supernatants were centrifuged (8000g/ 10min/ 4°C) and pellets re-suspended in equal volumes of PBS. For gavage, an aliquot of 100µl of caecal microbiota-suspensions of one donor mouse were given to recipients (approximately 1-5 x 10^8 cells per mouse, as determined by THOMA counting-chamber). For the colonization of GF recipients, each mouse was gavaged at the age of 8 weeks with caecal microbiota-suspensions of one (untreated) SPF-donor mouse. Mice were housed in group-specific isolators with mixed genotypes in each cage and sacrificed 4 weeks after colonization. To investigate the development of ileitis over time, additional mice were colonized as described above, and samples were collected 1, 2 and 4 weeks after colonization. For caecal-microbiota transplant experiments in conventionally reared animals, the donor mice (TNF^{deltaARE} or WT) were 12 weeks of age old, when the caecal luminal microbiota was sampled and processed as described above. One group of donor-TNF^{deltaARE} mice was pre-treated with VM as described before. Recipients were also treated with VM from the age of 8-12 weeks and received three transplants (always from the same donor) 24, 72 hours and 7 days after ending VM treatment. A control group received time and donor-matched transplants, though no VM pre-treatment. To control for influences of the gavage-procedure itself, a further control group received the same volume of PBS per gavage. Recipients were housed in donor- and pre-treatments specific cages till 18 weeks of age.

3.5 Cultivation of intestinal bacteria

According to caecal or ileal content weight measurement, ten-fold dilutions (w/v) were prepared by adding appropriate volumes of sterile PBS supplemented with peptone and cysteine (0.05 % w/v each, Fluka). Dilution series were prepared on Wilkins-Chalgren Agar supplemented with L-cysteine and dithioerythritol (0.05 % w/v each). For samples from the CONV facility, the medium was supplemented with 5 µg/ml pimaricin Sigma-Aldrich to prevent yeast growth.

3.6 Histopathology

Hematoxylin and eosin (H&E) stained terminal ileal and proximal colonic tissue sections were scored (blinded) by assessing lamina propria mononuclear cell infiltration, crypt hyperplasia, goblet cell depletion and architectural distortion resulting in a score from 0-12 as previously described.[198] Images were acquired using Digital microscope M8 (PreciPoint GmbH).

3.7 Immunofluorescence staining

Formalin-fixed paraffin-embedded tissue sections (3.5 µm) of the terminal ileum placed on Superfrost Plus slides (Thermo Scientific) were deparaffinised using a Leica ST5020 Multistainer system. Antigens were unmasked by boiling under pressure in sodium citrate buffer (pH 6, 900 W, 23 min). Tissue sections were allowed to cool down to room temperature (RT) and washed three times consecutively in deionized water and once in PBS (5 min each). Sections were incubated in blocking buffer (5 % normal goat or donkey serum and 0.3 % Triton X-100 in PBS) against the species of the secondary antibody (60 min; RT; in a humidified chamber). Primary antibodies (LY6G, 1:500, BD Pharmingen; lysozyme, 1:2000, Dako; Cleaved-caspase 3, 1:1000, Cell Signalling) were incubated overnight at 4°C. Fluorochrome-conjugated secondary antibodies (from Invitrogen, Dianova and Life Technologies) were diluted 1:200 and incubated for 1h at RT. Rhodamine-labeled UEA-I (*Ulex europaeus* agglutinin-1; Vector Laboratories) at a dilution of 1:1000 was used for detection of fucosylated cell-structures of Paneth and goblets cells. Nuclei counterstaining was done using DAPI (1:2000). Cryptdin 2 staining was performed by Aline Dupont in collaboration with the Institut for Medical Microbiology, RWTH University, Aachen, Germany. Paraffin-embedded tissue sections were deparaffinized and rehydrated. Antigen retrieval was performed using 0.01M citrate sodium buffer, pH 6.0. Slides were blocked with 5% BSA, 10% normal donkey serum (Dianova) and stained using a rabbit polyclonal antiserum against synthetic cryptdin 2 (LRDLVCYCRTRGCKRRERMNGTCRKGHLMYTLCCR; gift from Mats Andersson, Karolinska institute, Stockholm) and a mouse anti-E cadherin antibody (BD Bioscience), followed by Rhodamine Red-X-conjugated donkey anti-rabbit and AF647-conjugated donkey anti-mouse secondary antibodies (Dianova). Fluorescein-labelled wheat germ agglutinin (WGA; Vector) was used as a counterstaining. Samples were immediately sent to us and analysed within three days. Sections

were visualized using a confocal microscope (Olympus Fluoview 1000; FV10-ASW software. Ly6G-positive cells per microscopic field area were counted using the Volocity® 5.51 software (Perkin Elmer). For each individual mouse, 3 microscopic fields at a 60-fold magnification were quantified for mean Ly6G-positive cells per mm^2. Lysozyme and UEA-1 signals were quantified by counting numbers of positive cells per crypt. A minimum of 12 crypts per mouse, randomly picked and distributed all over the distal ileal cross-section, were assessed at 120-fold magnification.

3.8 Gene expression analysis

RNA of total distal ileal tissue was isolated (NucleoSpin® RNAII kit; Macherey-Nagel GmbH & Co. KG). Complementary DNA was synthetized from 500ng RNA using random hexamers and M-MLV RT Point Mutant Synthesis System (Promega). Quantification was performed using the LightCycler® 480 Universal Probe Library System (Roche). Primers and probes used were: *tnf* (5´-tgcctatgtctcagcctcttc-3´; 5´-gaggccatttgggaacttct-3´; Probe #49); *gapdh* (5'-tccactcatggcaaattcaa-3´; 5'-tttgatgttagtggggtctcg-3´; Probe #9); *ang4*; 5´-ccccagttggaggaaagc-3´; 5'-cgtaggaatttttcgtacctttca-3´; Probe #106); *defa-5* (5'-ttttgggacctgcagaaatc-3'; 5'-cagagccgatggttgtcat-3'; Probe #84); *reg3γ* (5'-accatcaccatcatgtcctg-3'; Probe #51).). Calculations (2-ΔΔCt method [199] were done normalized to *gapdh.*

3.9 ELISA analysis

Concentration of cytokines (TNF and IL-17) in EDTA-blood-plasma was determined via ELISA kits (eBiocience) according to manufacturer´s instructions.

3.10 16S ribosomal RNA (rRNA) gene sequence analysis

600µl DNA stabilization solution (STRATEC biomedical), 400 µl Phenol:Chloroform:IsoAmyl alcohol (25:24:1; Sigma-Aldrich) and 500 mg autoclaved glass beads (0.1mm; Roth) were added to frozen caecal samples (approx. 200mg). Microbial cells were disrupted by mechanical lysis using a FastPrep®-24 (3 x 30 sec at maximum speed) (MP Biomedicals) fitted with a 24 x 2 ml cooling adaptor. After heat treatment (95°C, 5 min) and centrifugation (15000xg/5 min/4°C), supernatants were treated with RNase (0.1 µg/µl; Amresco) for 30 min at 37°C. Metagenomic DNA was purified using gDNA columns (Macherey-Nagel) following the manufacturer's instructions. Concentrations and purity were checked by NanoDrop® (Thermo Scientific) and samples were stored at 4 °C during library preparation and at -20 °C thereafter for longer storage. The V3/V4 region of 16S rRNA genes was amplified (25 cycles) from 12 ng of metagenomic DNA using the bacteria-specific primers 341F and 785R [200] following a 2-step procedure to limit amplification bias [201]. Amplicons were purified using the AMPure XP system (Beckmann), pooled in an equimolar amount, and sequenced in paired-end mode (PE275) using a

MiSeq system (Illumina Inc.). A final DNA concentration of 10 pM and 15 % (v/v) PhiX standard library was used. Raw read files were demultiplexed (allowing a maximum of 2 errors in barcodes) and each sample was processed using USEARCH [202] following the UPARSE approach. [203] First, all reads were trimmed to the position of the first base with quality score <3 and then paired. The resulting sequences were size filtered, excluding those with assembled size <380 and >440 nucleotides. Paired reads with expected error >3 were further filtered out and the remaining sequences were trimmed by 10 nucleotides on each side to avoid GC bias and non-random base composition. For each sample, sequences were de-replicated and checked for chimeras with UCHIME.[204] Sequences of all samples were merged, sorted by abundance, and operational taxonomic units (OTUs) were picked at a threshold of 97 % similarity. Finally, all sequences were mapped back to the representative sequences resulting in one OTU table for all samples. Only those OTUs with a relative abundance above 0.5% total sequences per sample were kept. The final OTU counts were normalized to the sample with the lowest number of sequences. For each OTU, the most detailed taxonomic classification among Silva [205], RDP [206], and Greengenes [207], was manually assigned as final OTU taxonomy. In case of different predictions, Silva was preferred over RDP, and RDP over Greengenes. For OTUs showing significant differential abundance between groups, the EzTaxon classification [208] was used to identify the closest described species. For estimation of diversity within samples (alpha-diversity), the Shannon index was calculated and transformed to the corresponding effective number of species as described by Jost.[209] For comparisons across samples (beta-diversity), phylogenetic trees across all OTU representative sequences were constructed using the maximum likelihood method in Mega6 [210] and used for calculation of samples distances with generalized Unifrac (package GUniFrac in R; [211]).

3.11 Metaproteome analysis

The metaproteome analysis was performed by Sven Bastiaan Haange, Nico Jehmlich and Martin von Bergen in collaboration with the Helmholtz-Centre for Environmental Research - UFZ, Department of Proteomics, Leipzig, Germany. Colonic content was diluted in 1 ml of filter-sterilized PBS containing Protease- and Phosphatase-Inhibitor (Roche) and lysed using a FastPrep®-24 (3x40 sec at 6.5 M/s (MP Biomedicals). After centrifugation (16.000xg/4°C/5min), supernatants were frozen at -80°C until use. A total amount of 100 µg protein lysate was separated by SDS-PAGE. After electrophoresis, each lane was cut into three slices and processed by in gel proteolytic cleavage using trypsin.[212] Peptides were analyzed using an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific) coupled to a TriVersa NanoMate (Advion, Ltd.). A volume of 5 µL peptide lysate was separated with a Ultimate 3000 nano-LC system (Dionex, now Thermo Fisher Scientific) on a C18 reverse phase column (25 cm; 100 min linear gradient from 2% acetonitrile (ACN) to 55% ACN in 0.1% formic acid). Raw data files were searched with Proteome Discoverer (v1.4, Thermo Fisher Scientific) using the Mascot algorithm (v2.3

in-house server) against the taxonomy of mouse, bacteria, green plants and archaea of the NCBInr database. Only rank 1 peptides were considered to be identified with a threshold of FDR <1%. "PROteomics results Pruning & Homology group ANotation Engine" (PROPHANE) [213] was used to assign proteins to their taxonomic and functional groups. Label free quantification of protein groups were performed using the TOP-3 peptide abundance.

3.12 Metabolomics

The metabolome analysis was performed together with Alesia Walker and Philippe Schmitt-Kopplin in collaboration with the Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München.

Bile acid (BA) analysis: Standard stock solutions of bile acids (BAs) and three C15:0 fatty acids were prepared (1 mg/ml; 1000 ppm). Following BAs were used for studying BAs metabolism as standard library such as Chenodeoxycholic acid (CDCA; Sigma Aldrich, St Louis, MO, USA), Cholic acid (CA; Sigma Aldrich, St Louis, MO, USA), Deoxycholic acid (DCA; Sigma Aldrich, St Louis, MO, USA); Hyodeoxycholic acid (HDCA; Sigma Aldrich, St Louis, MO, USA), Lithocholic acid (LCA; Sigma Aldrich, St Louis, MO, USA), Taurochenodeoxycholic acid (TCDA; Sigma Aldrich, St Louis, MO, USA), Tauroursodeoxycholic acid (TUDCA; Calbiochem, Billerica, MA, USA), Taurocholic acid (TCA; Sigma Aldrich, St Louis, MO, USA), Taurodeoxycholic acid (TDCA; Sigma Aldrich, St Louis, MO, USA), Taurolithocholic acid (TLCA; Sigma Aldrich, St Louis, MO, USA), Ursodeoxycholic acid (UDCA; Sigma Aldrich, St Louis, MO, USA), alpha Muricholic acid (α-MCA; Steraloids, Newport, RI, US), beta Muricholic acid (β-MCA; Steraloids, Newport, RI, US), omega Muricholic acid (ω-TMCA; Steraloids, Newport, RI, US), alpha Tauromuricholic acid (α-TMCA; Steraloids, Newport, RI, US), beta Tauromuricholic acid (β-MCA; Steraloids, Newport, RI, US) and omega Tauromuricholic acid (ω-TMCA; Steraloids, Newport, RI, US). The C15:0 fatty acids were pentadecanoic acid (Sigma Aldrich, St Louis, MO, USA), isopentadecanoic acid (13-methylmyristic acid; Sigma Aldrich, St Louis, MO, USA) and antiisopentadecanoic acid (12-Methyltetradecanoic acid; Sigma Aldrich, St Louis, MO, USA). A mixture of all BAs was prepared with a concentration of 50 ppm of each BA and diluted with MEOH to 1 ppm (of each) which was used for subsequent UPLC-MS analysis. A mixture of 1 ppm of all C15:0 FAs was diluted in MEOH.

Short chain fatty acids (SCFA): Acetic acid, butyric acid, propionic acid, lactic acid, and succinic acid were all purchased from Sigma (Sigma Aldrich, St Louis, MO, USA) and were prepared in MEOH to a stock solution of 1000 ppm. All were diluted to 50 ppm as working dilution.

Acidic hydrolysis for plasmalogen analysis: A 32% HCL was used to prepare 4 M HCL. 50 µL of 4 M HCL was added to 50 µL of caecal extract, which was incubated for 30 min., at room temperature. Afterwards, 1 ml of milliQ H_2O was added and evaporated. Control samples were treated with 50 µL of pure milliQ H_2O and treated in the same way. Dried samples were re-constitued with 50 µL MEOH.

Derivatization of SCFAs: AMP+ Mass Spectrometry Kit was purchased from Cayman Chemical (Cayman Chemical, Ann Arbor, MI, USA). SCFAs were derivatized and subsequently analyzed by UPLC-MS in positive mode (+). Eight µL of each standard (5 ppm final concentration) and caecal methanol extracts were used for all mice. All other processing is described in the protocol of the AMP+ Mass Spectrometry Kit. After derivatization, 200 µL of MEOH was added to a final concentration of ~1.5 ppm.

Metabolite extraction: Around 10-20 mg (wet weight) of caecal content of each mouse was taken and weighted. Original samples were stored on dry ice. The weighted samples were transferred into sterile ceramic bead tubes (NucleoSpin® Bead Tubes, Macherey-Nagel, Dueren, Germany) primed with a stainless metal bead (5 mm, Qiagen, Hilden, Germany) and 1 mL of cold MEOH was added (-20 °C, LC-MS CHROMASOLV®, FLUKA, Sigma Aldrich, St Louis, MO, USA). Samples were homogenized in a Tissue Lyser II (Qiagen, Hilden, Germany) for 5 min at 30 Hz, centrifuged 10 min at 21,000 g and 4 °C. Supernatants were transferred into sterile Eppendorf tubes and stored at -80°C before mass spectrometry analyses.

High resolution mass spectrometry – ICR-FT/MS: Metabolite analysis was performed using ESI (-) ICR-FT/MS mode in direct infusion using an autosampler system (Gilson, Inc., Middleton, WI, USA) with a flow rate of 2 µL/min. Internal calibration of ICR-FT/MS was done before analyses by using 3 mg/L L-arginine, diluted in MEOH (following theoretical mass signals were used for calibration 173.10440, 347.21607, 521.32775 and 695.43943). ESI parameters were as followed: capillary of 0.5 kV, source temperature of 120 °C, drying gas temperature of 180°C, drying gas flow rate of 4.0 L/min, nebulizer gas pressure was 1.0 bar and capillary voltage of 4000 V. The mass range was set to acquire the m/z range 147.4-1000 with a number of scans of 450. Time domain was 2 megaword. Ion accumulation and ion cooling time was set 0.500 sec and 0.050 sec, respectively. Time of flight was set to 0.8 msec. After acquisition, raw spectra were calibrated with reference lists of fatty acids ($C_{13}H_{25}O_2$; $C_{15}H_{29}O_2$; $C_{17}H_{33}O_2$; $C_{19}H_{37}O_2$; $C_{21}H_{41}O_2$; $C_{23}H_{45}O_2$; $C_{25}H_{49}O_2$; $C_{27}H_{53}O_2$; $C_{29}H_{57}O_2$; $C_{31}H_{61}O_2$ - 213.186004; 241.217304; 269.248604; 297.279904; 325.311204; 353.342504; 381.373804; 409.405105; 437.436405; 465.467705). Calibrated mass spectra were exported with a signal to noise ratio of 4 and relative intensity threshold (base peak) of 0.01 % to asc. files, containing m/z values and their intensities values. The alignment of asc. files, was done with an in-house software (Matrix Generator) within a window of 1 ppm. The mass signals found within this window were averaged and their respective intensities of each sample were integrated into a data matrix. Afterwards, only mass signals were included in further data analysis, which were annotated in MassTRIX. All annotated isotopes were excluded from further data analysis. Moreover, a frequency count of 50% cut-off of each group (n = 6) for each mass signal was performed. All mass signals below, were removed from data analysis. This data was normalized to the total ion count (TIC) and auto-scaled before performing multivariate data analysis.

UPLC-MS analysis using (-) TOF MS/MS: A reversed-phase separation was applied using a C8 column (C8: 1.7 µm, 2.1 x 150 mm, Acquity™ UPLC BEH™, Waters, Milford, MA, USA). Elution of derivatized SCFAs was ensured by following solvent system of ammonium acetate (5 mM, Sigma Aldrich, St Louis, MO, USA) combined with acetic acid (0.1 %,pH 4.2, Biosolve, Valkenswaard, NL) in water (A) (milliQH_2O, Milli-Q Integral Water Purification System, Billerica, MA, USA) and acetonitrile (B) (LC-MS CHROMASOLV®, FLUKA, Sigma Aldrich, St Louis, MO, USA). The flowrate was 0.3 mL/min, injection volume of 1 µL and column temperature was 40°C.

A gradient profile was applied by starting at 1% B for 1 min., increasing to 95%B within17 min. The 95%B was hold for 2.0 min, returning to initial 1%B within further 0.2 min and holding 2 min with a total run time of 22 min. A pre-run time of 2 min was also included.

Peak areas were elaborated with QuantAnalysis (Bruker, Daltonics, Bremen, Germany). Mass signals for derivatized SCFAs were as following in positive mode: acetic acid ($[M]^+$: 227.1184); propionic acid ($[M]^+$: 241.1341); butyric acid ($[M]^+$: 255.1492), , lactic acid ($[M]^+$: 257.128454) and succinic acid ($[M]^{2+}$:226.1106).

Non-targeted metabolomics: MEOH extracts of cecum were subsequently used for reversed-phase separation. The gradient was optimized for bile acids separation. The optimized gradient was applied in a non-targeted metabolomics way.

Cecal extracts (methanol supernatants) were separated using a reversed-phase column (C8: 1.7 µm, 2.1 x 150 mm, Acquity™ UPLC BEH™, Waters, Milford, MA, USA) applying flow rate of 350 µl*min^{-1} with an injection volume of 5 µl and column temperature of 60°C, respectively. Elution of BAs and other compounds was ensured by following solvent system of ammonium acetate (5 mM, Sigma Aldrich, St Louis, MO, USA)) combined with acetic acid (0.1 %,pH 4.2, Biosolve, Valkenswaard, NL in water (A) (milliQH_2O, Milli-Q Integral Water Purification System, Billerica, MA, USA) and acetonitrile (B) (LC-MS CHROMASOLV®, FLUKA, Sigma Aldrich, St Louis, MO, USA). A gradient profile was applied by starting at 10% B for 1 min., increasing to 22%B within 2 min., followed by increase to 27%B within 4 min., accompanied by a linear gradient to 95%B within 13 min. The 95%B was hold for 2.5 min, returning to initial 10%B within further 2.5 min with a total run time of 25 min.

The UPLC was coupled to maXis (qq-TOF-MS). Internal Calibration of mass spectrometer was done with 5 ppm L-arginine (MEOH dilution, Sigma Aldrich, St Louis, MO, USA). External Calibration of mass spectrometer was ensured by injecting ESI-L Low Concentration Tuning Mix 100 mL (Agilent, Santa Clara, CA, United States) in front of each sample, in the first 0.5 min of LC-MS run, by a switching valve. Tuning mix was diluted (1:4) in pure MEOH and injected with a flow rate of 0.2 mL/h in 10 mL syringe. Derived LC-MS data were processed using the Genedata Refiner MS software (Genedata GmbH, Munich, Germany). The data consisted of m/z and their respective retention time (RT) and summed

intensity (Cluster_nr). After statistical data elaboration, important clusters were evaluated in terms of identification via MS/MS. Standards matching were only done for BAs and C15:0 FAs.

UPLC-TOF MS/MS analysis –Fragmentation: Data dependent MS/MS analysis (Auto MS/MS) was performed on a representative sample of each group (in total 6 LC-MS/MS, based on TIC of each group). MRM-MS was done for selected clusters such asAcyl-PGs. Annotation was done by means of MassTRIX (±0.005) and METLIN (10 ppm) as first indicators of compound class.[214, 215] FTICRMS data is based solely on annotation with MassTRIX. In terms of UPLCMS data, identification was done by elaboration of MS/MS spectra. Metabolites, which were identified after MS/MS elaboration, were named. Annotated fatty acids were labelled as following; the number of carbons (C) and number of double bonds (C:nr). All other (annotated or unknown) were treated as mass signals with their respective RT (Cluster_nr). Several databases such as MetFrag, METLIN and MyCompoundID were used for MS/MS analysis.[216])[217] Spectra were observed visually for existing fragments.

3.13 Statistics

Statistical analyses were performed with R or Sigma Plot 11.0 using ANOVA followed by pairwise comparison testing (Holm-sidak test). Graphics were created using GraphPad Prism version 5.00. Unless otherwise stated, data are presented as mean ± SD and p-values below 0.05 were considered to be significant. The Benjamini-Hochberg method was used for adjustment after multiple testing. For visualization of the relationships between bacterial profiles, non-parametric multiple dimensional scaling (NMDS) plots were computed using the packages vegan and ade4. Multivariate data analysis of metabolome was performed with SIMCA-P 9.0. Ileitis score values and TIC normalized metabolome data values were auto-scaled before analysis. Principal component analysis (PCA) and partial least square regression (PLS) or discriminant analysis (PLS-DA) was applied by using metabolome and ileitis scores-values or dummy variables for PLS modeling. VIP values were used for further data analysis.

Pearson correlation was calculated between ileitis scores and metabolites and furthermore all metabolites with correlation values of $r>0.5$ and $r<-0.5$ were considered to be important. Student's t-test was performed in Microsoft Office Excel 2010. Two-way ANOVA was performed with Sigma Plot 12.0 (genotype and treatment, Tukey test).

4 RESULTS

4.1 Inflammation in TNF^{deltaARE} mice is linked to changes in gut bacterial communities

4.1.1 The intestinal ecosystem in the presence of inflammation

To assess the role of the gut microbiota in CD-like inflammation, TNF^{deltaARE} mice and WT-littermates were cohoused in a CONV hygienic environment. At the age of 12 weeks TNF^{deltaARE} mice developed tissue pathology in the terminal ileum without cage- or sex-specific differences (**Figure 4A-B**).

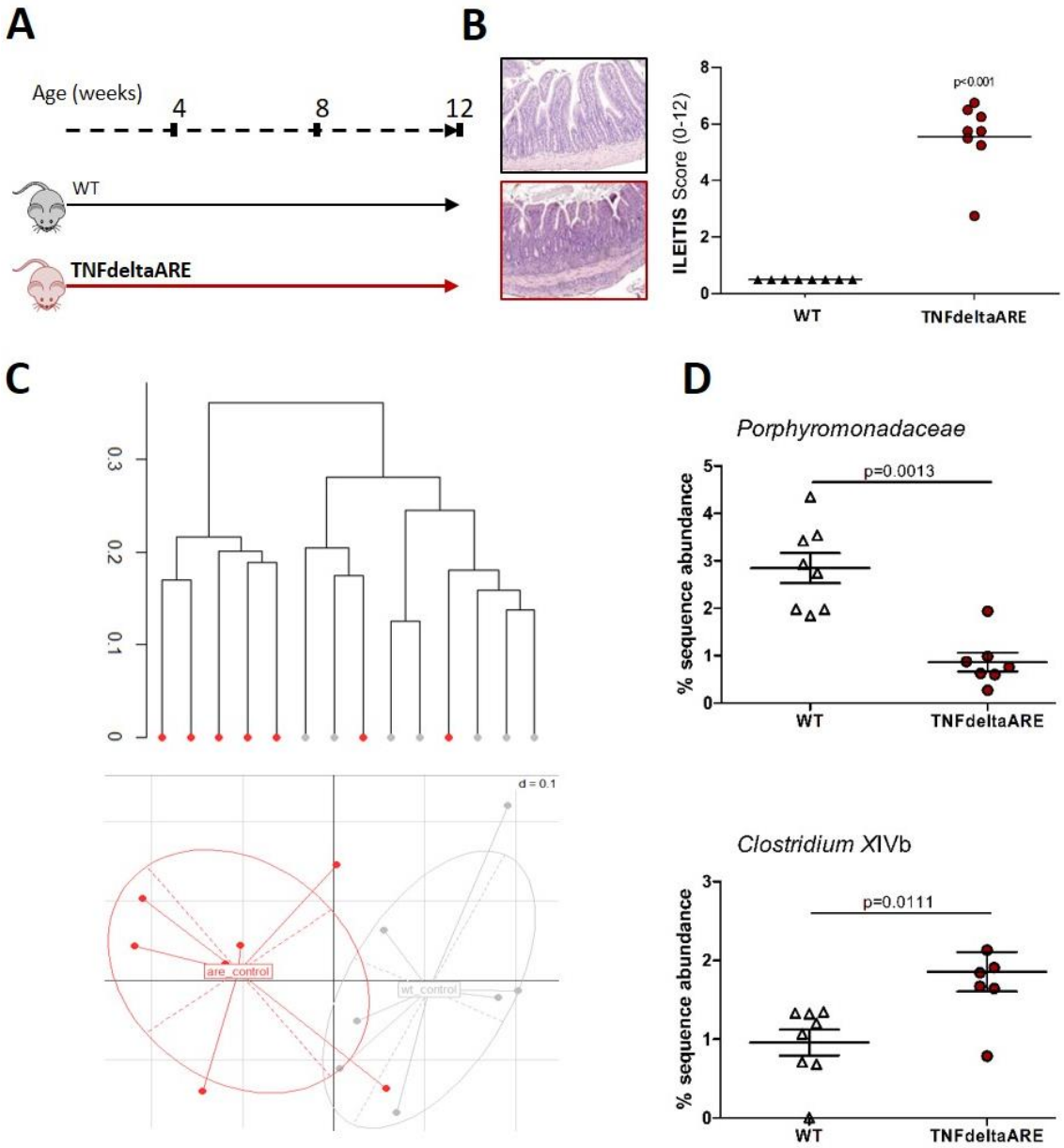


Figure 4: Inflammation in TNF^{deltaARE} mice is associated with changes in the caecal microbiota
(A) Experimental setup: TNF^{deltaARE} and WT-littermates were cohoused till the age of 12 weeks. **(B)** Left: Respective H&E stained terminal ileal sections. Black: WT; Red: TNF^{deltaARE}. Right: Ileitis scores in the terminal ileum. **(C)** Dendrogramm and NMDS plot (below) showing variance of caecal bacterial profiles of TNF^{deltaARE} (red) and WT (grey) mice. 16S ribosomal RNA gene amplicons of the V3/V4 region (450bp) in faeces. **(D)** Sequence abundance in % of inflammation related bacterial taxa. Student´s t-test.

To describe inflammation-dependent differences in the caecal microbiota, 16S microbial profiling (sequencing of 16S ribosomal RNA gene amplicons of the V3/V4 region (450bp)) was performed. Though mice were cohoused and showed coprophagic behaviour, slight genotype-induced separation was observed (**Figure 4C**). This clearly indicated the influence of an inflammatory milieu on the phylogenetic makeup in the luminal ecosystem. Thus, the analysis of taxonomic composition (on classification level of "family") revealed significant reduction in *Porphyromonadaceae* in inflamed animals. In contrast, *Helicobacteraceae* and *Clostridium XIVb* were increased in inflamed animals.

Beside alterations in the bacterial pattern, changes in the caecal bacterial metabolism may also be affected by the presence of inflammation. By non-targeted metabolomics with direct infusion FT-ICR-MS and UPLC-ToF-MS, metabolite profiles were assessed in collaboration with the Research Unit *Analytical BioGeoChemistry* at the *Helmholtz Zentrum* (Munich). Partial least squares (PLS) analysis revealed separation of genotypes according to ileitis severity (y-axis) and metabolite profile (**Figure 5A**). Further analysis was done for UPLC-ToF-MS data, as the derived model was better compared to FT-ICR-MS. The metabolites with highest impact on the regression are given in **Figure 5B** by a very important projection (derived from the PLS-model; VIP-values are indicated by dots).

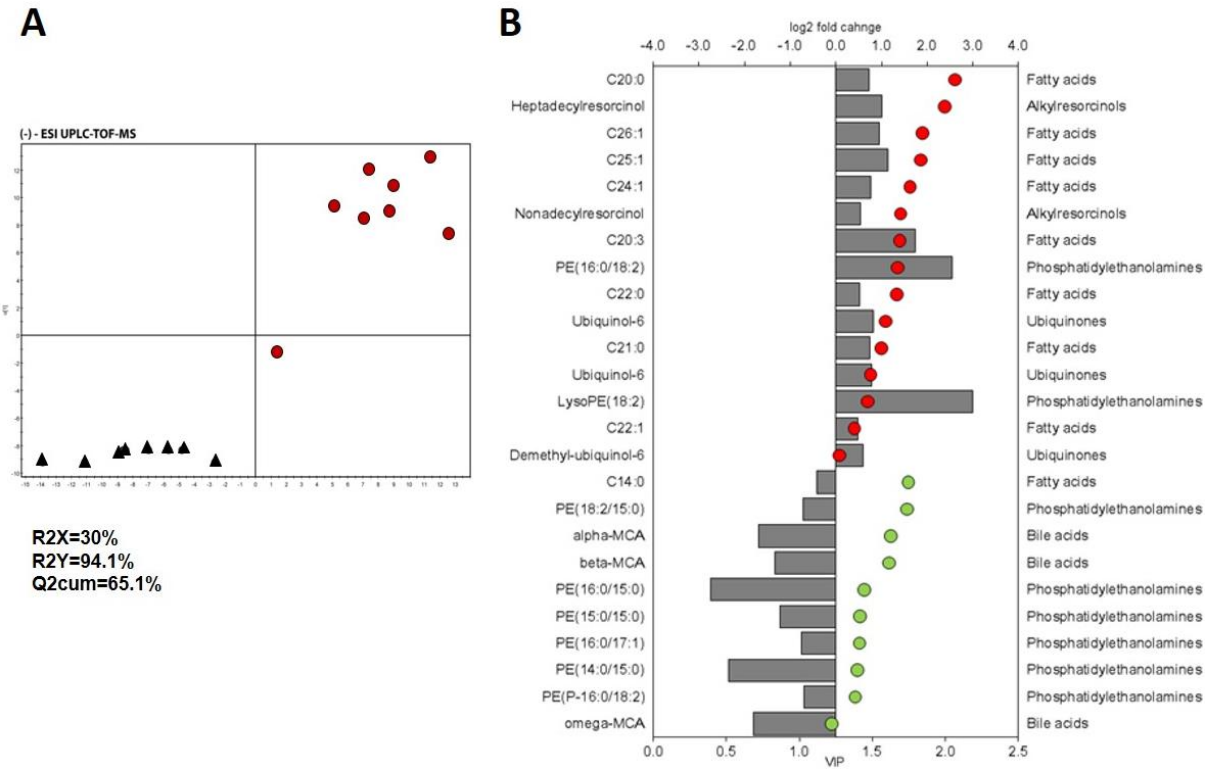


Figure 5: Inflammation is associated with shifts in the intestinal metabolite profile
(A) PLS analysis of TNF^{deltaARE} and WT mice. Divergence of clusters according to metabolite profiles (x-axis) and ileitis-severity (y-axis). **(B)** Metabolites with highest fold-change (scale-bars) and resulting VIP-values (shown as dots; lower x-axis) which are upregulated in TNF^{deltaARE} (red) or WT mice (green). Respective chemical classes are shown on the right.

Many of the 25 most regulated metabolites with high VIP-value (1.2 - 2.1) were fatty acids, suggesting that the metabolism of fatty acids seems to be altered by the presence of inflammation. 15 metabolites were increased (eight different fatty acids, three ubiquinones, two alkylresorcinols, one phosphatidylethanolamine (PE(16:0/18:2)) and one LysoPE(18:2)). In contrast, 10 metabolites (six different PEs, one middle chain fatty acid (C14:0), and α-, β- and ω-MCA) were decreased in TNF^{deltaARE} mice compared to WTs. **These data clearly demonstrate minor changes in the bacterial composition and metabolite profile in an early phase of inflammation – even in a situation of continuous interaction with non-inflamed individuals.** To confirm these observations and reduce mixture of genotype-specific microbial patterns, an additional cohort was housed in genotype-specific cages. In this setup, faecal bacterial diversity and composition was observed over time. We previously showed that ileitis develops gradually in CONV-TNF^{deltaARE} mice and reaches maximum levels at 12 weeks.[218] Therefore faecal samples were collected at 4, 8 and 12 weeks of age (**Figure 6A**).

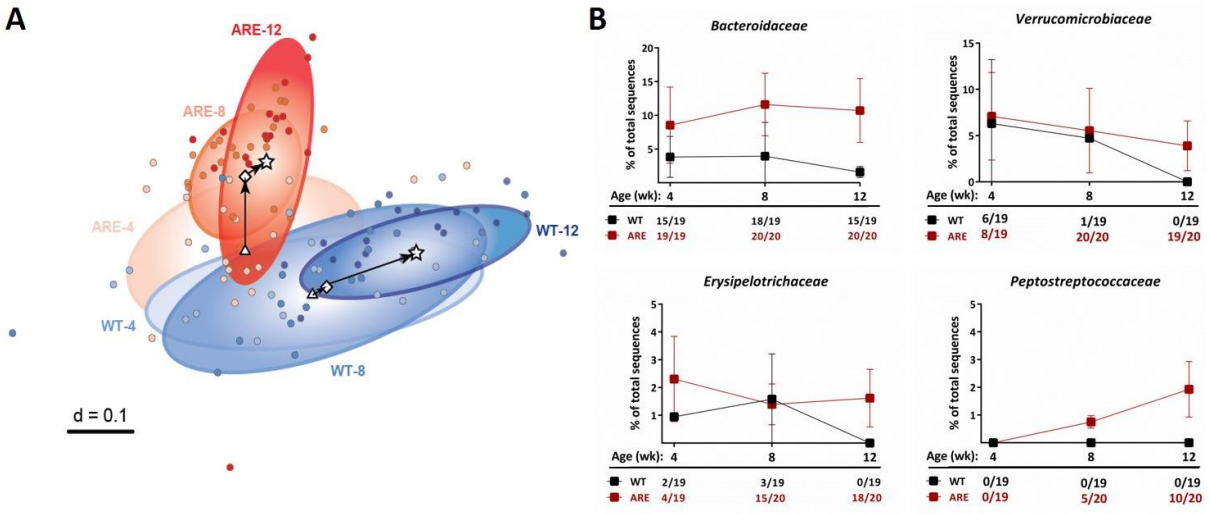


Figure 6: Divergence of faecal bacterial ecosystems are associated with increasing inflammation
(A) NMDS plot showing shifts of centroids (indicated by arrows) and variance (circled areas) of faecal bacterial profiles of CONV-TNF^{deltaARE} (ARE; red) and WT (blue) mice at 4, 8 and 12 weeks of age. 16S ribosomal RNA gene amplicons of the V3/V4 region (450bp) in faeces **(B)** Relative abundances of bacterial sequences (%) in WT and ARE mice at 4, 8 and 12 weeks of age. The numbers of mice positive for the respective bacteria are given below, as quotient of all mice tested. Two-way ANOVA; Holm-Sidak.

In the absence of inflammation (age of four weeks), bacterial phylogenetic makeups in TNF^{deltaARE} and WT mice were clearly overlapping. Shifts in community structure started to be distinct at the age of eight weeks, when tissue pathology reaches significance. Parallel to the age-related increase in inflammation, microbial communities from WTs diverged from TNF^{deltaARE} mice and inter-individual differences decreased within genotypes. Main significant differences in composition included increased abundance of *Bacteroidaceae*, *Erysipelotrichaceae*, *Peptostreptococcaceae*, and *Verrucomicrobiaceae* in TNF^{deltaARE} mice ($p<0.001$). The increase in *Bacteroidaceae* was primarily due to three OTUs closely related to *Bacteroides acidifaciens* and *Bacteroides sartorii* (**Figure 6B**).

The divergent intestinal ecosystems in inflamed TNF^{deltaARE} and non-inflamed WT mice clearly shows the presence of dysbiosis in the presence of inflammation. Increasing inflammatory conditions may lead via various mechanisms (e.g. reduction in AMP production), to separation of the microbial community structure. Nevertheless, to investigate a putative causal relationship between microbial triggers and ileitis development, pathology development was tested in the absence of microbial triggers.

4.1.2 The presence of bacterial stimuli is a prerequisite for ileitis

To assess the role of the gut microbiota in the development of CD-like inflammation, we generated GF-TNF^{deltaARE} mice. At the age of 18 weeks, we compared inflammation development to age matched CONV housed mice. Under CONV but not GF housing, TNF^{deltaARE} mice developed tissue pathology in the terminal ileum, showing necessity of microbial stimuli for pathology induction (**Figure 7A**).

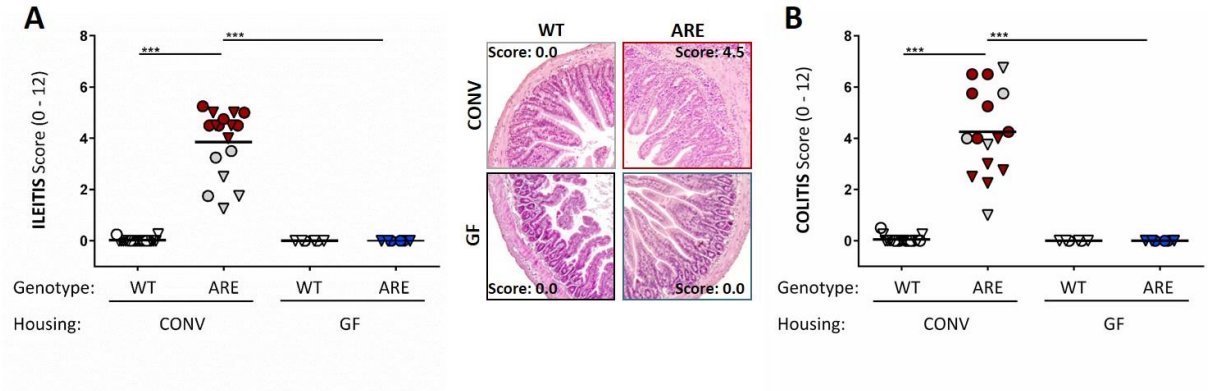


Figure 7: Inflammation in TNF^{deltaARE} mice is dependent on microbial triggers
(A) Ileitis scores (left) and representative H&E-stained sections of the distal ileum in 18-week-old TNF^{deltaARE} (ARE) and WT littermates in CONV or GF housing. Ileitis-score groups are color-coded according to inflammation-severity: score = 0 (blue); score <4 (grey); score >4 (red). Male mice are displayed as triangles, females as circles. **(B)** Colitis cores of the proximal colon.

Although TNF^{deltaARE} mice are known as model for CD-like ileitis [111], inflammation of the proximal colon was observed in this cohort of CONV-TNF^{deltaARE} mice (**Figure 7B**). Neither cage effects, nor correlation between ileitis and colitis severity were observed, as several mice with a medium ileitis score (Score 1-4; grey) developed severe colitis (Score >4). In the absence of microorganisms, GF-TNF^{deltaARE} mice were also free of colonic disease. **The lack of intestinal pathology in the complete absence of microbial stimuli emphasizes the crucial role of bacterial triggers in this mouse model of CD-like inflammation.** However, GF animal models have limitations in translating some mechanisms of microbe-host interaction, as they are devoid of a fully maturated immune system or mucus layer. Therefore targeted alteration of bacterial stimuli in colonized mice and the impact on inflammation severity was tested.

4.2 Targeted changes in the intestinal microbiota attenuate inflammation

4.2.1 Antibiotics are capable to prevent development of ileitis

The microbiota was shown to be associated to ileitis severity in TNF^{deltaARE} mice. Consequently, the potential of targeted alteration of the intestinal microbiota to prevent disease-onset, was assessed. Therefore, different antibiotic mixtures were applied to TNF^{deltaARE} and WT mice before development of inflammation. CONV housed TNF^{deltaARE} and WT mice were treated with antibiotics from the age of 4 weeks on – when inflammation is not yet visible (**Figure 8A**). At the age of eight weeks, TNF^{deltaARE} mice in the control group showed higher ileitis (3.92±0.52), compared to intervention groups (**Mix** (2.14±1.0) and Vancomycin/Metronidazol **VM** (1.85±0.7); **Figure 8B**)**.** Treatment with Norfloxacin (**Nor**) did not lead to significantly attenuated inflammation (2.79±0.8; p>0.05).

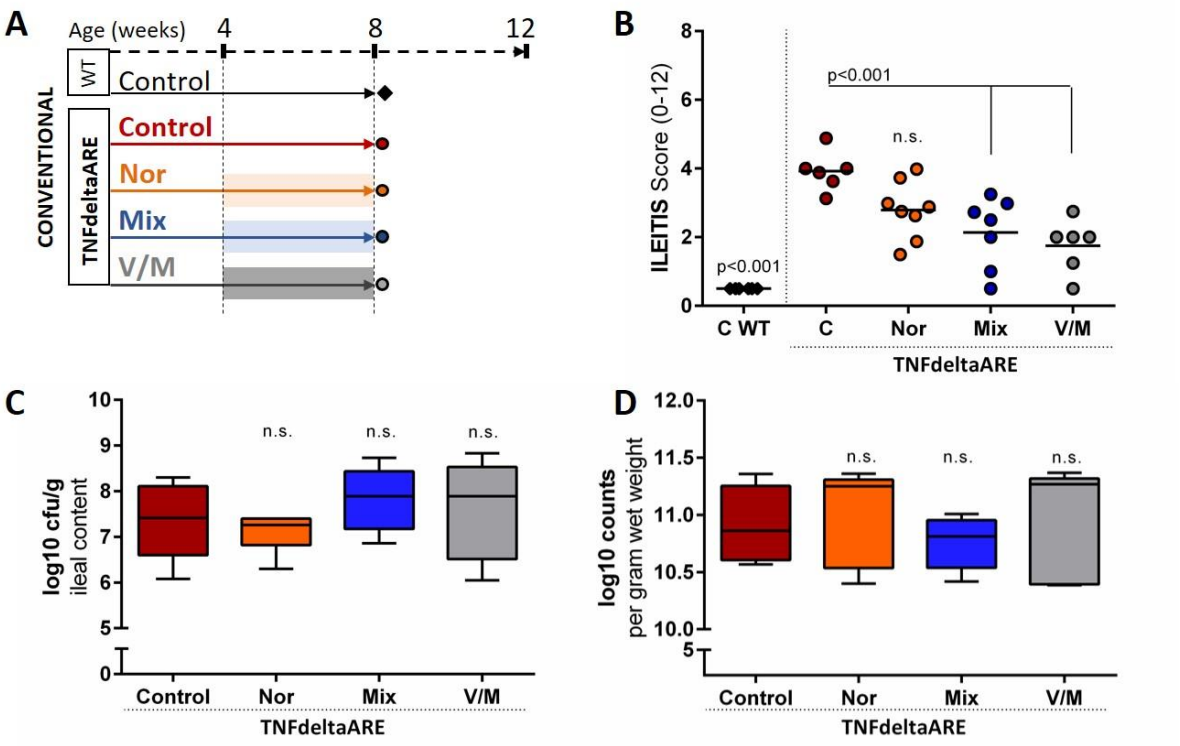


Figure 8: Different effectiveness of antibiotic regimes to prevent onset of pathology
(A) Treatment scheme. TNF^{deltaARE} and WT littermates were cohoused in CONV housing. Antibiotics (Norfloxacin, or vancomycin, metronidazole, norfloxacin and neomycin (Mix), or vancomycin and metronidazole (VM) were administered for 28 days, starting at 4 weeks of age. **(B)** Ileitis scores of Control WT (C WT) and TNF^{deltaARE} mice. **(C)** Ileal bacterial density of cultivable anaerobes (as log 10 of colony forming units (cfu) per gram ileal content; n = 3-6). **(D)** Total cell counts of ileal luminal content (as determined using a THOMA counting chamber; n = 5-8). Two-way ANOVA; Holm-Sidak.

Even though all mice were housed in our CONV-facility, no colitis was observed at this time of experiments. Comparison of FELASA-reports depicted no differences in the presence of murine pathogens between different experiment times (**Table 2**). To test, whether bacterial quantity is causative for antibiotic-effectiveness, the bacterial load was assessed by quantification of cultivable anaerobes (on WCA medium) and total cell counts of ileal luminal content (as assessed by THOMA counting chamber). Interestingly, after 4 weeks of treatment, there was no reduction in bacterial

density, hinting towards an overgrowth of resistant bacterial groups during the intervention phase (**Figure 8C and D**). Bacterial density in TNF^{deltaARE} mice was comparable to WTs (data not shown).

As antibiotics were shown to effectively protect from pathology, it was tested whether targeted changes in microbial communities can also antagonize established inflammation. Therefore, inflamed TNF^{deltaARE} mice were treated with different antibiotics from the age of 8 to 12 weeks. After antibiotic intervention, ileitis was significantly attenuated by VM and Mix (**Figure 9A-B**). **Again, antibiotic intervention did not reduce total cell counts, which indicates that the protective effects are mediated by changes in the microbial composition rather than reduction of bacterial numbers** (**Figure 9C**).

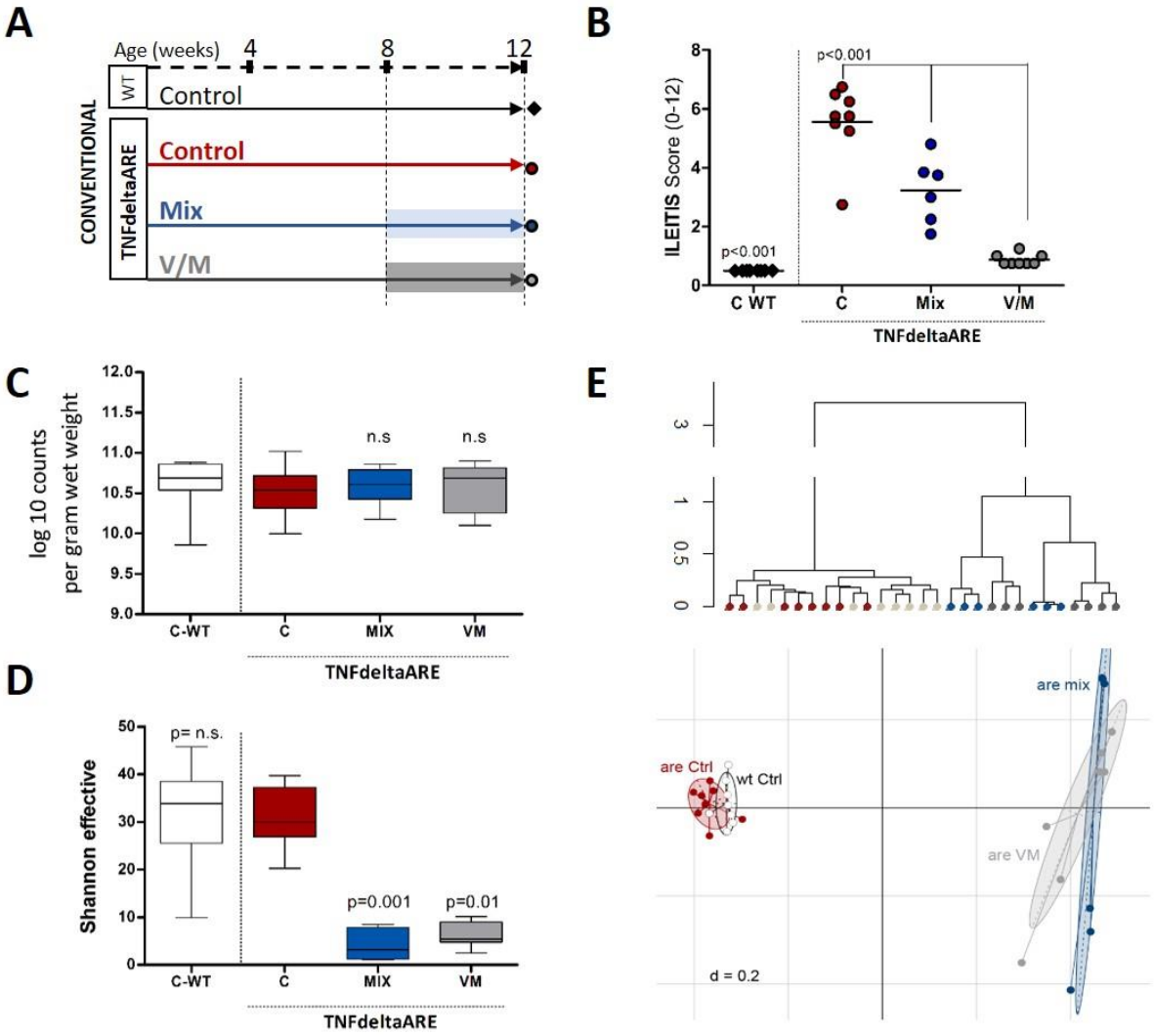


Figure 9: Antibiotic-induced shifts in the intestinal ecosystem reduce ileitis severity
(A) Treatment scheme. TNF^{deltaARE} (ARE) and WT littermates were cohoused in CONV-housing. Antibiotics (vancomycin and metronidazole (VM) or vancomycin, metronidazole, norfloxacin and neomycin (Mix) were administered for 28 days, starting at 8 weeks of age. **(B)** Ileitis scores of Control WT (C WT) and TNF^{deltaARE} mice. **(C)** Total cell counts of ileal luminal content (as determined using a THOMA counting chamber; n = 5-8). **(D)** Shannon effective species counts in WT and TNF^{deltaARE} mice. One- or Two-way ANOVA; Holm-Sidak**. (E)** Upper part: Dendrogramm of TNF^{deltaARE} (red dots) and WT mice (black dots) in the control group and TNF^{deltaARE} mice after Mix- or VM-intervention (blue and grey dots, respectively). NMDS plot showing caecal bacterial profiles. 16S ribosomal RNA gene amplicons of the V3/V4 region (450bp) of caecal content were sequenced on a MiSeq platform.

To assess for shifts in the phylogenetic makeup after antibiotic treatment, 16S profiling was performed. According to expectations, antibiotics diminished bacterial diversity dramatically (**Figure 9D**).

Beta-diversity was assessed and revealed clear separation of controls and different antibiotic treatment groups (**Figure 9E**). As visible on the NMDS plot, the MIX and VM treated profiles cluster closer together, reasoned by the fact that MIX treatment also contains VM and therefore targets similar bacterial groups. The antibiotic-induced shift in the caecal ecosystem is already evident at phylum level (**Figure 10A**).

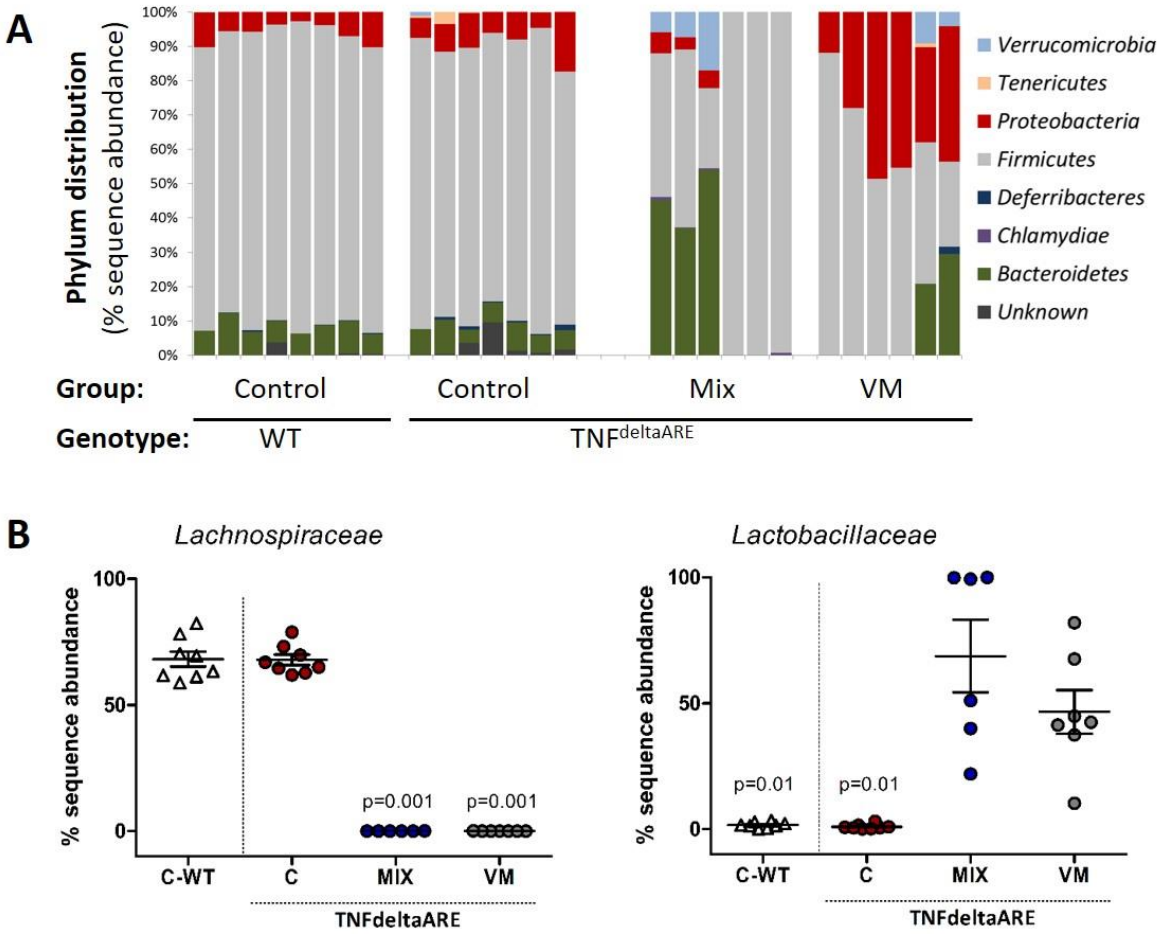


Figure 10: Antibiotics induce compositional alterations in the caecal microbiota
(A) Changes in caecal bacterial composition (relative abundance of total sequences) at the phylum level. **(B)** Most regulated bacterial taxa at family-level. Two-way ANOVA; Holm-Sidak**.**

In untreated TNF^{deltaARE} and WT mice, *Firmicutes* and *Bacteroidetes* were the dominant phyla. After VM treatment a reduction of *Bacteroidetes* and a bloom in *Firmicutes* (*Lactobacillus* spp.) and *Proteobacteria* (*Escherichia coli*) was observed in TNF^{deltaARE} mice. The high number of antibiotic-induced changes at lower taxonomic levels is shown in **supplementary Table 1.** Most OTUs that were eliminated to abundances below detection level, were of the family of *Lachnospiraceae* (n = 20; **Figure 10B**) or *Ruminococcaceae* (n = 6). Some OTUs are increased upon antibiotic treatment and are therefore regarded as indifferent to ileitis development or even protective. While MIX and VM induced

a bloom in *Lactobacillaceae* (**Figure 10B**), a distinct increase in *Enterobacteriaceae* was observed for VM treated mice only. Furthermore, MIX increased the abundance of an unknown member of the order of *Clostridiales.*

Antibiotics were shown to induce large changes in the intestinal community structure and not only single bacterial species. Therefore, also great shifts in the metabolite profile can be expected.

4.2.2 Antibiotic treatment affects the caecal metabolome

To assess variations in the metabolite profile associated to alterations in the microbiota, caecal luminal metabolites of antibiotic treated TNF^{deltaARE} mice were measured. As shown before, inflammation *per se* induced shifts in the metabolite profile **(Figure 5)**. Therefore, we focused in TNF^{deltaARE} mice for metabolites, which are changed in their abundance and putatively associated to inflammatory settings. In fact, **both antibiotics fundamentally modified the caecal metabolome in TNF^{deltaARE} mice**, as visualized by the principal component analysis of UPLC-TOF-MS data in **Figure 11A**. A total of 142 metabolite-clusters depicted a VIP-value above 1.2. Not all of them could be identified due to low abundance, insufficient MS/MS fragmentation or deisotoping. For that reason, only significantly regulated and identified metabolites, with the highest fold-change upon intervention are displayed in **Figure 11B** and **supplementary Table 2**. Antibiotic treatment clearly depleted several lipids, e.g. phosphatidylglycerides (PGs) or phosphatidylethanolamines (PEs) and phosphatides (PAs) with different fatty acid composition. Interestingly, we could also detect another class of lipids with m/z values higher than 900 Da that were decreased by antibiotics ([M-H]; 955.7317; 941.7077). Further MS/MS derivation classified them into acylphosphatidylglycerols (Acyl-PGs; PG(P-18:1/14:0/16:0)), which were described in *Salmonella Typhimurium* first.[219] Also other metabolite classes such as bile acids (including DCA, α-MCA, LCA and 7-Sulfocholic acid) were reduced by antibiotics and will be discussed in more detail below. In contrast cholesterolsulfate, galactosylglycerol, different sulfated flavonoids (apigenin sulfate), fatty acids, taurocholic acid (TCA)-sulfate and three ubiquinons (UbQs) increased under antibiotic intervention. Also for PE(16:0/17:1) higher levels were detected. PE(16:0/17:1) was previously reported to be a putative membrane constituent of *E. Coli* [220], this would back up the observation of increased levels of *E. Coli* in antibiotic (especially VM) treated mice.

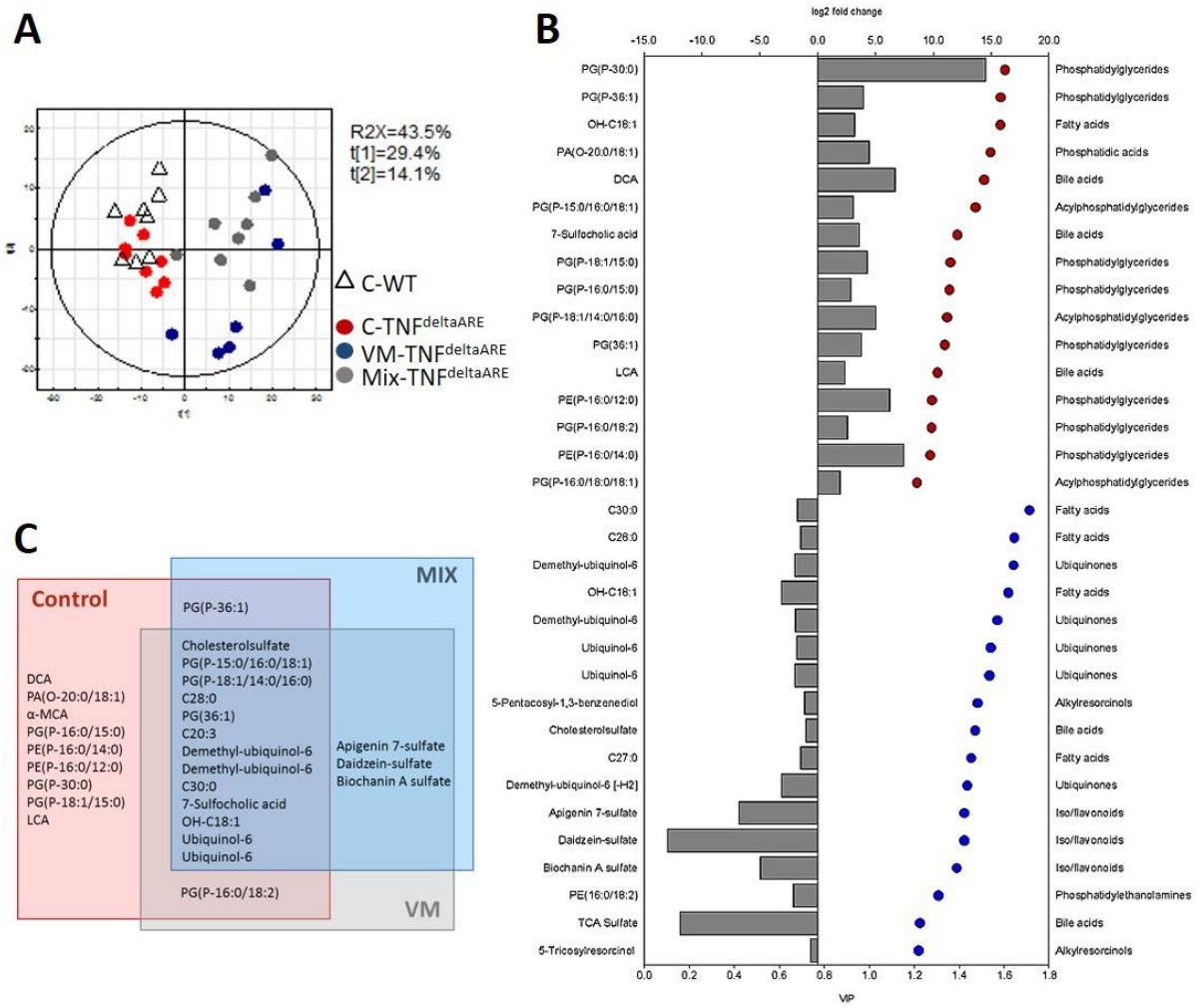


Figure 11: Antibiotic induced differences in metabolite cluster
(A) Principal component analysis (PCA) of caecal metabolite profiles in TNF^{deltaARE} (circles) and WTs (triangles) were generated by (-)-UPLC-TOF-MS. Antibiotic treated mice (VM: grey; MIX: blue) cluster apart from controls (red and white). **(B)** Metabolites with highest fold-change (scale-bars) and resulting VIP-values (shown as dots; lower x-axis) which are higher in control TNF^{deltaARE} (red) or antibiotic treated mice (blue). Respective chemical classes are shown on the right. **(C)** Venn Diagram of significantly regulated and identified metabolites, which are unique for each group.

Additionally, some fatty acids including C30:0 and C28:0, as well as different ubiquinones and sulfated iso/flavonoids like apigenin-7-sulfate or daidzein-sulfate were increased by VM and MIX. Hereby, several metabolites were unique for one of the experimental groups. As shown in the Venn-Diagramm in **Figure 11C,** 12 metabolites were unique for untreated TNF^{deltaARE} mice. The bile acids DCA, LCA, ω-MCA and PA(O-20:0/18:1) were found in controls, though they were absent in antibiotic treated mice. In contrast, apigenin 7- sulfate, daidzein-sulfate, TCA sulfate and biochanin A sulfate were exclusively found after antibiotic treatment. Galactosylglycerol was found exclusively in VM treated TNF^{deltaARE} mice. This hints towards the loss of bacterial mediated metabolism of flavonoids or increased sulfate conjugation in liver or loss of bacterial mediated de-sulfation of excreted liver metabolites.

These changes in the metabolite profile were traced back to antibiotic induced modulation in the phylogenetic composition. Hereby, inflammation relevant bacterial taxa can be correlated to the

metabolite profile to unveil microbe-metabolite interactions. The strongest antibiotic-microbe-metabolite-coherences are given in **Table 3.**

Table 3: Significant shifts in the metabolite profile are associated to OTU-abundances.
OTUs and their taxonomic assignment were positively or negatively correlated to the quantity of significantly regulated and identified metabolites.

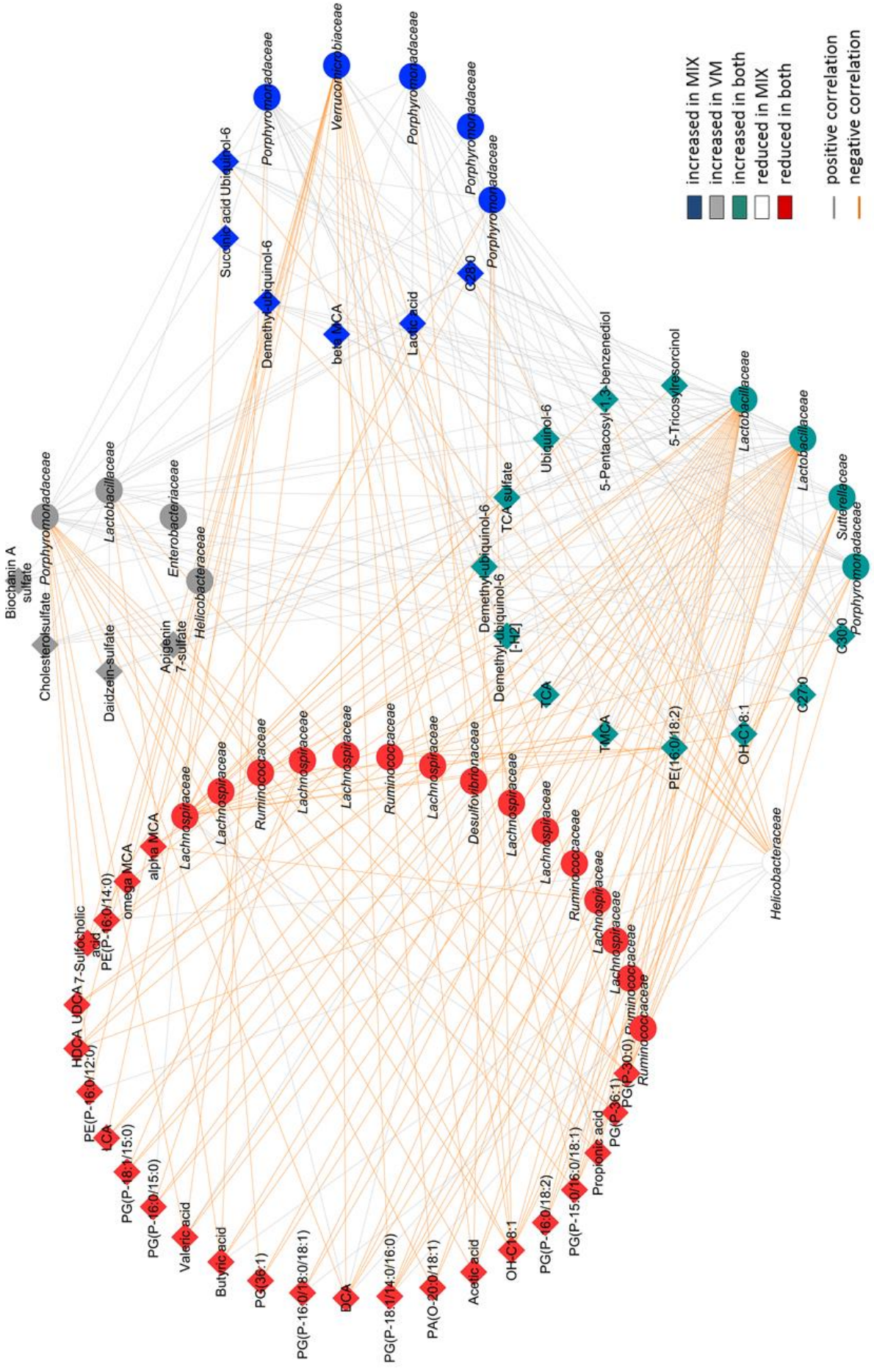
OTU-number	Taxonomic assignment (family)	OTU-abundance	Metabolite Name	Correlation
OTU_80	*Porphyromonadaceae*	↑ MIX	Lactic acid	0.92
			Ubiquinol-6	0.9
			Demethyl-ubiquinol-6 [-H2]	0.87
			OH-C18:1	0.87
			Demethyl-ubiquinol-6	0.87
OTU_54	*Porphyromonadaceae*	↑ MIX; ↑VM	OH-C18:1	0.93
			C30:0	0.92
			PE(16:0/18:2)	0.91
OTU_6	*Porphyromonadaceae*	↑ MIX	beta MCA	0.9
			TMCA	0.86
OTU_73	*Porphyromonadaceae*	↑ MIX; ↑VM	Acetic acid	-0.84
OTU_61	*Lachnospiraceae*	↓ MIX; ↓VM	Lactic acid	-0.9
OTU_14	*Verrucomicrobiaceae*	↓ MIX; ↓VM	PA(O-20:0/18:1)	-0.96
			DCA	-0.93
			OH-C18:1	-0.9
			LCA	-0.87
			PE(P-16:0/12:0)	-0.87
			PG(P-18:1/14:0/16:0)	-0.85
			Propionic acid	-0.83
			PE(P-16:0/14:0)	-0.83

Furthermore, a network analysis was performed to visualize OTU-metabolite correlations. On phylum level, *Firmicutes* are dominating the correlations, with 137 connections to metabolites (in comparison: *Proteobacteria* 33; *Bacteroidetes* 87; **Figure 12**).

The network-nodes represent metabolites (squares) and OTUs (circles), which were modulated in their abundance by VM or MIX treatment. The network depicts positive correlations (e.g. secretion or cell component) by grey connections and negative ones (e.g. degradation of this metabolite by the respective bacteria) in orange.

Next page:

Figure 12: Network analysis of significantly affected metabolites and bacteria
Network analysis showing significantly regulated metabolites of $TNF^{deltaARE}$ mice. Taxonomic assignment was done (if possible) to family level. Grey arrows indicate positive correlations, orange ones indicate negative ones.


increased in MIX
increased in VM
increased in both
reduced in MIX
reduced in both
positive correlation
negative correlation
Biochanin A sulfate
Cholesterolsulfate
Porphyromonadaceae
Lactobacillaceae
Daidzein-sulfate
Enterobacteriaceae
Apigenin 7-sulfate
Helicobacteraceae
Succinic acid
Ubiquinol-6
Demethyl-ubiquinol-6
Verrucomicrobiaceae
beta MCA
Lactic acid
C28:0
TCA sulfate
Demethyl-ubiquinol-6 [-H2]
TCA
Ubiquinol-6
5-Pentacosyl-1,3-benzenediol
5-Tricosylresorcinol
Sutterellaceae
C30:0
C27:0
OH-C18:1
PE(16:0/18:2)
TMCA
HDCA
UDCA
7-Sulfocholic acid
PE(P-16:0/12:0)
PE(P-16:0/14:0)
omega MCA
alpha MCA
LCA
PG(P-18:1/15:0)
PG(P-16:0/15:0)
Valeric acid
Butyric acid
PG(38:1)
PG(P-16:0/18:0/18:1)
DCA
PG(P-18:1/14:0/16:0)
PA(O-20:0/18:1)
Acetic acid
OH-C18:1
PG(P-16:0/18:2)
PG(P-15:0/16:0/18:1)
Propionic acid
PG(P-36:1)
PG(P-30:0)
Lachnospiraceae
Ruminococcaceae
Desulfovibrionaceae

Many of those metabolites, especially lipids such as PGs, PEs or fatty acids can be ascribed to the presence of bacterial cell layers. However, some of the regulated metabolites discussed above cannot be fully accredited to bacterial components or metabolism, though may be induced by inflammatory host responses or direct effects of antibiotics on host cells. Therefore, those effects have to be carefully controlled and will be discussed below. Some metabolites can be clearly assigned to bacterial activity. Secondary bile acids (deoxycholic acid (DCA), lithocholic acid (LCA) and Hyo-DCA), are exclusively generated by bacterial metabolism via 7alpha- dehydroxylation of primary bile acids (cholic acid (CA), chenodeoxycholic acid (CDCA) and muricholic acid (MCA)). The chemical assortment of the bile acid profile is even more varietized by the intestinal microbiota and thereby shifted by antibiotic treatment. Analysis of relative bile acid quantities revealed impaired generation of secondary bile acids in antibiotic treatment groups (DCA, HDCA, LCA, ω-MCA, UDCA; **Figure 13A**).

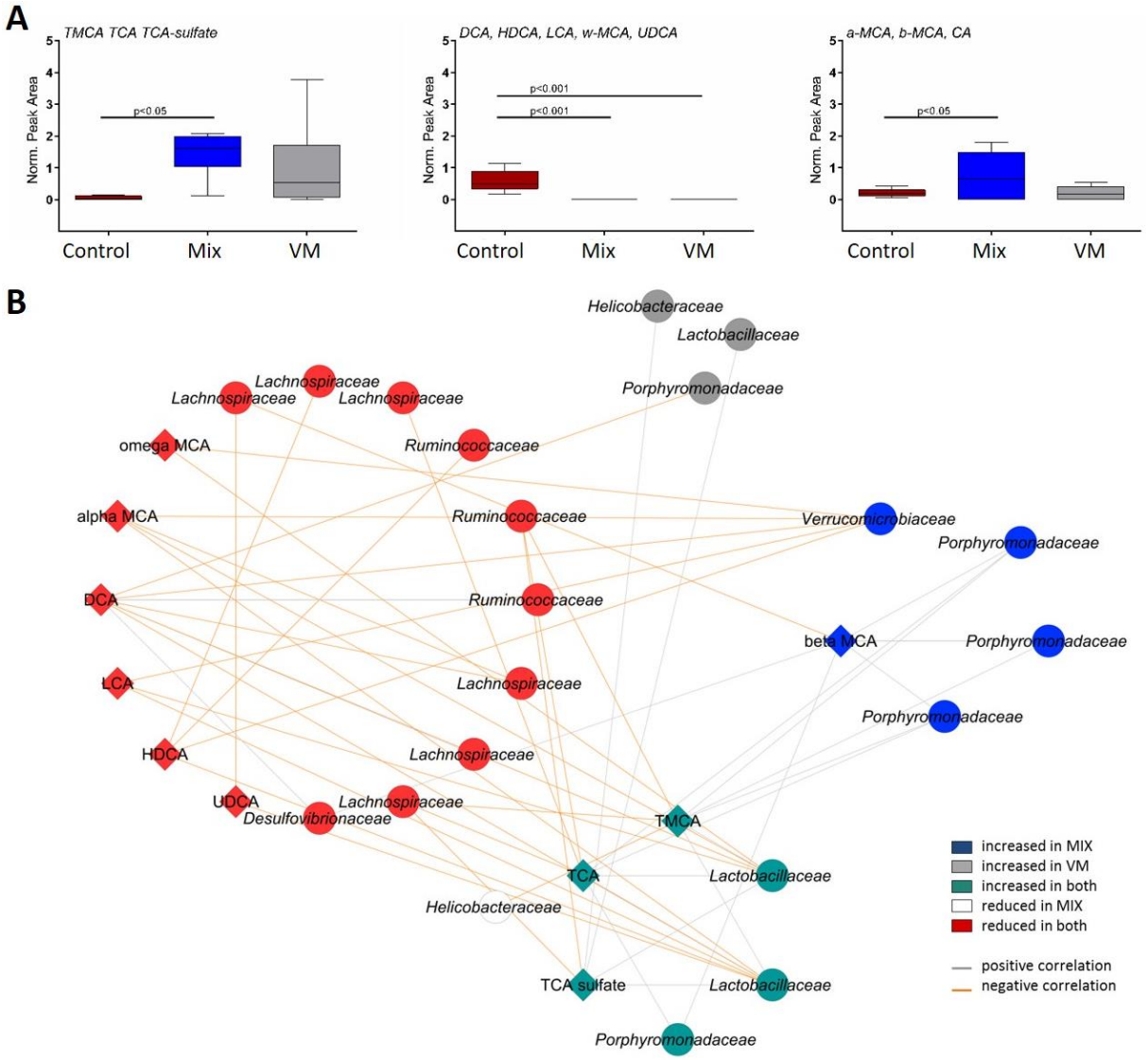


Figure 13: Antibiotic induced variation in bile-acid profile.
(A) Bile acids in TNF^{deltaARE} mice. TMCA=Tauromuricholic acid; TCA=Taurocholic acid/Sulfate; MCA=Muricholic acid (alpha, beta, omega); DCA=Deoxycholic acid; HDCA=HyoDCA; LCA=Lithocholic acid; UDCA=UrsoDCA. **(B)** The network analysis shows bile acids which were significantly regulated within the three treatment groups of TNF^{deltaARE} mice.

Simultaneously, primary bile and tauro-conjugated primary BAs accumulated in the caecal lumen of antibiotic treated mice. In contrast to VM, only MIX treated TNF^{deltaARE} mice displayed increased taurine conjugated primary BAs (TMCA, TCA and TCA-sulfate). **The increase in tauro-conjugated primary BAs shows loss of ability for deconjugation or dehydroxylation of primary bile acids, despite the presence of a diverse bacterial community.** Bile acids are direct targets of bacterial conversion. Therefore, correlations of single metabolites to members of the microbiota could be calculated and are displayed in the network in **Figure 13B**.

Another important group of bacteria-derived metabolites are SCFAs. The abundance of SCFA (shown as peak area in **Figure 14A**) is radically reduced by VM and MIX. Acetic, propionic and butyric acid were significantly decreased after AT, while lactic acid was increased. Again, correlations of single metabolites to members of the microbiota could be calculated and are displayed in the network in **Figure 14B**.

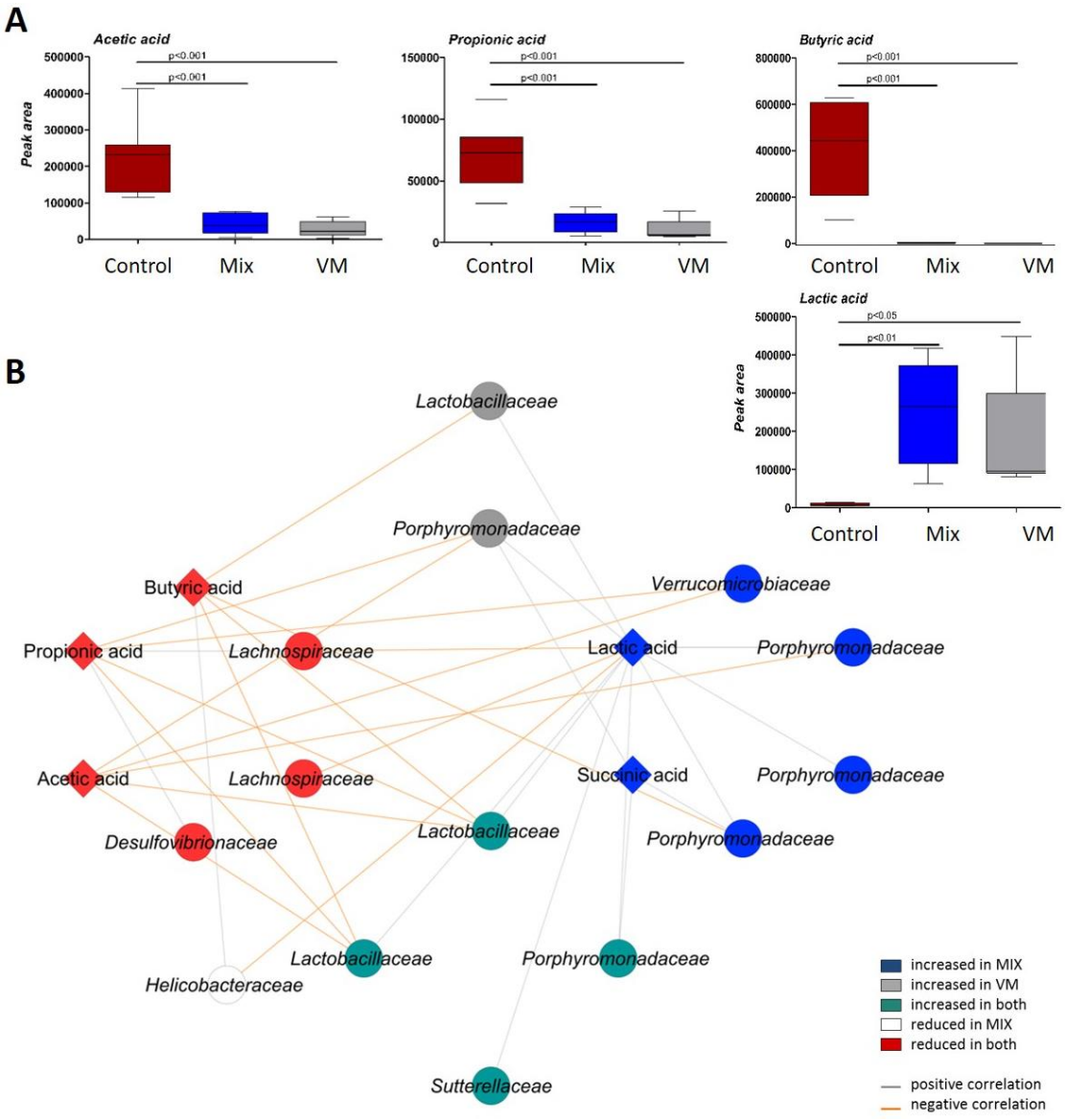


Figure 14: Antibiotic induced variation in SCFA profiles.
(A) Acetic acid, propionic acid, butyric acid and lactic acid in the caecal lumen were quantified in TNF^{deltaARE} and WT mice. **(B)** The network analysis shows SCFAs which were significantly regulated within the three treatment groups of TNF^{deltaARE} mice.

4.2.3 Recurrence of inflammation is subsequent to microbial alteration

The previously shown experiments demonstrate that antibiotics shift the intestinal bacterial and metabolite profile. These shifts were associated with attenuated inflammation. To consolidate the hypothesis of ileitis-attenuation by microbial alterations, the recurrence of inflammation after treatment cessation was monitored.

Therefore, TNF^{deltaARE} mice were treated with VM from the age of eight weeks on. After the four-week treatment-period, inflammation recurrence and microbiota-resilience was monitored over six weeks (**Figure 15A**). Once more, VM reduced ileitis severity during the intervention phase. After ending antibiotic treatment, complete relapse of ileitis was achieved six weeks later (**Figure 15B**). *Tnf*-expression levels in the terminal ileum reflected the dynamic changes in ileal disease activity (**Figure 15C**). Of note, this time the mice in CONV housing developed a colitis phenotype, which remained unaltered by VM **(Figure 15D**).

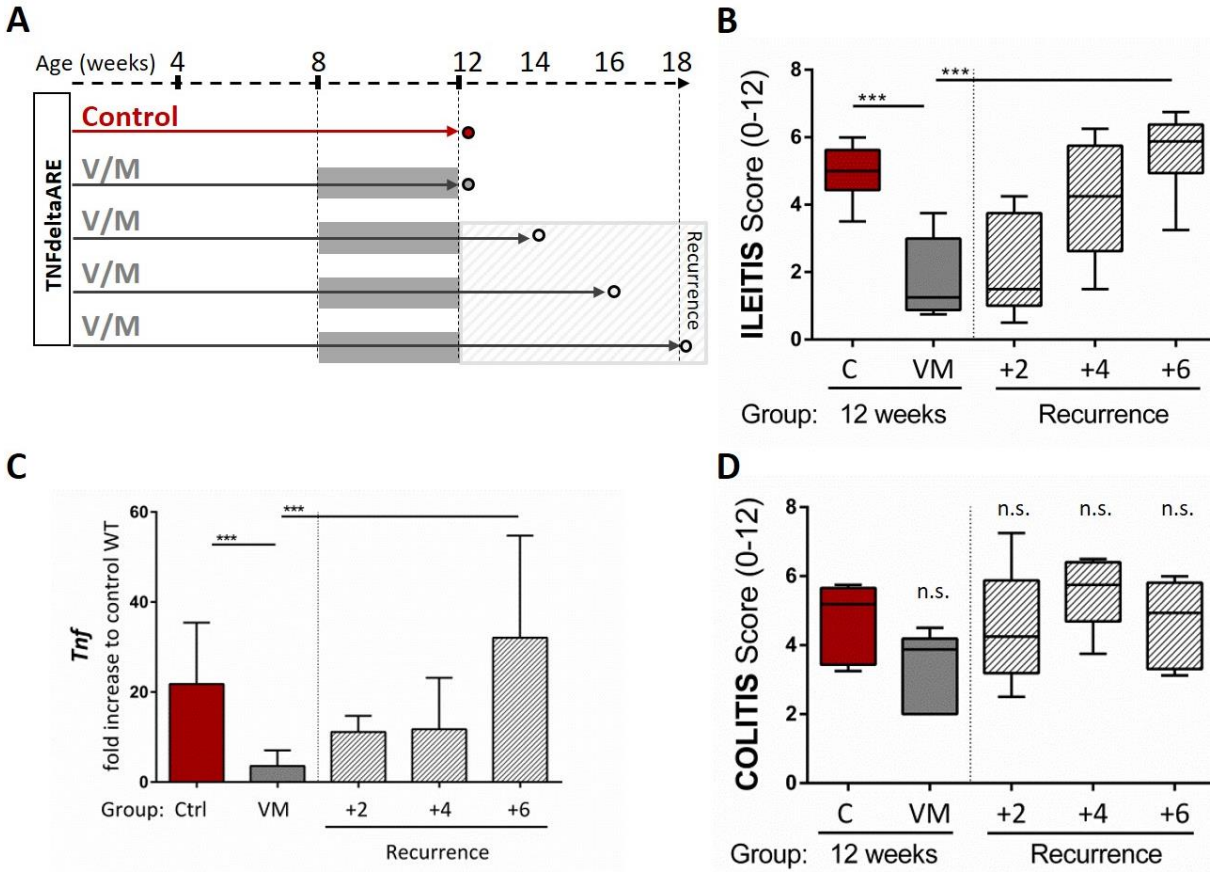


Figure 15: Recurrence of inflammation in antibiotic treated TNF^{deltaARE} mice
(A) Treatment scheme. TNF^{deltaARE} and WT mice were treated for 4 weeks with vancomycin and metronidazole (VM), starting at 8 weeks of age. Recurrence of ileitis after VM therapy was followed for 6 weeks (n = 5-6/group). **(B)** Ileitis scores and **(C)** Colitis scores in TNF^{deltaARE} mice. **(D)** Ileal expression of *Tnf* as fold-increase to CONV-WT mice (normalized to *Gapdh*). One-way ANOVA. Holm sidak. ***p<0.001; n.s. = not significant.

Again, numbers of colony-forming units grown under anaerobic conditions and total cell counts showed no change upon VM treatment, suggesting growth of VM-resistant bacterial species during intervention (**Figure 16A and B**). However, bacterial diversity was drastically reduced in VM-treated mice (**Figure 16C**).

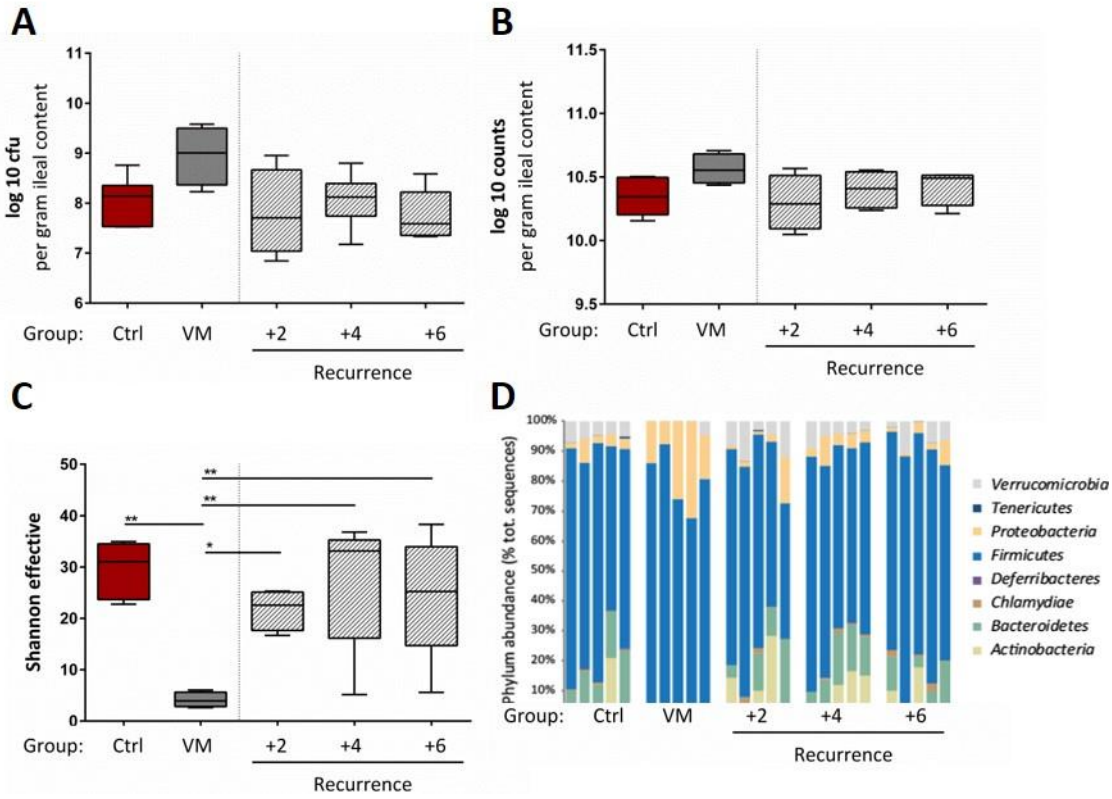


Figure 16: Targeted shifting of the intestinal microbiota attenuates ileitis, but not colitis
(A) Ileal bacterial density of cultivable anaerobes (as log 10 of colony forming units (cfu) per gram ileal content) and **(B)** log 10 counts. **(C)** Intestinal bacterial diversity (Shannon effective counts). **(D)** Changes in caecal bacterial composition (relative abundance of total sequences) at the phylum level. *p<0.05; **p<0.01; Two-way-ANOVA followed by Holm-Sidak.

Analysis of phyla distribution in TNF^{deltaARE} mice showed predominance of *Firmicutes* and *Bacteroidetes* in controls, whereas VM treatment led to a reduction of *Bacteroidetes* and a bloom in *Firmicutes* (*Lactobacillus* spp.) and *Proteobacteria* (*Escherichia coli*) (**Figure 16D**).

Interestingly, **bacterial community composition showed rapid resilience in overall composition, *i.e.* phyla distribution returned to pre-VM values two weeks after cessation of treatment, preceding the return of inflammation.**

Analysis at lower taxonomic level assigned OTU-dynamics into three categories: i) reduction upon VM treatment and subsequent recovery (**Figure 17A**); ii) increase upon VM treatment with reduction in the recurrence phase (**Figure 17B)** and iii) bloom of previous low-abundance OTUs which occupy vacant niches in the disturbed microbiota during the recurrence phase (**Figure 17C)**. Seven OTUs were especially sensitive to VM-treatment (*Blautia, Ruminococcus, Hungatella, Lactobacillus, unknown Bacteroidales and Bifidobacterium*). They sum up to 25% of the microbiota before VM treatment and are absent after four weeks of VM treatment. Interestingly, one OTU (OTU_24) was identified as

Lactobacillus, and Lactobacilli are regarded as resistant to VM. However, already two weeks after VM cessation, five of these OTUs recover to almost pre-treatment abundances. Of note, two OTUs identified as *Blautia* and *Ruminococcus* were not yet detectable two weeks after cessation, indicating some successive growth-behaviour. During VM treatment about 60% of the detected sequences are associated to two dominant OTUs of *Lactobacillus*. Further 30% of sequences were annotated to *Eryspelotrichaceae, Parasutterella, Cosenzaea,* and *Escherichia*. 16S-sequencing is not capable of distinguishing between *Escherichia* and *Shigella*, however, repeated isolation studies hint toward presence of *Escherichia*, as isolates were identified as *Escherichia* by biochemical methods. VM treatment leads to a distinct disturbance in the intestinal bacterial community and elimination of some VM sensitive taxa. Therefore, certain OTUs identified as *Chlamydia, Hungatella, Romboutsia, Lactonifactor, Ruminococcus, Butyrivibrio* and *Eryspelotrichaceae* were capable of occupying vacant niches. Remarkably, though the sum of those OTUs was below 3% of all sequences before treatment, they sum up to almost 30% of the microbiota in the late recurrence phase.

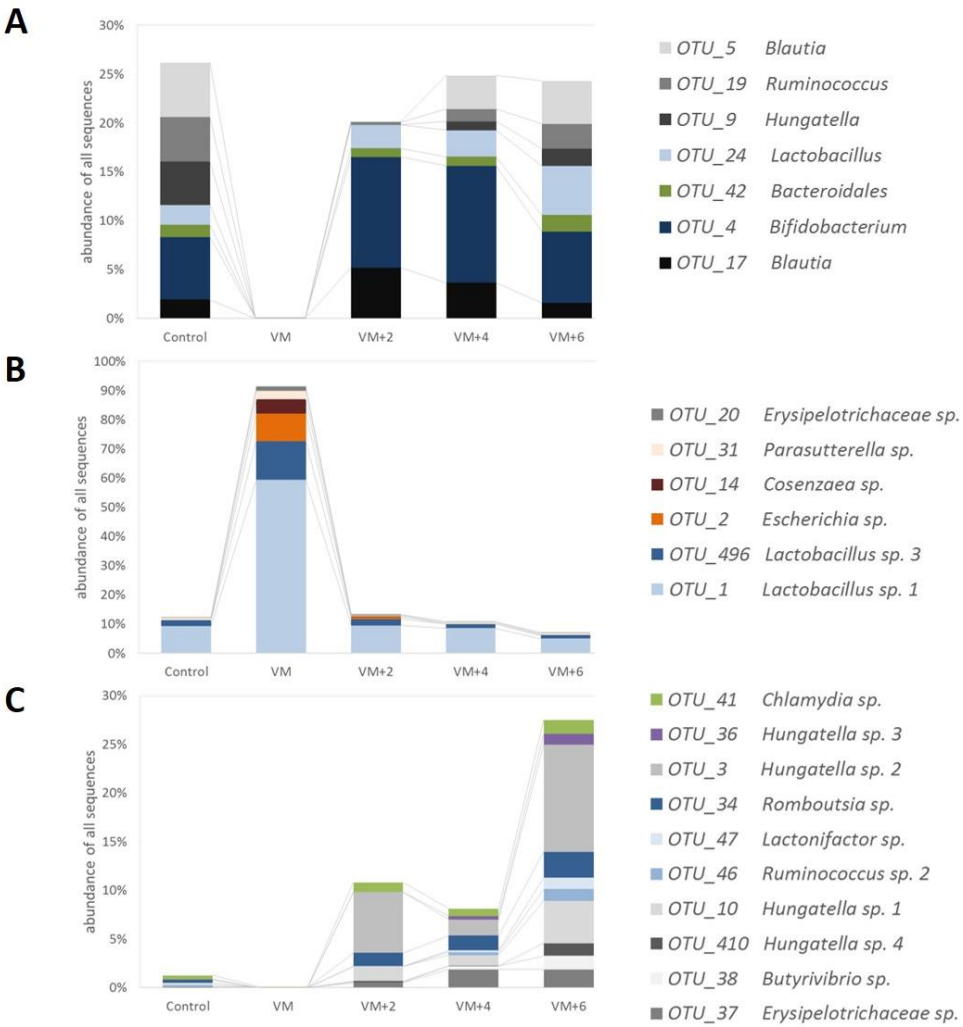


Figure 17: Dynamic regulation and bloom of bacterial taxa upon antibiotic treatment
Significantly regulated OTUs and their assignment, shown as percent of abundance of all sequences. **(A)** VM sensitive OTUs, which are reduced during VM treatment and increase after VM cessation. **(B)** VM resistant OTUs, which are overgrowing during VM therapy. **(C)** Bloom of OTUs with low abundance before and during VM treatment.

4.2.4 Metabolite profiles are not affected in antibiotic treated germ-free mice

The previously shown data clearly demonstrate alteration of the intestinal microbiota upon antibiotic perturbation. As discussed above, this microbial divergence is associated to changes in the metabolite profile. Antibiotics were given over a longer period of time, partly absorbed by the host and subsequently metabolized by the liver. Consequently effects on host cells may be expected. Therefore, the dependence of changes in the metabolite profile could also be assigned to antibiotic-induced effects on eukaryotic host cells. To exclude host-derived effects, VM was administered to GF mice to test for effects that were not mediated by changes in the bacterial community. The same treatment regime as mentioned above (VM via drinking water from the age of 8 weeks on for 28 days) was administered to TNF^{deltaARE} and WT mice in the absence of an intestinal microbiota (**Figure 18A**).

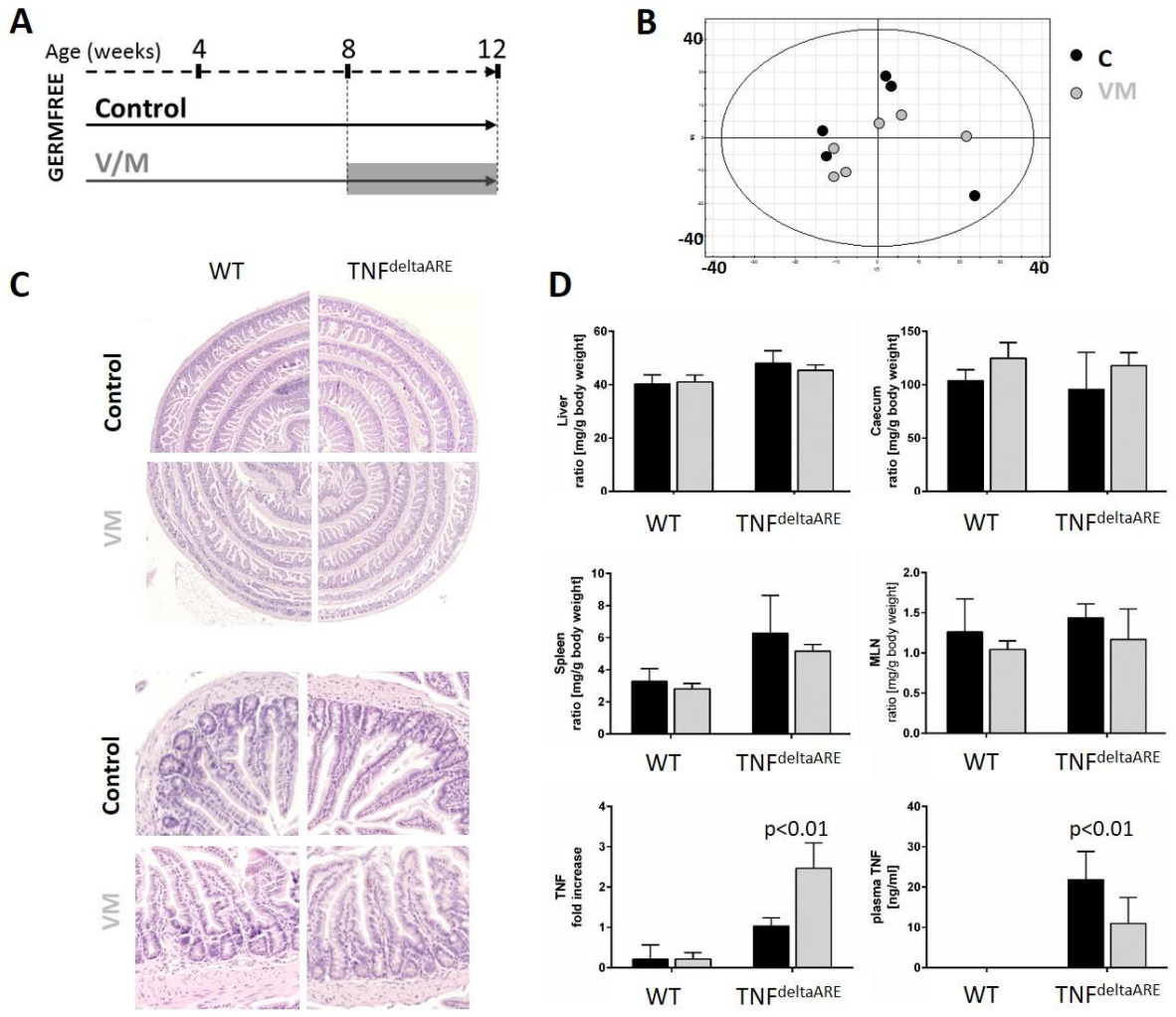


Figure 18: Discrepant inflammatory response in GF TNF^{deltaARE} mice upon antibiotic intervention
(A) Treatment scheme. **(B)** PLS analysis of TNF^{deltaARE} mice housed in GF conditions. TNF^{deltaARE} mice received VM (grey) or water (black) from living week 8-12. **(C)** Representative H&E-stained ileal sections. **(D)** Organ to body weight ratios for liver, caecum, mesenteric lymph nodes (MLN) and spleen. *TNF* expression in total ileal tissue (as fold increase to SPF WT mice) and TNF levels as ng/ml in EDTA plasma. In WT mice TNF levels were below detection level. Data are given as mean ± STD; Two-way ANOVA, Holm-sidak.

PLS analysis of the caecal luminal metabolite profile depicted no antibiotic induced shifts, clearly emphasizing the causality of microbial divergence **(Figure 18B).** At the age of 12 weeks, ileitis scores

showed no differences between controls and VM treated mice (**Figure 18 C**). When comparing organ-to-body-weight ratios (as surrogate markers for inflammation), no changes were detected **(Figure 18E).** Interestingly, in VM-treated GF TNF^{deltaARE} mice the TNF level in plasma was significantly lower, compared to controls. Surprisingly, in contrast to the TNF level in plasma, *Tnf*-expression in total ileal tissue segments was higher in VM-treated GF TNF^{deltaARE} mice **(Figure 18F).** Therefore, also direct mechanisms of antibiotics on host cells can be expected, though were beyond the scope of this work.

4.2.5 Microbiota transplantation has no effect on established inflammation

The shifts in the microbial ecosystem were clearly associated to disease amelioration in TNF^{deltaARE} mice. Therefore, in a translational approach, the transferability of this protection should be tested. A new clinical methodology in the therapy of *Clostridium difficile* induced diarrhoea is the re-establishment of a normal, health-associated microbiota by faecal microbiota transplantation (FMT). To test, whether the protective effect of an antibiotic-shaped microbiota is transferrable in the context of CD-like ileitis, microbiota from VM treated TNF^{deltaARE} mice was transplanted to inflamed recipients. Also the caecal microbiota from untreated TNF^{deltaARE} and WT mice was transferred into recipient mice. Donor-animals were housed in a CONV facility and received either water or VM from the age of 8 to 12 weeks (**Figure 19A**). The caecal luminal microbiota was collected, aliquoted and transplanted via gavage to recipient mice at day 1, 3 and 7 after ending the 8th living week (hexagons). To test whether the colonization efficacy is higher in a disturbed microbial ecosystem, one group of recipient mice was pre-treated with VM.

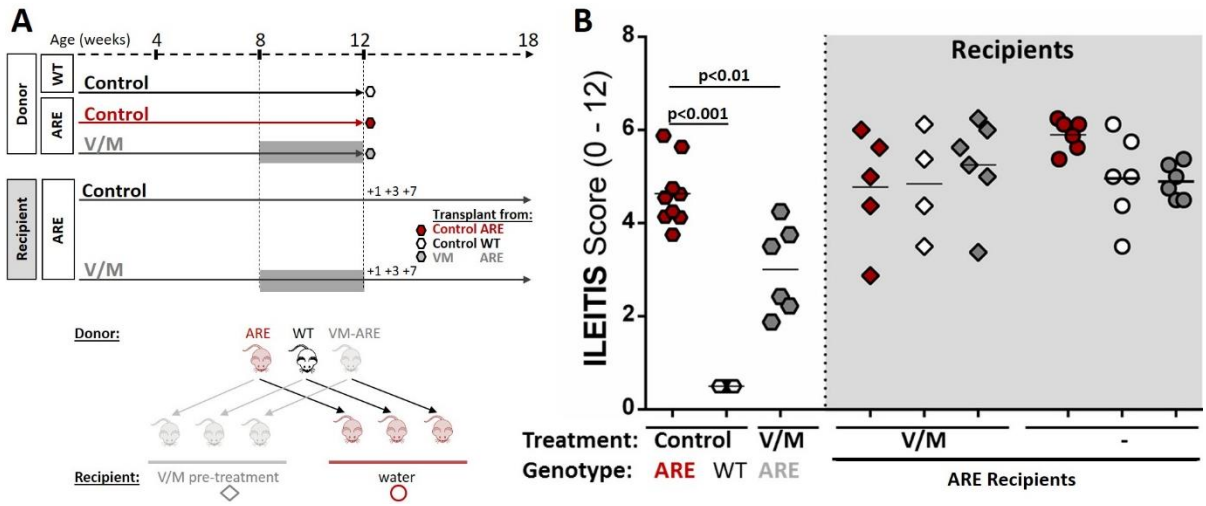


Figure 19: Microbiota transplantation is not reducing established or recurrent inflammation
(A) Treatment scheme: TNF^{deltaARE} (ARE) and WT donor mice (hexagon) received from living week 8 to 12 water (control) or VM. Recipients: One group of recipient mice was pre-treated with VM from week 8 to 12 (squares). The other group served as control (circles). After ending the 8th living week, mice were gavaged three times (one, three and seven days after ending living week 8) with donor microbiota from one donor. **(B)** Ileitis scores of donor mice (left) and TNF^{deltaARE} recipients (within grey square). Two-way ANOVA; Holm sidak. Within recipient groups no significant differences were detected.

At the age of 18 weeks ileitis was scored. The gavage of donor-microbiota (from untreated TNF^{deltaARE} (red), WT (white) or VM pre-treated TNF^{deltaARE} (grey) mice), did not prevent recurrence of ileitis in VM pre-treated recipients (squares; **Figure 19B**). In recipients without VM pre-treatment (circles), no reduction in ileitis was observed. To control for the gavage effect, PBS was transferred and showed no effect on inflammation (data not shown). Of note, also WT animals perceived the same treatment setup and were cohoused with TNF^{deltaARE} mice. Furthermore, no changes in inflammation were detected in WT-recipients (data not shown). The gavage of a microbiota that was associated to attenuated inflammation, did not induce protection from ileal pathology. This may be explained by a lack of donor microbiota-engraftment in an already populated ecosystem, as antibiotics did not reduce bacterial numbers. Further studies would be necessary to show the potential of colonization of the donor microbiota in the receiver´s intestine.

The comparison of CONV *vs* GF housed TNF^{deltaARE} mice depicted the dependency of gut inflammation on the presence of microbial triggers. Antibiotic treatment temporarily attenuated ileitis in CONV housed TNF^{deltaARE} mice without reducing bacterial numbers. This clearly emphasized the role of bacterial composition in the intestinal ecosystem. Colitis was unaffected by antibiotic interventions. However, colitis was observed only infrequently, therefore no consequent analysis of colitis could be performed. Based on sequencing, culture, and FELASA-recommended analyses, presence of *murine Norovirus*, *Helicobacter*, *Pasteurella*, *Syphacia*, *Trichomonas*, *Candida*, *Kazachstania*, and *Chlamydiae* spp. were revealed (**Table 2**). Therefore, the presence of murine pathogens may overwrite protective effects from microbiota transplant experiments. Hence, to study the role of microbial communities in a strictly controlled environment, CONV TNF^{deltaARE} mice were relocated by embryo-transfer to a SPF environment.

4.3 Ileitis severity is linked to dysbiosis

4.3.1 Housing conditions influence the spatial distribution of inflammation development

Interestingly and in contrast to age-matched CONV mice (**Figure 7**), SPF TNF^{deltaARE} mice developed CD-like ileitis but not colitis (**Figure 20A-B**). Furthermore, **SPF TNF^{deltaARE} mice displayed a gradient of ileitis severity**, including 3 main categories of TNF^{deltaARE} mice: i) 4 mice out of 20 showed no ileitis, hereon referred to as "non-responders" (NR); ii) 9 mice had an ileitis score >4 and were defined as "responders" (R); and iii) 7 mice with a score between 1 and 4 were defined as "low responders" (lowR). Of note, all non-inflamed mice were males and no cage effect was observed. Inflammation-related markers, such as cytokine expression and granulocyte infiltration were measured in the three ileitis phenotypes and compared to disease free GF TNF^{deltaARE} mice. Similar to CONV TNF^{deltaARE} mice, *Tnf* expression levels and granulocyte infiltration into the ileal mucosa reflected the gradual increase in inflammation activity in SPF TNF^{deltaARE} mice (**Figure 20C-D**). In comparison, *Tnf* transcript levels were reduced and Ly6G-positive granulocytes were totally absent in age-matched GF TNF^{deltaARE} mice. As shown above, alterations in the microbial profile were associated with ileitis developent. Therefore, further characterization of the intestinal ecosystem should give additional insights into differences between responder and non-responder mice.

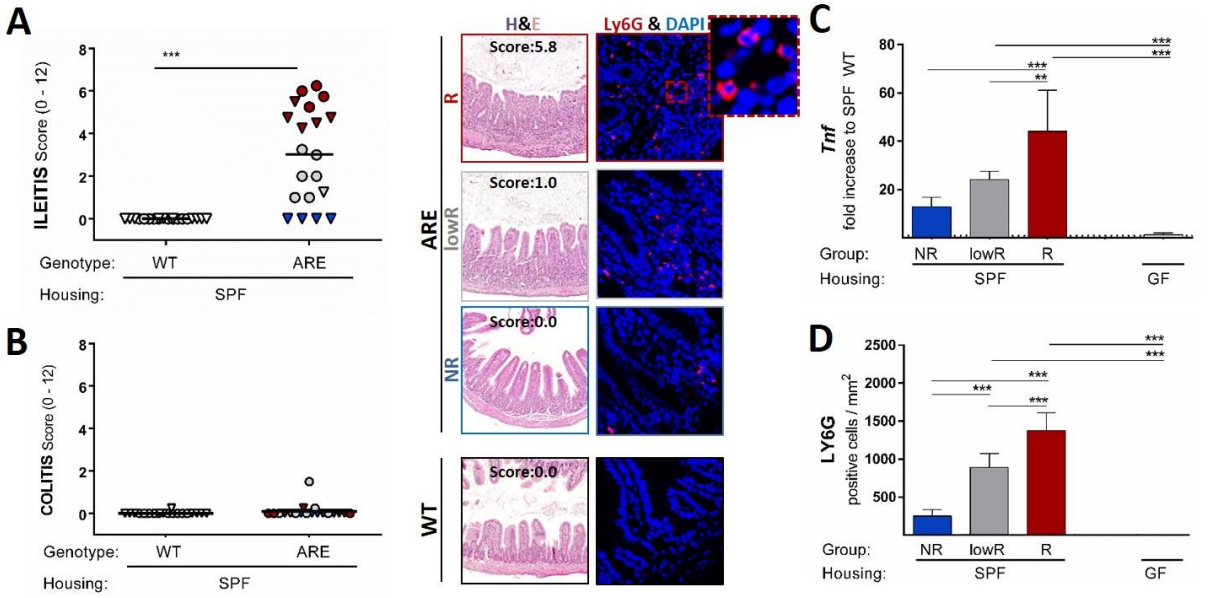


Figure 20: Ileitis in the SPF environment shows different grades of severity
(A) Ileitis and colitis scores in 18-week-old TNF^{deltaARE} (ARE) and WT littermates in the SPF facility. The three ileitis score groups are color-coded as "non-responders" in blue (score = 0), "low-responders" in grey (score <4) and "responders" in red (score >4). Male mice are displayed as triangles, females as circles. **(B)** Left panel: Representative H&E-stained sections of the distal ileum. Right panel: Representative terminal ileum sections stained against Ly-6G (red). **(C)** Ileal expression (normalized to *Gapdh*) of *Tnf* as fold-increase to SPF WT-mice (dotted line) in non-responders (NR), low-responder (lowR) and responders (R). **(D)** Quantification of Ly6G-positive granulocytes per mm^2 in tissue sections of the terminal ileum. *p<0.05; **p<0.01; ***p<0.001; Two-way-ANOVA followed by Holm-Sidak.

4.3.2 Ileitis severity is associated with changes in beta-diversity

To investigate the relationship between ileitis severity and the gut microbiota in SPF mice, 16S rRNA gene sequencing was performed. No change in *alpha*-diversity between the three ileitis phenotypes were detected (**Figure 21A).** However, *beta*-diversity analysis revealed clear separation of responder TNF^{deltaARE} mice and their WT littermates (**Figure 21B**). Most interestingly, **bacterial community structures of non-responder TNF^{deltaARE} mice clustered together with WTs but were separated from responder TNF^{deltaARE}-mice.** This clearly indicates a link between inflammation and bacterial communities. Relative abundance of taxonomic groups revealed that changes in inflammation severity were associated with alterations in composition (**Figure 21C**). Ileitis scores negatively correlated with relative abundances of *Porphyromonadacaeae* (r=-0.79; p<0.001). In contrast, unknown members of the order *Clostridiales* showed significantly higher proportions in responder mice and positively correlated with the degree of histopathology (r=0.48; p=0.016).

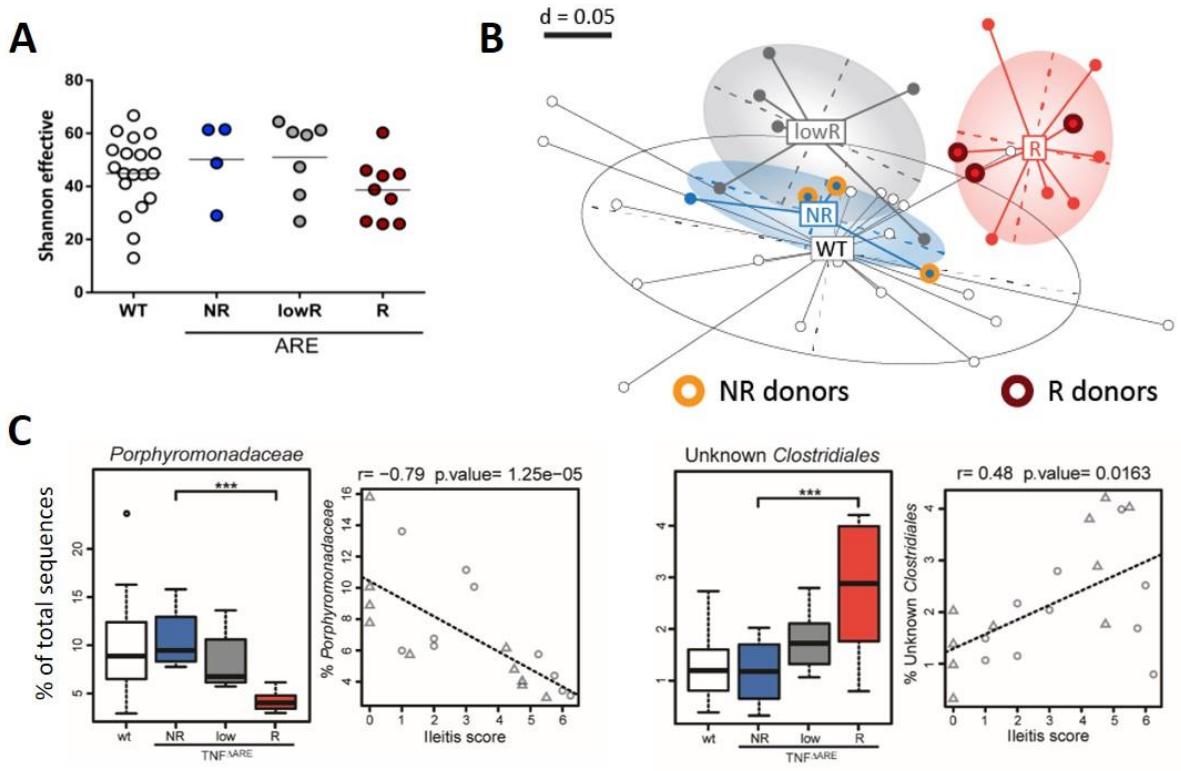


Figure 21: Bacterial profiles mirror ileitis severity
(A) Shannon effective species counts in SPF-WT and -TNF^{deltaARE} mice. **(B)** NMDS analysis showing separation of caecal bacterial communities according to genotype and ileitis severity. Responders (R; red) were clearly separated from TNF^{deltaARE} with low ileitis scores (lowR; grey) and from non-responders (NR; blue). Microbiota donor-mice used in colonization experiments are shown with halos. **(C)** Correlation of bacterial taxa with ileitis scores.

To exclude litter-, sex- or cage-dependent effects, the faecal microbiota of 4 week old TNF^{deltaARE} mice was analysed **(Figure 22)**. This additional sequence analysis of faecal samples directly after weaning demonstrated that **the transfer of microbiota from mother to offspring is not indicative for disease susceptibility later in life and inflammation-related changes in microbiota are independent of littermate-effects**. TNF^{deltaARE} mice of the same litter (indicated by same shape of data-points) were found in the three groups of ileitis-severity (indicated by colour of data-points). As visible in the lower left quadrant, the microbiota of mice in three different litters (triangle, circle, and square) was similar at weaning. Though, at the age of 18 weeks the mice displayed varied ileitis severity (R, NR and lowR). This emphasizes that dysbiotic or non-dysbiotic caecal microbiota profiles developed post-weaning.

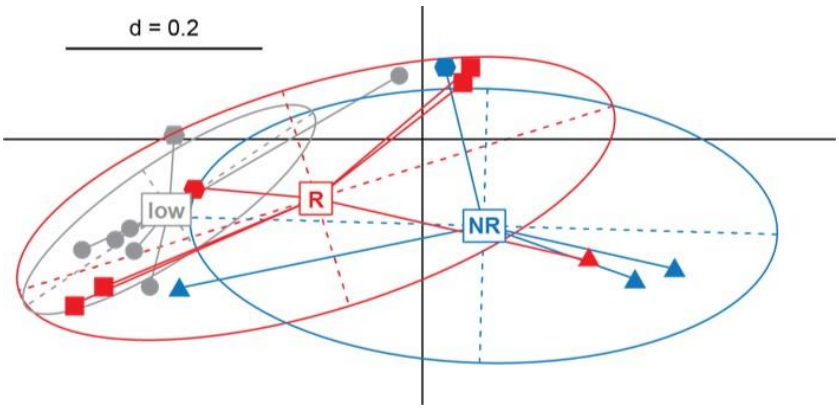


Figure 22: Disease related microbial changes are independent of littermate effects
The MDS analysis displays the phylogenetic distances between faecal bacterial communities of 4 week-old TNF^{deltaARE} mice according to litter (shape of symbols) and ileitis severity (colour of symbols). Responders (R; red) were not yet separated from TNF^{deltaARE} mice with low ileitis scores (lowR; grey) and from non-responders (NR; blue). Littermates are depicted with identical symbols, suggesting no inheritance of a dominant bacterial profile responsible for later ileitis severity, as TNF^{deltaARE} mice from the same litter were found in the three groups of ileitis-severity.

4.3.3 Metaproteome profiling reveals functional dysbiosis

The microbiota of highly inflamed TNF^{deltaARE} mice differed from non-inflamed mice. However, bacterial differences seem small compared to the huge variance in inflammation severity. These small differences in composition could be underlined by functional changes in microbial communities. Therefore, the functional metaproteome in colon samples from highly inflamed responder (R) and non-inflamed non-responder (NR) TNF^{deltaARE} mice was determined by LC-MS/MS metaproteome analysis in collaboration with the Helmholtz-Centre for Environmental Research (Leipzig). PLS-analysis showed a clear separation of responder and non-responder mice according to their protein function (**Figure 23A)**. Also, several protein pathways were significantly upregulated in responder compared to the non-responder microbiota (**Figure 23B).** Proteins were assigned to functions using Cluster of Orthologous Groups (COG). The sub-roles *translation ribosomal structure and biogenesis* (fold change (fc) 103, p=0.028), *carbohydrate transport and metabolism* (fc 59.8, p=0.018) as well as *amino acid transport and metabolis*m (fc 23.4, p=0.027) showed significant increase in the responder group.

Proteins that were significantly different between the two groups of mice within the categories *metabolism* or *cellular processes and signalling* are shown in **Figure 23C**.

Ten proteins were exclusively found in responders, while only two were specific for non-responders, clearly underlying the existence of microbial changes on a compositional as well as functional level in the microbiota of SPF TNF^{deltaARE} mice.

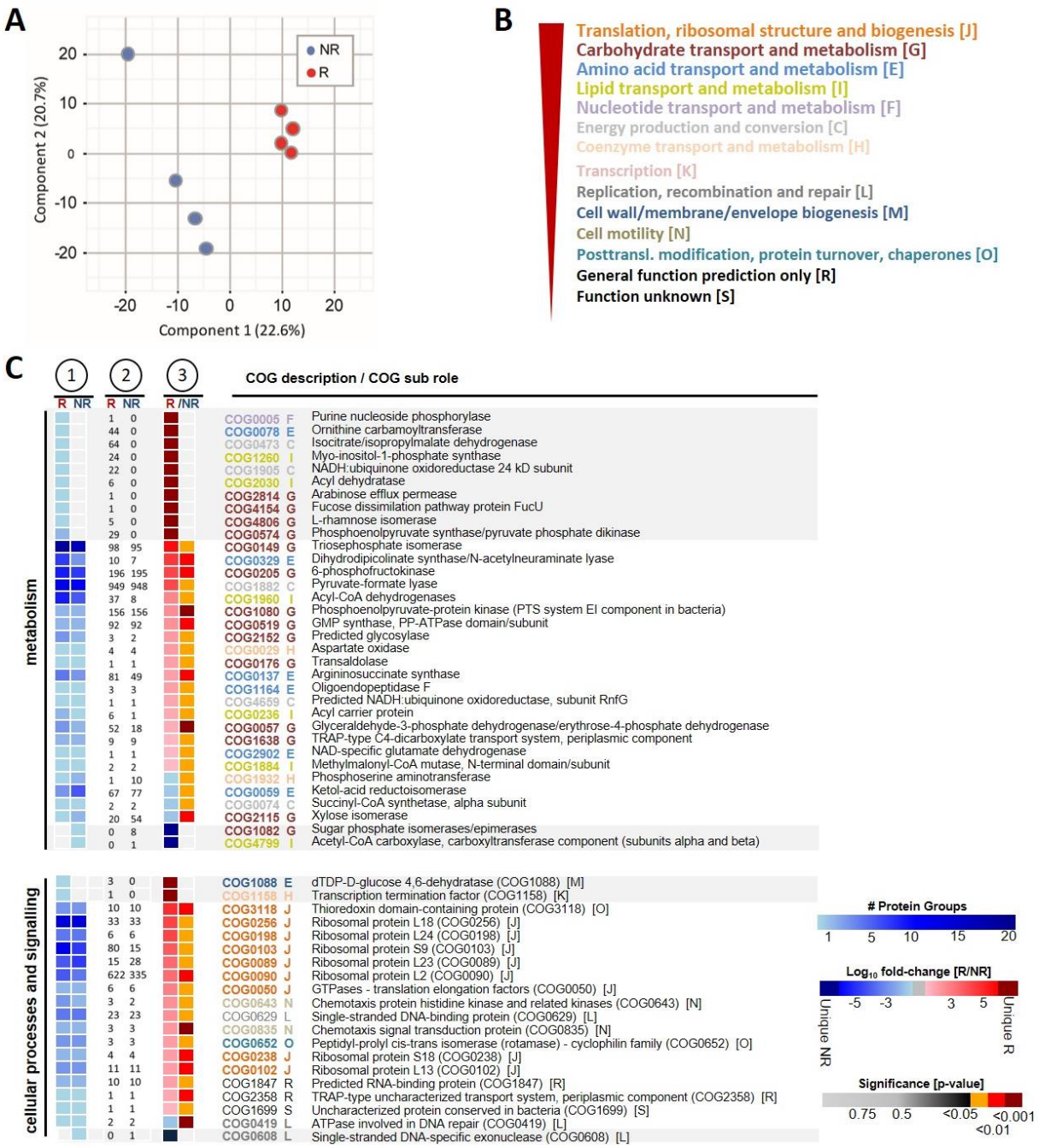


Figure 23: Dysbiosis is characterized by functional changes
(A) Metaproteome analysis of the colonic microbiota. Partial Least Squared (PLS) analysis differentiated responder (red) and non-responder (blue) TNF^{deltaARE} mice. **(B)** Significantly upregulated pathways: The five highest regulated pathways (upregulated in responder). **(C)** Significant changes in abundance of protein functions belonging to the Cluster of orthologous groups (COG) main role. The heatmap represents differences in the abundance of COGs. Protein group data were log10 transformed and normalized by median of bacteria protein data. Protein functions that are unique to either R or NR are shown in the grey squares. COG sub-roles: C, Energy production & conversion; E, amino acid transport & metabolism; F, nucleotide transport & metabolism; G, carbohydrate transport & metabolism; H, coenzyme transport & metabolism; I, lipid transport & metabolism.

4.3.4 Ileitis severity is associated with loss of Paneth cell function

As shown above, microbial composition was clearly associated to ileitis severity in antibiotic-treated mice. However, the causality of this high divergence in the bacterial ecosystem in SPF TNF^{deltaARE} mice was still unclear. Microbial divergence was not yet visible in the fecal microbiota in 4 week old mice. Therefore, separation must be induced by inflammation or host-derived signals prior to ileitis development. Paneth cells are known for modulation of intestinal communities by antimicrobial peptides (AMPs) and are connected to ileitis manifestation in CD patients.[87, 138] Hence, closer characterization of Paneth cell function in TNF^{deltaARE} mice should reveal putative mechanism of microbial divergence induction.

To assess Paneth cell function, lysozyme and alpha-fucosylated proteins (as detected by *Ulex europaeus agglutinin* 1 (UEA-1)) were stained in the ileal mucosa of SPF TNF^{deltaARE} and WT littermates (**Figure 24A**). Immunofluorescence analysis clearly demonstrated a significant loss of lysozyme but not UEA-1 positive cells in the crypt base of inflamed low-responder and responder SPF TNF^{deltaARE} mice compared to GF controls (**Figure 24B**). In contrast, numbers of lysozyme and UEA-1 positive cells in non-responder TNF^{deltaARE} mice were comparable to WTs (dotted line). **These data suggest a loss of Paneth cell activity but not Paneth cell number in the presence of inflammation.** Besides the loss of lysozyme expression, signals for Cryptdin 2-positive cells were also reduced in severe ileitis (**Figure 24 C-D**).

Consistent with a loss of Paneth cell function in responder mice, transcript levels of the antimicrobial peptide *Angiogenin 4* (Ang4) were significantly reduced compared to non-responders (**Figure 24E**). While *Cryptdin 5 (Defensin α5)* showed high inter-individual variations and a trend towards reduction under inflammation ($p>0.05$), Reg3γ expression remained unchanged. GF mice showed lower transcript levels of the tested antimicrobial peptides, which accords to previous publications.[221, 222] An increase in Paneth cell-apoptosis and therefore in the number of Caspase-3-positive cells in the ileal crypts could not be detected (**Figure 24F**).

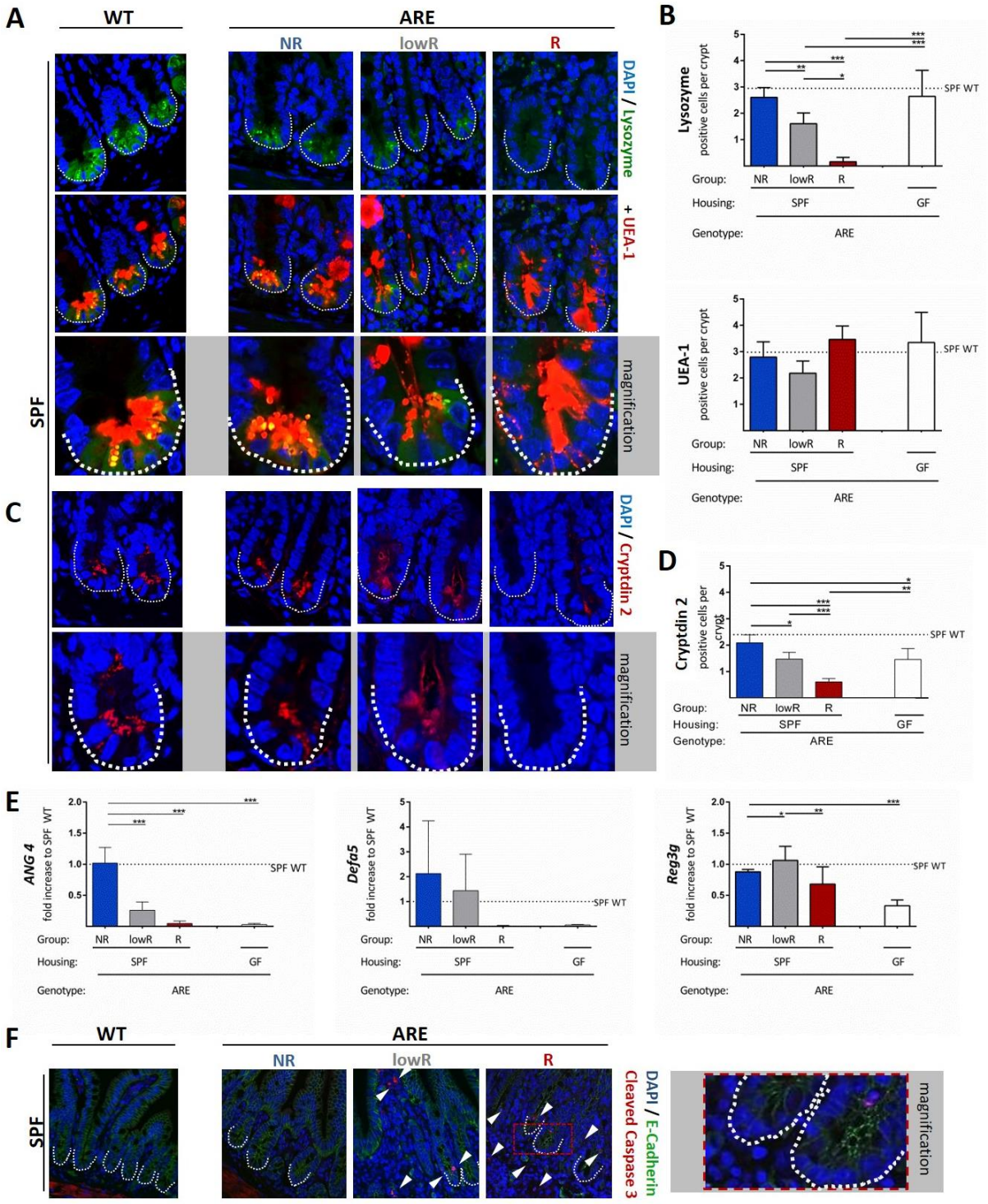


Figure 24: Disease severity is associated with Paneth cell function
(A) Paneth cell staining of lysozyme (green) combined with UEA-1 (red). A representative crypt is shown in the magnification. **(B)** Paneth cell staining of cryptdin 2 (red). **(C)** Quantification of lysozyme, UEA-1 and **(D)** cryptdin 2 positive cells per crypt base in ARE-mice compared to WT-mice (dotted line). **(E)** Ileal expression of a*ngiogenin 4 (ANG4)* and *defensin-α-5* and *Reg 3γ* as fold-increase to SPF-WT mice (dotted line) normalized to *Gapdh* (n=4-6). **(F)** Staining of cleaved caspase 3 positive cells in the ileal crypts of mice in SPF and GF housing. *p<0.05; **p<0.01; ***p<0.001; One-way ANOVA followed by Tukey´s.

4.4 Microbiota transplantation reveals transferability of phenotypes

4.4.1 Loss of Paneth cell function is secondary to development of inflammation

The above shown results clearly hint toward a causal role of dysbiotic microbial ecosystems in CD-like inflammation. Antibiotic-induced shifts in the microbiota were protective. Resilience of the microbiota toward a pre-treatment pattern preceded recurrence of inflammation. Furthermore, loss of Paneth cell function is observed in highly inflamed animals accompanied by a dysbiotic microbiota.

Therefore, microbiota-transplant experiments should show whether i) the inflammation observed in responder SPF TNF^{deltaARE} mice can be transferred to GF TNF^{deltaARE} mice and ii) whether loss in Paneth cell function is the first pathologic mechanism. This should elucidate, whether loss in Paneth cell function induces subsequent development of tissue-pathology and potential microbial divergence.

GF-TNF^{deltaARE} and -WT recipients were colonized at the age of 8 weeks with dysbiotic microbiota from responder SPF TNF^{deltaARE} mice for one, two and four weeks. As shown in **Figure 25A-B**, ileitis was absent after one week (Ileitis score 0.13±0.28), but increased with the duration of colonization in TNF^{deltaARE} mice. **Thereby, the causal role of microbes could be shown. WT littermates showed no signs of inflammation, supporting the fact that genetic susceptibility was required for the development of ileitis after transfer.**

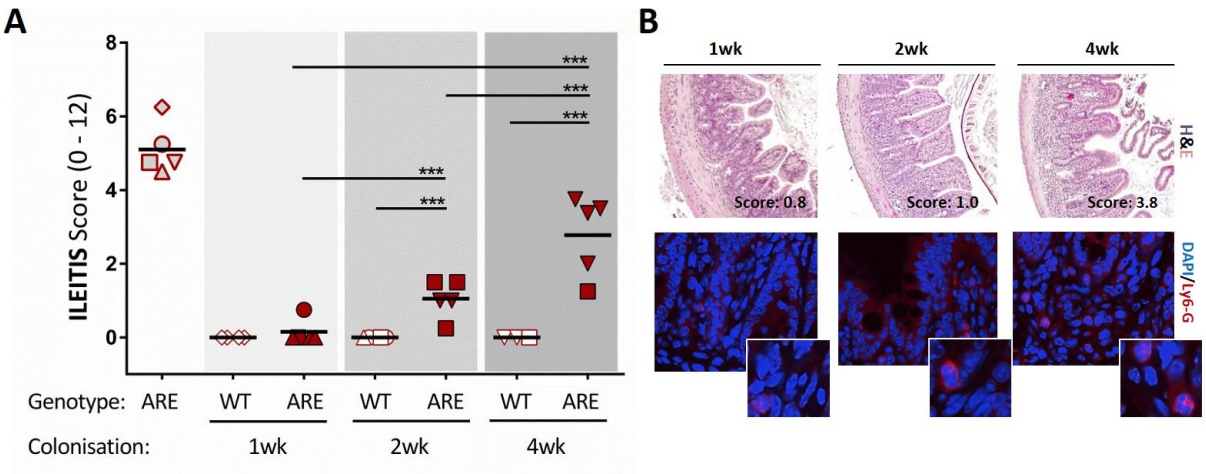


Figure 25: Transfer of dysbiotic microbiota induces tissue ileitis
(A) GF WT and TNF^{deltaARE} (ARE) mice were colonized at the age of 8 weeks with the complex caecal microbiota from inflamed SPF responder mice (open symbols). After 1, 2 and 4 weeks of colonization, ileitis was assessed. **(B)** Upper panel: Representative H&E stained terminal ileum sections of TNF^{deltaARE} representative terminal ileum sections stained against Ly6-G (red). ***p<0.001; Two-way ANOVA followed by Holm-sidak.

Tnf mRNA expression levels were already significantly increased relative to GF controls after 1 week of colonization (**Figure 26A**; p<0.001), whereas granulocyte infiltration and MLN-weight increased later, parallel to disease severity in TNF^{deltaARE} mice (**Figure 26B**). The influence of colonization on caecum-weight was already visible one week post colonization and was significantly reduced compared to GF mice (dotted line; **Figure 26D**).

Although, whether loss of Paneth cell function is prior to dysbiotic divergence and subsequent inflammation development, was not yet shown. Therefore, again Paneth cell function was assessed by staining for lysozyme, UEA-1 and cryptdin 2-positive cells. Numbers of UEA-1-positive crypt cells remained constant over the entire colonization period and were independent of colonization (**Figure 26E-F**).

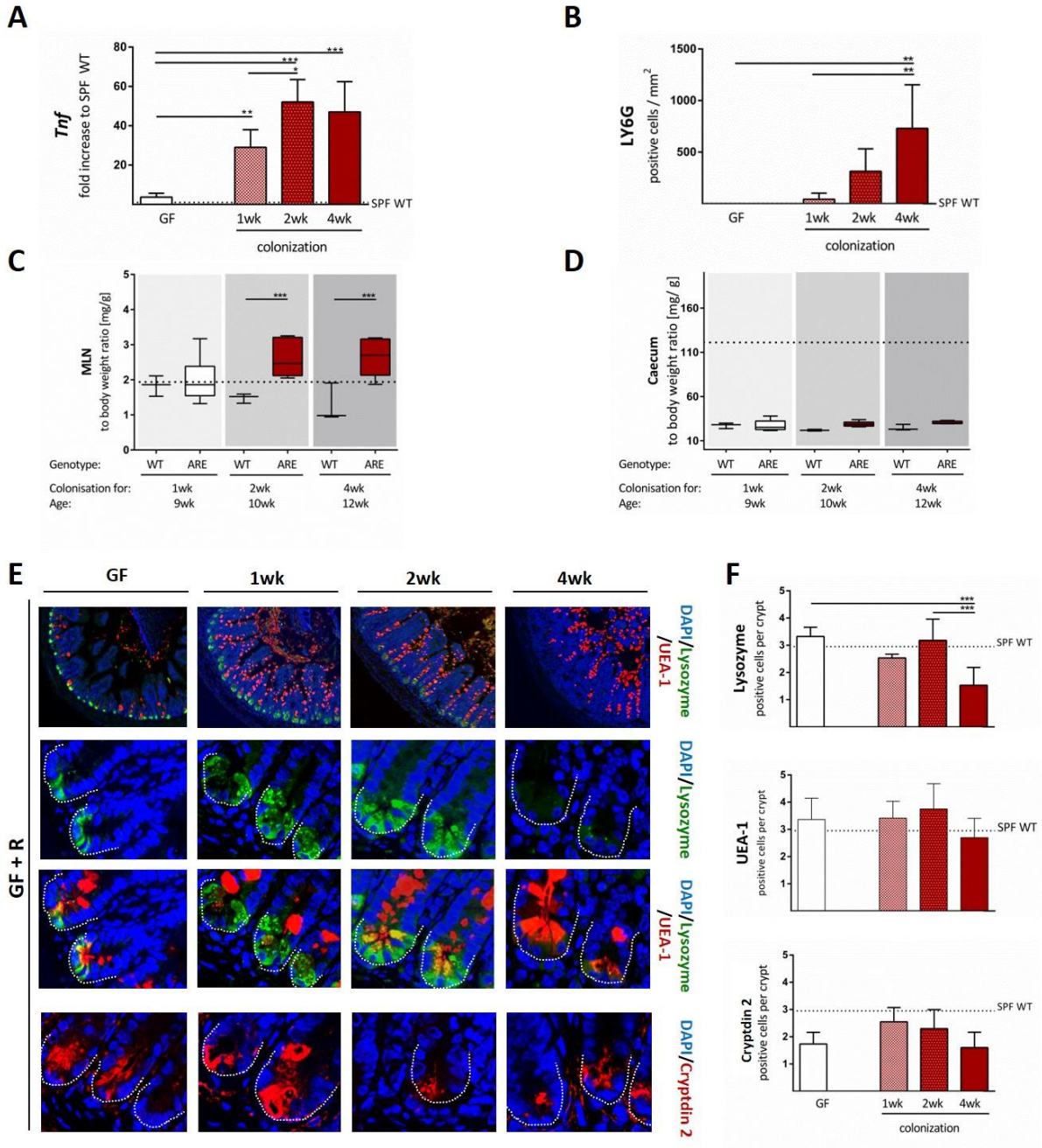


Figure 26: Loss of antimicrobial peptides is secondary to the development of inflammation
(A) Ileal *Tnf*-expression (normalized to *Gapdh*) as fold-increase to SPF WT mice. *Tnf* expression of GF TNF^{deltaARE} (ARE) mice is displayed as dotted line. Age matched GF TNF^{deltaARE} (left) served as controls. **(B)** Quantification of Ly6-G-positive granulocytes per mm^2 in ileal tissue sections after 1, 2 and 4 weeks of colonization with R microbiota. **(C)** MLN to body weight ratios of mice colonized with responder-microbiota for 1, 2 or 4 weeks. Groups that were significantly increased when compared to GF TNF^{deltaARE} mice are displayed as red boxes. The dotted line represents mean of respective data in GF TNF^{deltaARE} mice. **(D)** Caecum to body weight ratios. **(E)** Paneth cell staining of lysozyme, UEA-1, and Cryptdin 2, as labelled in the figure (upper panel, 10x magnification; others, 120x). **(F)** Quantification of lysozyme-, UEA-1- and cryptdin 2-positive cells per crypt base in TNFdeltaARE mice compared to WT mice (dotted line); *p<0.05; **p<0.01; ***p<0.001 (One-way-ANOVA followed by Tukey´s).

Already one week post microbiota transplantation the influence of microbial colonization was visible in mucus-fucosylation. In colonized animals, increased mucus-fucosylation across the crypt-villus axis could be detected. Most importantly, loss of lysozyme-positive crypt cells of recolonized TNF^{deltaARE} mice did not precede onset of tissue pathology (**Figure 26F**), suggesting that loss of Paneth cell function is associated but not causal for development of CD-like ileitis. Quantification of Cryptdin 2-positive cells per crypt (**Figure 26E-F; lower panels**) showed no reduction in Cryptdin 2 staining in mice after four weeks of colonization, despite the presence of moderate histopathology and inflammatory activation (increased *Tnf* expression levels and Ly6G-positive granulocyte infiltration). **This clearly emphasizes the observation of loss of Paneth cell function secondary to inflammatory processes in the TNF^{deltaARE} mouse model.**

4.4.2 Ileitis is dependent on a dysbiotic microbial ecosystem

Paneth cells do not seem to be the initiating factor in development of ileitis in TNF^{deltaARE} mice, as changes in Paneth cell function occur subsequent to first signs of tissue pathology. Transfer of inflammation-associated microbial ecosystems induced ileitis in TNF^{deltaARE} mice already within 4 weeks. The dependence of inflammation in TNF^{deltaARE} mice on microbial triggers was shown by the absence of intestinal pathology in GF-housing, compared to mice in CONV or SPF housing. Changing the microbiota composition without reducing bacterial quantity revealed the dependence of inflammation severity on the composition of the microbiota and its associated metabolites. Consequently, the causal relationship of a dysbiotic microbiota in the initiation of CD like ileitis in GF TNF^{deltaARE} mice should be compared to a non-dysbiotic microbiota.

Therefore, GF TNF^{deltaARE} and -WT mice were colonized with donor-microbiota from either responder or non-responder SPF TNF^{deltaARE} mice. Donor-microbiota is highlighted with halos (**Figure 27A)**. TNF^{deltaARE} mice colonized with dysbiotic microbiota from responders (+R; red) developed inflammation after 4 weeks, mimicking ileitis-severity of corresponding donors (recipient mice and respective donor are shown with same symbols; **Figure 27B**). WT littermates showed again no signs of inflammation, supporting the fact that genetic susceptibility was required for the development of ileitis after transfer. **Most remarkably, GF-TNF^{deltaARE} mice colonized with microbiota from non-responders also showed no signs of ileitis** (+NR; blue). *Tnf* transcript levels of terminal ileal tissue correlated with the induction of tissue pathology (**Figure 27C**). MLN to body weight ratios were significantly elevated in both recipient groups when compared to GF TNF^{deltaARE} mice (**Figure 27D**), suggesting the presence of mucosal immune cell activation independent of tissue pathology. GF mice were colonized with equal densities of bacteria in both recipient groups (approx. 10^9 cfu/g) and tissue maturation was confirmed by reduced caecum weight ratios (**Figure 27E**).

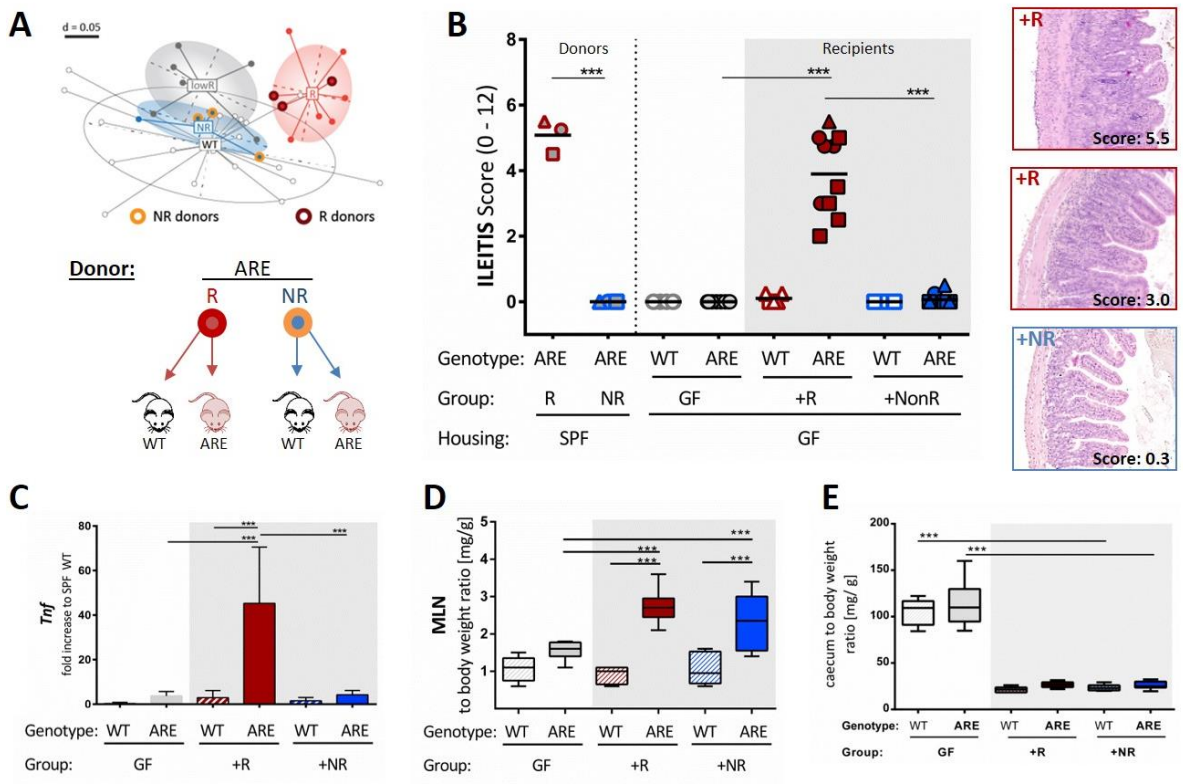


Figure 27: Ileitis is transmissible via dysbiotic gut microbiota
(A) GF-WT and -TNF^{deltaARE} (ARE) mice were colonized at the age of 8 weeks with the complex caecal microbiota from SPF responder (+R) or non-responder (+NR) donor-TNF^{deltaARE} mice (halo in Figure 4; each n = 3). Age and time-matched GF WT and -TNF^{deltaARE}-mice served as controls. **(B)** Ileitis was scored after 4 weeks of colonization. SPF-donor mice and respective recipients are shown with identical symbols. **(C)** Ileal expression (normalized to *Gapdh*) of *Tnf* as fold-increase to SPF WT mice. **(D)** Increase in mesenteric-lymph-node (MLN) weight. **(E)** Caecum to body weight ratios of WT and TNF^{deltaARE} mice in the GF housing and after four weeks of colonization with responder (+R) or non-responder (+NR) microbiota. ***p<0.001; Two-way ANOVA followed by Holm-sidak.

To verify the hypothesis that transmissible ileitis was due to the transfer of dysbiotic bacterial communities, we analysed caecal content in recipient mice using high-throughput sequencing. *Alpha*-diversity analysis showed no differences between genotypes and donor-groups (**Figure 28A**). *Beta*-diversity analysis displayed again a clear separation between inflamed and non-inflamed recipients **(Figure 28B).**

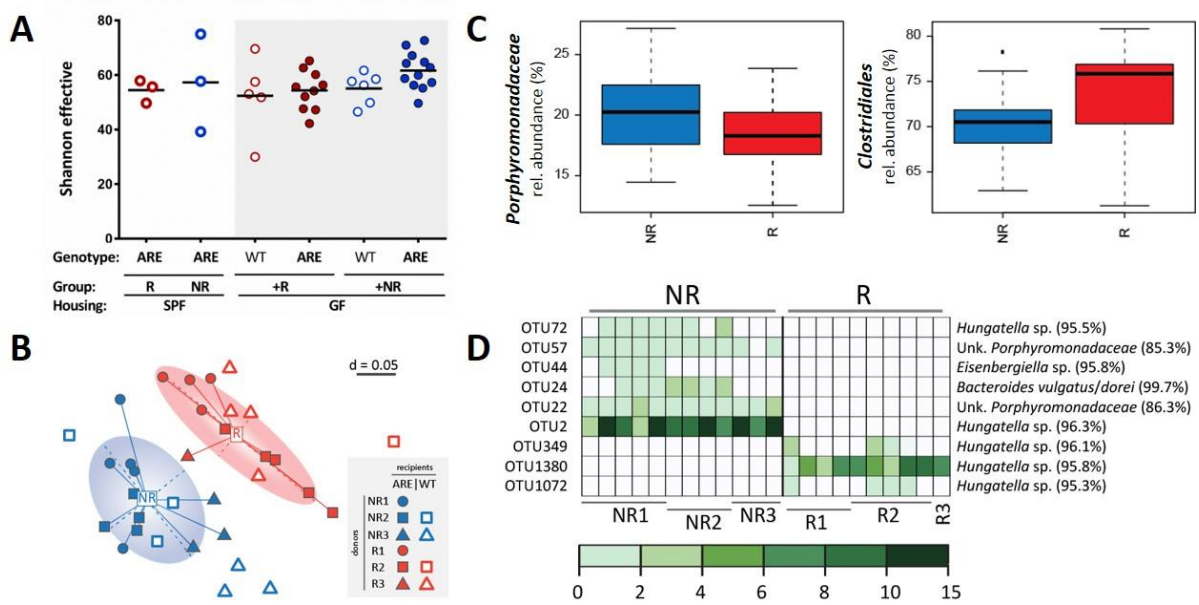


Figure 28: Differences in dysbiotic microbiota are found on very low taxonomic levels
(A) Shannon effective species counts were not different between recipient and donor mice. Two-way ANOVA followed by Holm-sidak. **(B)** NMDS plot showing clear separation of R (red) and NR (blue) recipients according to respective donor (R1-3 or NR1-3; donor and recipient are displayed using identical symbols). TNF^{deltaARE} and WTs are shown as filled and open symbols, respectively. **(C)** Differences of bacterial taxa in responder (R) and non-responder (NR) recipients. **(D)** OTUs significantly associated with R or NR colonization. Each column represents one recipient. Donors and percentages of total sequences (coloured scale) are indicated below.

Differences within the recipient groups could still be assigned to the respective donors including WT and TNF^{deltaARE} mice. The increase in *Clostridiales* spp. and decrease in *Porphyromonadacaeae* observed in inflamed donor SPF TNF^{deltaARE} mice also occurred in recipients, but results did not reach significance **(Figure 28C).** Differential abundance analysis identified two OTUs classified as *Hungatella* spp. exclusively present in non-responders, with relative abundance of up to 15%. Three other OTUs of the same genus were only present in responders **(Figure 28D),** indicating species-specific differences between responder and non-responder mice. **These observations again indicate small compositional changes in bacterial composition between a dysbiotic and non-dysbiotic microbiota and point toward a role of functional alterations of the microbiota**.

5 DISCUSSION

Microbial triggers are essential for the development of intestinal pathology

In the present work for the first time the development of CD-like ileitis was examined in GF TNF^{deltaARE} mice. In the absence of microbial triggers, TNF^{deltaARE} mice are protected from intestinal inflammation. This clearly demonstrates the essential role of microbial triggers in an experimental model that shares main clinical features of CD. In clear contrast to the abundant number of colitis models, there are only few IBD-related ileitis models, including TNF^{deltaARE}, SAMP1/YitFc or the SKG mouse. In the SKG-mouse model, curdlan-induced pathology was dependent on the presence of microorganisms. In the absence of intestinal microbial triggers, GF SKG mice developed mild spondylitis, though no ileitis.[110] Contrary to GF TNF^{deltaARE} mice, which are free of intestinal inflammation throughout life, high aged SAMP1/YitFc mice developed mild ileitis.[113] In several models of colitis (e.g. IL-10$^{-/-}$ and TRUC mice, as well as in HLA/B27-β2m transgenic rats), the pathology-free status under GF conditions has been shown.[101, 102, 223] Yet, in most studies on the contribution of the microbiota on intestinal pathology, only a comparison between microbial presence and absence is drawn. Information to the specific contribution of certain microbial communities in the development of ileitis is not yet reported.[108, 224]

A dysbiotic intestinal ecosystem is required for development of intestinal pathology

The pathology free status of animal models in GF housing provides a valuable tool to investigate colonization-induced mechanisms of microbe-host-interaction. In SPF housing, inflamed and non-inflamed TNF^{deltaARE} mice with compositionally and functionally diverse microbial ecosystems (though the same genetic background) were observed. To this end, the pathology-initiative potential of these dysbiotic *vs.* non-dysbiotic microbial patterns should be compared. The transfer of these ecosystems to GF TNF^{deltaARE} and WT mice should elucidate the causal relationship of dysbiosis and initiation of ileitis. Inflammation occurred only in TNF^{deltaARE} mice engrafted with dysbiotic microbiota. Importantly, GF WT recipient mice were not inflamed, supporting the necessity of genetic susceptibility for ileitis development, as well as the non-infectious nature of the dysbiotic microbial community. Strikingly, after 4 weeks of colonization, the non-inflammatory microbiota from non-responder donors failed to induce ileitis in colonized TNF^{deltaARE} recipients. This clearly demonstrates the inflammation-related specificity of the dysbiotic microbiota. The overall community structure between recipients of responder and non-responder microbiota were very distinct. No cage-effects were observed and differences within the groups could still be assigned to the respective donors, though recipients with different donor microbiota were cohoused and showed coprophagic behaviour. OTU-based analysis identified *Porphyromonadaceae* to be increased in non-responders. Also *Hungatella* spp. was significantly different. Of note, the phylogenetic classification of *Hungatella* is not yet well established

and delineated from other taxonomic groups and members within the Clostridium cluster XIVa were recently reclassified into the new genera of *Blautia* or *Hungatella*.[225] Therefore, according to reference database or time of analysis, regulated OTUs were identified as *Hungatella* sp, *Blautia* sp.. However, different representatives of *Hungatella* were found in higher abundances in both responder and non-responder $TNF^{deltaARE}$ mice. This hints at dysbiotic conditions characterized by subtle differences in bacterial profiles down to at least species level. There is rising evidence that pathology or protection associated microbiota traits are strain- or species specific, like the gelatinase production of *Enterococcus faecalis*, or PSA-production of *Bacteroides fragilis*.[118, 226]

There are only a few published reports on the disease-initiating potential of transferring pathology via dysbiotic microbiota. Of these, none is relevant for ileitis and neither used donor-animals of same genetic background. Garrett *et al.* demonstrated in TRUC mice that a genotype-specific microbiota transferred colitis to $RAG2^{-/-}$ recipients. However, WT cage-mates were also inflamed, suggesting a rather pathogenic than dysbiotic trait.[102, 227] Interestingly, Powell *et al*. identified *Helicobacter typhlonius* as key driver of pathogenesis in TRUC mice.[123] All results point towards a community effect of the complex microbiota and loss of aggressive, or gain of protective mechanisms, rather than the selection of aggressive pathobionts. In addition, inflammation occurred only after transfer of disease-related microbiota in GF $TNF^{deltaARE}$ and not in GF WT recipient mice, illustrating that genetic susceptibility is a prerequisite for ileitis development. Nonetheless, in the present study, inflammation clearly affected the phylogenetic composition of the intestinal ecosystem. More to the point, presence of a dysbiotic ecosystem was essential in initiating intestinal pathology.

Antibiotics as tool to unravel mechanisms of microbe-host-interactions

However, studies in GF animals have their limitations. The presence of bacterial stimuli is a prerequisite for different defence mechanisms (e.g. mucus-maturation, antimicrobial peptide production, IgA-secretion). Therefore some observations made in GF conditions may be over interpreted. Also only comparisons between presence and absence of microbial triggers can be made. To study the role of the phylogenetic makeup of inflammation associated ecosystems, other strategies are needed. Therefore, antibiotics pose an attractive approach to assess the consequences of shifts in the intestinal ecosystem for the health of the host.

Interestingly, the applied antibiotics attenuated ileitis without reduction in bacterial density. Antibiotics are assumed to reduce bacterial numbers in the gut and thereby induce temporal removal of inflammatory triggers. Even though in the present experiments, simple and complex antibiotic treatments were applied, none of the treatment schemes eliminated luminal bacterial numbers. Others before reported no reduction in intestinal bacterial density after a combined antibiotic

mixture.[228] Even at high dosages the combined usage of ampicillin and norfloxacin induced only a minor attenuation of anaerobe numbers. In contrast, the number of aerobes was reduced in a dose-dependent manner.[229] In humans receiving broad spectrum antibiotic-therapy, a shift of the intestinal bacterial composition was reported by Panda *et al*..[230] Hereby, no reduction in bacterial density was visible. In the IL-10$^{-/-}$ mouse model, two antibiotic studies established the colitis preventive, as well as therapeutic effect of different antibiotics, independent of reduction in bacterial density.[189, 229] These observations hint towards overgrowth of resistant species after a short lag phase. This emphasises that not the quantity of the microbial stimuli, but the microbial composition determines intestinal pathology.

In the present experiments antibiotic induced changes in the microbiota were associated to alleviated ileitis. Antibiotics (VM and MIX) induced great shifts within the phylum of *Firmicutes*. Especially the order of *Clostridiales,* many OTUs of *Lachnospiraceae* and *Ruminococcaceae* were eliminated, accompanied by an increase in *Lactobacillaceae.* Higher abundance of *Enterobacteriaceae* was detected in VM treated mice. *Enterobacteriaceae* are often concomitant to higher risk for development of IBD.[23, 26, 52, 55, 231] This stands in contrast to the here presented results, in which *Enterobacteriaceae* were linked to protection. However, it is still under debate whether higher sequential abundance of *Enterobacteriaceae* is really increasing the IBD risk previously to onset of disease. Increased presence of *Enterobacteriaceae* may also be secondary to inflammation, as *Enterobacteriaceae* may be better equipped to thrive within inflammatory conditions.[23, 58]

The perturbation of the intestinal ecosystem by the here applied antibiotics was temporal. After cessation of treatment, relapse of ileitis was observed. This model of recurrent inflammation is similar to observations made in patients.[232] Through transient changes in the intestinal ecosystem, partial re-establishment of the phylogenetic makeup and dynamics of recolonization could be demonstrated. While several species recovered within two weeks after treatment cessation, others recuperated later. This hints towards mechanisms of competitive exclusion or the necessity of microbe derived nutrients. The bloom of several species (e.g. *Chlamydiae*) revealed the opportunistic acquisition of vacant niches in the absence of competing taxa. Consequently, the microbial profile six weeks after VM-cessation did not fully resemble its pre-treatment organisation. This points to long lasting compositional disturbances and loss of resilience upon massive disruption of the intestinal community. Croswell *et al.* observed rapid recolonization already within one week after antibiotic intervention. Here, alterations in the microbial profile were still visible for several weeks.[233] In humans, Jacobsson *et al.* report persistence of antibiotic-induced community-changes up to even 4 years.[234] These long-lasting shifts in the microbiota are also discussed to be a risk factor for development of IBD.[235, 236]

However, knowledge about resilience of the intestinal ecosystem, its functional capacity and associated metabolites, is still scarce.

Antibiotics as tool to elucidate mechanisms of microbe-metabolite-interactions

Antibiotic intervention induces a distinct perturbation in the overall intestinal ecosystem. Accompanied shifts in the intestinal metabolite profile may also influence pathology. After antibiotic intervention, a marked reduction in caecal PGs and PAs was observed. In contrast, PE, cholesterolsulfate, sulphated flavonoids, fatty acids, TCA-sulphate and UbQs increased during treatment. Though, their role in IBD is still not well described and further studies are expected. However, changed patterns in phospholipids were detected in faecal samples of CD patients before.[237] Several bile acids, including α-MCA, DCA, LCA and 7-Sulfocholic acid, were depleted by antibiotics. Due to their hydrophobic and detergent-like attributes, BAs are considered as cytotoxic and associated to liver and colon cancer. Especially DCA and LCA are assumed to be toxic to epithelial cells.[238, 239] Thereby, ablation of secondary bile acids and other metabolites may display a secondary protective effect of antibiotics.

The question, which bacterial taxa are responsible for generation of secondary bile acids is still poorly understood. In the present experiments, the number of microorganisms in the gut was not reduced and cultivation of intestinal luminal contents did not indicate hampered viability of aerobic and anaerobic bacteria. This hints towards a loss in bacterial taxa with a central role in conversion of primary bile acids. Kang *et al* showed that the ability of bile acid 7-dehydroxylation is limited to a small number of intestinal anaerobes, e.g. *Clostridiales*.[240] Ablation in these taxa will hamper the generation of secondary bile acids. Importantly, the order of *Clostridiales* was almost depleted after VM as well as MIX treatment, explaining the observed reduction in secondary bile acids. Though, pathways depending on the collaboration of several bacterial species are also discussed.[240, 241] In the present experiments, metabolite- as well as bacteria-profiles were assessed in the same homogenous luminal content of the caecum. Therefore, correlations of metabolites to certain bacterial taxa could be shown for *Clostridiales* and other bacterial taxa. Within the order of *Clostridiales*, *Lachnospiraceae* were diminished upon antibiotic treatment and showed negative correlation to primary taurine conjugated bile acids. For *Porphyromonadaceae,* another interesting correlation between microbe, metabolite and inflammation was shown. *Porphyromonadaceae* were positively correlated to lactic acid and taurine-conjugated bile acids (TMCA, beta-MCA, TCAsulfate and TCA). This taxonomic family within the order of *Bacteroidales* seems to be of importance in inflammation development. A drop in the abundance of *Porphyromonadaceae* was repeatedly observed in inflamed $TNF^{deltaARE}$ mice in the CONV as well as SPF housing (compared to WTs or non-inflamed $TNF^{deltaARE}$ mice). Recently, reduction in *Porphyromonadaceae* was also associated with reduced colonization resistance

against *Clostridium difficile* in humans and pathology in a murine model of inflammation driven tumorigenesis.[242, 243] Therefore, ileitis-attenuation by intestinal ecosystem-modulation may be supported by changed abundance of bile acids. Along with a reduction of secondary bile acids, SCFA concentrations were reduced, suggesting the loss of microbial communities involved in bile acid metabolism and SCFA synthesis. Immune-regulatory effects of SCFA were described previously.[157, 158, 244] SCFAs are moreover discussed to serve as a surrogate marker for symbiotic microbiota composition.[159]

Recently, the vital impact of microbial derived metabolites in dysbiotic settings were shown in a mouse model of DSS-colitis. Here, Levy *et al*. could picture dependency of IL-18 generation upon microbiota-derived histamine, spermin or taurine.[245] They showed that bacteria-derived metabolites modify inflammasome signalling and downstream antimicrobial peptide profiles. Alteration of antimicrobial peptide production subsequently induced dysbiosis.[245] Therefore, targeted manipulation or monitoring of microbe-metabolite-correlations will be important to therapeutically influence the intestinal ecosystem.[69] However, microbe-metabolite-networks cannot depict causal relationships, but illustrate correlations only. These observed correlations may suggest increased production/degradation by microbial metabolism. They could also indicate enhanced resistance of those taxa, as e.g. bile acids are also discussed to shape the intestinal community.[246] Devkota *et al* showed the TCA-associated bloom of the bile-tolerant pathobiont *Bilophila wadsworthia*, which induced colitis in $IL10^{-/-}$ mice.[247] To prove the real existence and characteristics of metabolic correlations, further studies with defined microbial consortia, would be of importance.

While bile acids and SCFAs can be clearly ascribed to microbial metabolism, other metabolites may be of eukaryotic source as well. Direct, *i.e.* bacteria-independent effects of antibiotics on host cells must not be neglected.[182, 187, 248] Most antibiotics are administered orally and are subsequently metabolized by host cells. Though, their effect on these host cells is poorly described in literature. The same antibiotic treatment regime as used for CONV mice was applied to $TNF^{deltaARE}$ and WT mice in GF housing. No significant differences in the metabolite profile were observed, clearly ascribing observed metabolite changes to bacterial metabolism. The intestinal *Tnf*-expression was enhanced upon antibiotic treatment in GF $TNF^{deltaARE}$ mice. Conversely, plasma TNF-levels were reduced. Macrolide-antibiotics were previously shown to have an effect on immune cells. As summarized by Kanoh *et al*., macrolide-treatment diminished proinflammatory cytokine production, as well as neutrophil-infiltration.[249] These antibiotic-independent effects also led to the development of macrolides with solely immunomodulatory effects.[250] *In vitro*, the aminoglycoside gentamicin reduced TNF and MCP1 on protein level, despite increased expression levels for those cytokines.[251] Further *in vitro* studies from Ziegeler *et al.* report reduced cytokine levels in blood after pre-incubation with different

antibiotics.[248] Han *et al.* showed antibiotic-dependent attenuation of small intestinal inflammation in TRAF6^{deltaDC} mice, which was independent of bacterial presence.[252] These results clearly show a direct effect of antibiotics on host cells with potential systemic consequence. Consequently, further bacteria-independent effects of other antibiotic classes should be analysed. For vancomycin, as well as metronidazole, pharmaceutical effects beyond the alteration of the intestinal ecosystem have not yet been reported. Though, due to the high absorption rate of metronidazole, these effects should not be excluded.[187]

The intestinal ecosystem influences the spatial distribution of inflammation development

Different antibiotics target different bacterial taxa. While metronidazole mainly reduces the number of anaerobes, vancomycin targets gram positive bacteria. Therefore the extent of compositional changes may vary in the different gut segments. How the intestinal microbiota affects the spatial distribution of inflammation is poorly understood. In several murine models of intestinal inflammation, different antibiotic-regimens depicted site-specific effects of treatment success.[188, 189] This goes in parallel to our observations of VM-induced attenuation of ileitis, though not colitis. Unfortunately, in other experimental setups, the effect on colitis could not be validated, due to colitis-free status of CONV TNF^{deltaARE} mice during later experiments. Closer examination of FELASA-reports did not reveal differences in murine pathogens in the CONV facility. A comparison of 16S-profiles of TNF^{deltaARE} mice with and without colitis development may be of special interest. Though, this could not be performed here (not yet assessed variance and reproducibility of different sequencing-runs of these samples). During all animal experiments, neither modification in food-type nor –provider was made. However, between consecutive cohorts embedding, enrichment devices, as well as animal number per cage had to be changed. Also the microbiota may have been exposed to different microbes by adoption of new mice from external facilities. The sum of these factors indicate the multitude of possibilities leading to divergence of colitis-severity. This highlights current proclamations to more strict and continuous monitoring of microbiota composition in the respective facilities. This is essential, to ensure more reliable reproducibility of biomedical research. [98, 270] On the other hand, strict hygienic conditions may not induce full immune-maturation in the used IBD-models. Therefore, translation into human IBD-mechanisms may be limited. Importantly, also non-bacterial triggers - like different phages, yeasts or worms – should not be neglected as triggers for development of IBD. The presence and diversity of other microbial kingdoms in the gut, as well as trans-kingdom interactions, are only starting to be recognised.

Beside site-specific effects of antibiotics, also housing TNF^{deltaARE} mice in different hygienic conditions, affects disease location. The transfer of CONV TNF^{deltaARE} mice to a barrier-controlled SPF environment - free of infectious traits – ablated the colitis phenotype. Therefore, it would be of high interest to

unravel mechanisms of microbe-host interaction which lead to development of site-specific inflammation in IBD.

Loss of Paneth cell function is subsequent to TNF-driven development of inflammation

Interestingly, SPF housed TNF^{deltaARE} mice developed distinct ileitis-phenotypes. Approximately 50% of SPF TNF^{deltaARE} mice showed severe inflammation, whereas 20% remained ileitis-free, even at the age of 18 weeks. Variation in ileitis severity of SPF housed TNF^{deltaARE} mice correlated with inflammatory tissue activation, measured at the level of granulocyte-infiltration and *Tnf* expression. Furthermore, significant reduction in lysozyme-positive Paneth cells per crypt was shown. Paneth cells play an important role in small intestinal mucosal defence and were shown to be functionally impaired in IBD, especially in CD patients.[143, 253-255] Genetic risk variants like autophagy-associated ATG16L1-T300A and endoplasmic reticulum stress responses contribute to Paneth cell dysfunction, and recently the generation of tissue-specific knock-out models, *i.e.* Caspase-8 or ATG16L1 and XBP-1 epithelial cell deficient mice, allowed a detailed mechanistic understanding of CD-like disease phenotypes. Lineage-specific ablation of Paneth cells showed no specific IBD-associated phenotype.[256] However, mouse-models with endoplasmic reticulum stress- and autophagy-related loss of Paneth cell functions revealed inflammatory pathologies in the small intestine.[114-116] Interestingly, these newly generated mouse models share loss of Paneth cell functions as an important mechanism of ileal pathogenesis. Considering that Paneth cells influence microbial composition, disruption of this homeostasis might lead to the development of pathology-related dysbiosis and increased penetration of bacterial triggers into immune-sensitive tissue areas.[87, 114, 115, 257] A role for TNF in regulatory pathways of antimicrobial peptide expression has been suggested, but mechanisms through which TNF-driven inflammation induces Paneth cell failure are still unclear.[258] Loss of A20, the feedback mechanism of NF-kB-related signal-transduction, sensitized the epithelium towards apoptosis and development of intestinal inflammation, including ileitis. In this A20-KO model, Paneth and goblet cell loss was associated with microbial dysbiosis.[259] In addition, the hypothesis that inflammation-associated mediators abrogate Paneth cell functions, is supported by the fact that Paneth cell-derived antimicrobial peptide-release in organoids is controlled by the Th1 cytokine IFN-γ.[260] The link between ileitis severity and Paneth cell function rather than numbers was shown by using a combination of lysozyme and UEA-1 staining in SPF TNF^{deltaARE} mice. A significant reduction in lysozyme-positive cells per crypt was found to be correlating with disease activity and inflammatory tissue activation, measured at the level of granulocyte infiltration and induction of TNF expression.

Interestingly, SPF TNF^{deltaARE} mice with severe ileitis and Paneth cell failure were characterized by a taxonomically and functionally dysbiotic gut microbiota. Analysis of intestinal communities showed increased relative abundance of unknown *Clostridiales* and reduced abundance of the family

Porphyromonadaceae (order of *Bacteroidales*) in ileitis-related microbiota. While members of the order of *Clostridiales* were found in lower abundance in CD patients, the abundance of *Clostridiales* was increased in TNF^{deltaARE} ileitis and TM-IEC-C1galt1$^{-/-}$ colitis-models, indicating model-specific traits of this very broad taxonomic group.[32, 261] Metaproteomics revealed that, among other proteins, myo-inositol-1-phosphate synthase and fucose-utilizing enzyme (fucU) were characteristic for the dysbiotic ecosystem. These proteins were found to be abundant in members of the order *Clostridiales*, though also enteropathogens.[262] Consistent with changes in the metaproteome analysis, shotgun metagenomic analysis of CD and UC patients showed "extreme functional shifts" especially in ileal CD, driven by pathways of *carbohydrate transport and mechanism* as well as *amino acid biosynthesis.*[65] In SPF housed mice, ileitis-severity was strongly associated to changes in the caecal microbiota.

The transfer of ileitis associated microbiota to GF TNF^{deltaARE} mice verified the causal relationship of dysbiosis and initiation of disease. Interestingly, by time-series analysis of inflammation, it was demonstrated that loss of Paneth cell function was subsequent to the TNF-driven development of inflammation in recipient mice. Therefore, lysozyme produced by Paneth cells might contribute, but seems not to be causal for the divergence of disease-related bacterial communities or development of dysbiosis.

In summary, different housing conditions and antibiotic treatments of TNF^{deltaARE} mice clearly established the importance of non-infectious microbial communities in the development of CD-like ileitis. A dysbiotic pattern of the intestinal ecosystem was required for development of pathology. By changing the intestinal ecosystem or engrafting non-disease associated microbiota, ileitis severity was altered in the susceptible host. Therefore, establishing a protection-associated, non-dysbiotic microbiota in susceptible recipients may state a possible treatment strategy.

Translation into IBD therapy

The potential of therapeutical microbiota transplantation was assessed by transferring caecal microbial communities to CONV TNF^{deltaARE} mice. VM-treated TNF^{deltaARE} mice with attenuated ileitis severity served as donor animals. However, no attenuation of ileitis was observed in transplant-recipients. This hints towards a lack of engraftment of the donor microbiota. Creation of vacant bacterial niches by antibiotic treatment did not improve microbiota engraftment, contrary to the currently often proclaimed hypothesis. Manichanh *et al.* analysed the degree of microbiota adoption after transplantation.[263] Interestingly they observed the possibility to reshape the intestinal ecosystem by exogenous transplantation, independent of previous antibiotic perturbation.

This observation is exceptionally interesting for clinical practice. For CD, the benefit of microbiota-modulating-therapy, *i.e.* antibiotics, probiotics or FMT, is still discussed. Meta-analysis data show no

clear advantage of probiotics in CD, though hint towards a placebo-superior effect of antibiotics.[264] Newer studies go from single-probiotic strains or mixtures with low defined numbers to more complex setups. A new treatment approach is currently tested for efficacy in treatment of IBD: The therapeutic transfer of a complex faecal microbiota (FMT) from healthy donors to IBD patients. In *Clostridium difficile*-induced infection, the transfer of a complex, unknown microbial community via FMT seems to be a very promising approach, and even more effective than antibiotic treatment regimens.[265] Although the bacterial taxa associated with a successful FMT-donor-microbiota have not been discovered yet, the subsequent increase in the recipient´s intestinal alpha-diversity, is in most cases associated with recovery.[266] Despite the fact that FMT was highly effective in *C. difficile* infection, its therapeutic implementation in IBD is unclear. A recent study by Moayyedi *et al*. showed a placebo superior effect of FMT in UC patients, though this effect is donor and time-dependent.[267] However, in a study from Rossen *et al*., there was no statistically significant difference in clinical and endoscopic remission in UC patients after FMT.[268] Yet, due to the novelty of this very promising treatment approach, decent cohort numbers and well controlled trials are to be provided in the future. Safety control with regard to the donor-microbiota is of utmost importance but time consuming. Therefore, the generation of biobanks for frozen donor-microbiota (e.g. OpenBiome (Microbiome Health Research Institute Inc.)), or the identification of analogously effective but defined bacterial mixtures may be of enormous advantage. Furthermore, a future prospect of FMT is the capsulized applicable therapy form, which is currently developed.[269] By now, no study with decent cohort numbers of ileal-CD patients is available. Though, a separated investigation on ileal and colonic disease manifestation is necessary, as the microbiota may also impact the location of inflammation development.

6 CONCLUSION AND OUTLOOK

In summary, the present work clearly identifies dysbiosis as a main trigger in TNF-driven chronic CD-like ileitis. Inflammation was transmitted by a dysbiotic microbiota only, thereby emphasizing the causal and crucial role of the intestinal community. Genetic susceptibility was a prerequisite for inflammation, and loss of Paneth cell function was found to be subsequent to ileitis development. This inflammatory milieu, plus alternated antimicrobial peptide-profile will finally lead to more pronounced divergence of microbial communities. For the $TNF^{deltaARE}$ mouse model, dysbiotic microbial communities were successfully identified and transferred. Whether these dysbiotic microbial patterns induce the same phenotypes in other models with susceptibility to intestinal inflammation, has to be tested. Also, translation into models of extra-intestinal pathology will be of great interest. This would give interesting knowledge into mechanisms of disease initiation. Furthermore, insight on the correlation of microbial colonization and arthritis-development in the $TNF^{deltaARE}$ model is eagerly awaited.

The present results also put emphasis for a more comprehensive characterization of ecosystems, to gain deeper insight into microbe-host mutualism. In future, beyond 16S-profiling techniques, metatranscriptomics, -viromics or -proteomics will be important to unravel trans-kingdom interactions as well as functional-dependencies. Studies in monocolonized and gnotobiotic animal models will show the underlying molecular pathways and triggers and will help to shed light onto important mechanisms of microbe-host interaction. Though GF-animals pose an excellent tool to unravel specific mechanisms, a disadvantage is their immature immune and tissue development. This may lead to over-interpretation or negligence of certain functional mechanisms. Therefore, targeted changes of the intestinal ecosystem in conventionally raised animals will still be an important proof of principle. Nevertheless, clear and strict control of hygienic conditions are of utmost significance. Furthermore, different animal models of intestinal inflammation are needed and will give deeper knowledge of microbe-host-mutualism. This knowledge will be crucial to clinically implement IBD-treatments which aim at reshaping the intestinal ecosystem. Faecal microbiota transplantation seems already a promising treatment-route. Though, its establishment in IBD will need further research to optimize pre-treatment conditions and choice of donor-microbiota to reliably and successfully cure IBD.

APPENDIX

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Supplementary Table 1: Antibiotic induced shifts in bacterial profiles

OTU abundances (mean of percent of all sequences ± standard deviation) which are significantly reduced or increased by antibiotic treatments. Taxonomic assignments are given at the order or family level. Only OTUs with abundances over 1.5% in at least one of the groups are shown. OTUs are ordered by decreasing abundance in the control group.

OTUs with significant interaction to antibiotic sensitive metabolites are marked with **#**.

OTUs with genotype-dependent different abundance (control-WT *vs.* inflamed control-TNF^{deltaARE} mice) are marked with *.

Abundance	Genotype	CONTROL	MIX	VM	Taxonomy
decreased by antibiotics	OTU_7	8 ± 8.4	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_11*	7.1 ± 4	0 ± 0	2.6 ± 3.3	*Helicobacteraceae*
	OTU_16	5.7 ± 6.8	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_27	4.7 ± 1.7	0 ± 0	0 ± 0	*Rikenellaceae*
	OTU_21	4.3 ± 4.1	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_12	2.4 ± 3.8	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_322	2.2 ± 2.7	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_28	2.1 ± 3.3	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_41	2.1 ± 2.5	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_15	2.1 ± 3	0 ± 0	0 ± 0	unknown Bacteria
	OTU_184	2 ± 2.1	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_43	2 ± 2	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_77*	1.8 ± 0.6	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_25	1.7 ± 1	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_79	1.5 ± 0.5	0 ± 0	0 ± 0	*Ruminococcaceae*
	OTU_51	1.4 ± 2.1	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_24	1.3 ± 1.4	0 ± 0	0 ± 0	*Ruminococcaceae*
	OTU_29	1.3 ± 0.8	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_61#	1.3 ± 0.7	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_50	1.2 ± 0.2	0 ± 0	0 ± 0	*Ruminococcaceae*
	OTU_75	1.1 ± 0.4	0 ± 0	0 ± 0	*Ruminococcaceae*
	OTU_35	1.1 ± 1.7	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_40	1.1 ± 1.1	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_37*	1 ± 1.5	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_42	1 ± 0.7	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_372	0.9 ± 0.7	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_17	0.8 ± 1.2	0 ± 0	0 ± 0	*Lachnospiraceae*
	OTU_36	0.8 ± 0.6	0 ± 0	0 ± 0	*Desulfovibrionaceae*
	OTU_53	0.4 ± 0.4	0 ± 0	0 ± 0	*Ruminococcaceae*
	OTU_45	0.4 ± 0.4	0 ± 0	0 ± 0	*Ruminococcaceae*
increased by antibiotics	OTU_2	0.3 ± 0.2	65.8 ± 30.8	40.9 ± 19.2	*Lactobacillaceae*
	OTU_62	0.3 ± 0.3	2.4 ± 1.3	3.6 ± 2.6	*Lactobacillaceae*
	OTU_14#	0.2 ± 0.4	5.2 ± 6.3	2.1 ± 3.1	*Verrucomicrobiaceae*
	OTU_76	0.2 ± 0.2	1.7 ± 2.2	0 ± 0	*Porphyromonadaceae*
	OTU_48	0.2 ± 0.2	0 ± 0	1.9 ± 2.7	*Lactobacillaceae*
	OTU_26	0.1 ± 0.1	2.1 ± 2.2	5.7 ± 2	*Sutterellaceae*
	OTU_6#	0 ± 0	7.5 ± 10.7	0 ± 0	*Porphyromonadaceae*
	OTU_22	0 ± 0	6.3 ± 8.1	0 ± 0	*Porphyromonadaceae*
	OTU_20	0 ± 0.1	2.5 ± 3.9	2.8 ± 3.7	*Porphyromonadaceae*
	OTU_54#	0 ± 0	1.4 ± 1.5	1.3 ± 2.2	*Porphyromonadaceae*
	OTU_73#	0 ± 0	1.2 ± 1.6	0.2 ± 0.5	*Porphyromonadaceae*
	OTU_5	0 ± 0	0 ± 0	19.5 ± 9.6	*Enterobacteriaceae*
	OTU_31	0 ± 0	0 ± 0	4.4 ± 3.5	*Enterobacteriaceae*
	OTU_18	0 ± 0	0 ± 0	3.9 ± 5.2	*Helicobacteraceae*
	OTU_30	0 ± 0	0 ± 0	3.9 ± 3.7	*Paenibacillaceae 1*

Supplementary Table 2: Antibiotic-induced changes in caecal metabolite profiles

Metabolites responsible for the regression were sorted according to their respective R-value and VIP value. The metabolite ratios from the controls to the respective treatment groups are given on the right. n.d. = not determined.

Color code: red = high intensity; blue = low intensity; color coded bars in the "ratio" columns approximate fold-increase to the respective treatment group.

Metabolite			TNF^{deltaARE} mice			ratio		
Decreased after AT	**R**	**VIP**	**Control**	**VM**	**MIX**	**CN / VM**	**CN / MIX**	**VM / MIX**
PG(P-18:0/18:1); PG(P-20:1/16:0); PG(P-16:0/20:1)	0.8	1.5	0.2	0.0	0.0	29.8	12.2	0.4
PG(P-14:0/16:0); PG(P-16:0/14:0)	0.8	1.5	0.1	0.0	0.0	15183.2	n.d.	n.d.
PG(16:0/20:1); PG(18:0/18:1)	0.8	1.4	0.6	0.1	0.0	9.7	90.5	9.3
OH-C18:1	0.8	1.4	0.3	0.0	0.0	6.8	17.2	2.5
PA(O-20:0/18:1)	0.7	1.4	0.1	0.0	0.0	n.d.	n.d.	n.d.
PE(P-16:0/12:0)	0.7	1.3	0.1	0.0	0.0	45.4	n.d.	n.d.
DCA	0.7	1.3	0.5	0.0	0.0	n.d.	n.d.	n.d.
PG(P-15:0/16:0/18:1)	0.7	1.3	1.1	0.1	0.1	9.2	8.8	1.0
PG(P-18:1/15:0)	0.7	1.3	0.0	0.0	0.0	18.7	23.3	1.2
PG(P-18:1/14:0/16:0)	0.6	1.3	2.1	0.1	0.0	24.5	50.0	2.0
ω-MCA	0.7	1.3	0.0	0.0	0.0	n.d.	n.d.	n.d.
PE(P-16:0/14:0)	0.6	1.3	0.1	0.0	0.0	102.8	n.d.	n.d.
Heptadecylresorcinol	0.7	1.3	0.1	0.0	0.1	2.7	1.6	0.6
LCA	0.7	1.3	0.0	0.0	0.0	n.d.	n.d.	n.d.
PG(P-16:0/16:0/18:2)	0.5	1.3	8.6	2.3	1.3	3.8	6.5	1.7
PG(P-16:0/18:2)	0.6	1.2	0.4	0.1	0.1	7.2	4.1	0.6
PG(P-16:0/18:0/18:1)	0.6	1.2	0.7	0.1	0.2	5.4	3.0	0.6
PG(P-16:0/18:2/18:1)	0.5	1.2	0.8	0.1	0.3	6.7	2.7	0.4
α-MCA	0.6	1.2	0.1	0.0	0.0	50.5	2.5	0.0
LysoPE(18:2)	0.6	1.1	0.1	0.0	0.0	10.6	9.0	0.8
7-Sulfocholic acid	0.6	1.1	0.2	0.0	0.0	6.0	20.5	3.4
C20:3	0.5	1.0	0.3	0.1	0.2	2.2	1.9	0.9
PG(P-16:0/15:0)	0.6	1.0	0.1	0.0	0.0	4.2	n.d.	n.d.
Increased after AT	**R**	**VIP**	**Control**	**VM**	**MIX**	**CN/VM**	**CN/MIX**	**VM/MIX**
PE(16:0/17:1)	-0.7	1.8	0.1	1.0	0.1	0.1	1.5	17.8
Cholesterolsulfate	-0.8	1.8	0.5	1.4	1.0	0.4	0.5	1.4
Galactosylglycerol	-0.6	1.5	0.0	0.1	0.0	0.0	0.0	7.3
Biochanin A sulfate	-0.7	1.5	0.0	0.3	0.1	0.0	0.0	3.0
Daidzein-sulfate	-0.6	1.3	0.0	0.7	0.4	0.0	0.0	1.7
Apigenin 7-sulfate	-0.6	1.3	0.0	0.8	0.5	0.0	0.0	1.5
C30:0	-0.6	1.2	0.1	0.2	0.2	0.4	0.3	0.8
C28:0	-0.5	1.1	0.1	0.3	0.4	0.5	0.3	0.7
UbQ1*	-0.6	1.1	0.0	0.0	0.0	0.1	0.1	0.8
Demethyl-ubiquinol-6	-0.6	1.1	0.1	0.2	0.3	0.3	0.2	0.7
TCA Sulfate	-0.5	1.1	0.0	0.2	0.2	0.0	0.0	1.4
OH-C18:1	-0.6	1.0	0.0	0.2	0.3	0.2	0.1	0.7
Demethyl-ubiquinol-6	-0.6	1.0	0.1	0.2	0.3	0.4	0.3	0.7
Ubiquinol-6	-0.5	0.9	0.0	0.1	0.2	0.4	0.3	0.7
Ubiquinol-6	-0.5	0.9	0.1	0.1	0.2	0.4	0.3	0.7

ABBREVIATIONS

Acyl-PG	acylphosphatidylglycerol
AMP	antimicrobial peptide
Amp	ampicillin
ARE	adenosine uracil rich element
BA	bile acid
CA	cholic acid
CCL21	chemokine (C-C motif) ligand 21
CD	Crohn´s Disease
CD	cluster of differentiation
CDCA	chenodeoxycholic acid
CFU	colony forming units
COG	Cluster of Orthologous Groups
CONV	conventional
DC	dendritic cell
DCA	*deoxycholic acid*
E.	*Escherichia*
EEN	exclusive enteral nutrition
EGF	epidermal growth factor
ELISA	enzyme linked immunosorbent assy
ER	endoplasmic reticulum
FELASA	federation of Laboratory Animal Science Associations
FMT	faecal microbiota transplant
FTICRMS	fourier transformation-ion cyclotron resonance-mass spectrometry
Foxp3	forkhead box P3
FUT2	fucosyltransferase 2
FXR	farnesoid X receptor
GALT	gut associated lymphoid tissue
GF	germfree
H&E	hematoxylin & eosin
HLA	human leukocyte antigen
HMP	human microbiome project
IBD	inflammatory bowel disease
IEC	intestinal epithelial cells
IEL	intraepithelial lymphocytes
ILC	Innate lymphoid cells
IFN	interferon
Ig	immunoglobulin
iMP	intestinal mononuclear phagocytes
iNKT	invariant natural killer T cells
IP-10	interferon inducible protein 10
IL	interleukin
KC-1	keratinocyte chemoattractant
LC MS MS	liquid chromatography mass spectrometry mass spectrometry
L.	*Lactobacillus*
LCA	lithocholic acid
LPS	lipopolysaccharide
α-MCA	alpha-muricholic acid
MAPK	mitogen-activated protein kinases
MΦ	macrophage
MCP1	monocyte chemoattractant protein-1

MDP	muramyldipeptide
MEOH	methanol
Mix	antibiotic mixture combined of Van / Met / Neomycin and norfloxacin
MLN	mesenteric lymph nodes
MYD88	myeloid differentiation primary response 88
NCF4	neutrophil cytosolic factor-4
n.d.	not detectable
NOD	nucleotide-binding oligomerization domain-containing protein 2
Nor	norfloxacin
o.n.	over night
OTU	operational taxonomic unit
PAMP	pathogen associated microbial pattern
PC	paneth cell
PCA	principal component analysis
PCR	polymerase chain reaction
PE	phosphoethanolamine
pIgR	polymeric immunoglobulin receptor
PLS	partial least squares
PSA	capsular polysaccharide A
PRR	pattern recognition receptors
Reg	regenerating islet-derived genes
RT	room temperature
S.	*Salmonella*
SAA	serum amyloid A
SCFA	short chain fatty acid
SFB	segmented filamentous bacteria
sIgA	secreted immunoglobulin A
SNP	single nucleotide polymorphism
SPF	specific pathogen free
STAT3	signal transducer and activator of transcription factor 3
TCA	taurocholic acid
TCR	T cell receptors
TLR	toll like receptor
TNF	tumor necrosis factor
TRAF6	TNF receptor associated factor 6
TRIFF	TIR-domain-containing adapter-inducing interferon-β
TSLP	thymic stromal lymphopoetin
UbQs	ubiquinons
UC	ulcerative colitis
UEA-1	ulex europaeus agglutinin 1
UPLC-TOF-MS	ultra-performance liquid chromatography time-of-flight *mass* spectrometry
VIP	very important projection
VM	vancomycin/metronidazole
XBP-1	X-box binding protein 1

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PUBLICATIONS AND PRESENTATIONS

PEER-REVIEWED ORIGINAL MANUSCRIPTS AND REVIEWS

Authors marked with * contributed equally to the work

2015

I. Schaubeck M*, Clavel T*, Calasan J, Lagkouvardos I, Haange S.B., Jehmlich N, Basic M, Dupont A, Hornef M, von Bergen M, Bleich A, Haller D
Dysbiotic gut microbiota causes transmissible Crohn's disease-like ileitis independent of failure in antimicrobial defence
Gut, 2016. **65**(2): p. 225-37.

II. Schaubeck M; Haller D
Reciprocal interaction of diet and microbiome in inflammatory bowel diseases
Curr Opin Gastroenterol. 2015 Nov;31(6):464-70

III. Butto L*, Schaubeck M*, Haller D
Mechanisms of microbe-host-interaction in Crohn´s disease: dysbiosis vs. pathobiont selection
Front Immunol. 2015 Nov 19;6:555

IV. Hörmannsperger G; Schaubeck M; Haller D
Intestinal microbiota in animal models for inflammatory diseases
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V. Matthias Friedrich, Torsten Olszak, Monika Schaubeck, Felix Hiltwein, Jessica I. Grill, Frank T. Kolligs, Marlon R. Schneider, Karl Sotlar, Florian Beigel, Dirk Haller, Stephan Brand
IL-36γ-IL-17A-IL-17C cross-regulation in chronic intestinal inflammation sustains a self-amplifying, pro-inflammatory and pro-apoptotic program in intestinal epithelial cells
Under revision in the Journal of Immunology

VI. Alesia Walker, Barbara Pfitzner, Mourad Harir, Monika Schaubeck, Jelena Calasan, Thomas Clavel, Dirk Haller, David Endesfelder, Wolfgang zu Castell, Michael Schmid, Anton Hartmann, Philippe Schmitt-Kopplin
Sulfonolipids as novel metabolite markers of Alistipes and Odoribacter affected by high-fat diets
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VII. Monika Schaubeck*, Alesia Walker*, Thomas Clavel, Lagkouvardos Ilias, Martinez Ines, Walter Jens, Philippe Schmitt-Kopplin and Dirk Haller
Dysbiosis and connected intestinal metabolites in Crohn´s disease like ileitis
In preparation

2013:

VIII. Hörmannsperger G; Schaubeck M; Haller Dirk
Die Bedeutung des intestinalen Mikrobioms für die Gesundheit des Menschen
E&M – Ernährung und Immunologie 2013; 28: 105-109
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IX. von Schillde MA, Hörmannsperger G, Weiher M, Alpert CA, Hahne H, Bäuerl C, van Huynegem K, Steidler L, Hrncir T, Pérez-Martínez G, Kuster B, Haller D
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Preparation of passion fruit-typical 2-alkyl ester enantiomers via lipase-catalyzed kinetic resolution.
J Agric Food Chem. 2010 May 26;58(10):6328-33

PUBLISHED ABSTRACTS

DDW 2015: ***Transmissive Crohn's Disease-Like Ileitis Is Caused by Functional Dysbiosis in the Intestinal Microbiota Independent of Inflammation-Driven Paneth Cell Failure***
Schaubeck M*, Clavel T*, Lagkouvardos I, Calasan J, Bastiaan Haange S, Jehmlich N, von Bergen M, Basic M, Bleich A, Haller D; Gastroenterology, Vol. 148, Issue 4, S-61; Published in issue: April 2015

ECCO 2015: **OP001.** ***Transmissive Crohn's disease-like ileitis is caused by functional dysbiosis in the intestinal microbiota independent of inflammation-driven Paneth cell failure***
Schaubeck M*, Clavel T*, Lagkouvardos I, Calasan J, Bastiaan Haange S, Jehmlich N, von Bergen M, Basic M, Bleich A, Haller D; Journal of crohn´s and colitis DOI: http://dx.doi.org/10.1093/ecco-jcc/jju027.001 S1 First published online: 2 February 2015

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Schaubeck M, Clavel T, Waldschmitt N, Wehkamp J, Martinez I, Walter J, Kollias G, Haller D; Gastroenterology, Vol. 146, Issue 5, S-836; Published in issue: May 2014

ORAL PRESENTATIONS

2016:

"XXIII Course "Miquel Angel Gassull" on Inflammatory Bowel Diseases" (Barcelona)

Dysbiosis – Cause or consequence?

Schaubeck M*

2015:

Digestive Disease week (Washington)

Transmissive Crohn's Disease-Like Ileitis Is Caused by Functional Dysbiosis in the Intestinal Microbiota Independent of Inflammation-Driven Paneth Cell Failure

Schaubeck M*, Clavel T*, Lagkouvardos I, Calasan J, Haange SB, Jehmlich N, von Bergen M, Basic M, Bleich A, Haller D

Kongress der Deutschen Gesellschaft für Ernährung (Halle-Leipzig)

Transmissive Crohn's disease-like ileitis is caused by functional dysbiosis in the intestinal microbiota independent of inflammation-driven Paneth cell failure

Schaubeck M*, Clavel T*, Lagkouvardos I, Calasan J, Haange SB, Jehmlich N, von Bergen M, Basic M, Bleich A, Haller D

8th Seeon Conference and Science Camp (Seeon)

Transmissive Crohn's disease-like ileitis is caused by functional dysbiosis in the intestinal microbiota independent of inflammation-driven Paneth cell failure

Schaubeck M*, Clavel T*, Lagkouvardos I, Calasan J, Haange SB, Jehmlich N, von Bergen M, Basic M, Bleich A, Haller D

European Crohn´s and Colitis Organization (Barcelona)

Transmissive Crohn's disease-like ileitis is caused by functional dysbiosis in the intestinal microbiota independent of inflammation-driven Paneth cell failure

Schaubeck M*, Clavel T*, Lagkouvardos I, Calasan J, Haange SB, Jehmlich N, von Bergen M, Basic M, Bleich A, Haller D

2014:

United European Gastroenterology Week (Vienna)

Causative role of the intestinal microbiota in Crohn´s disease-like ileitis using germ-free and antibiotic-treated TNFΔARE mice

Schaubeck M, Clavel T, Waldschmitt N, Wehkamp J, Martinez I, Walter J, Roulis M, Kollias G and Haller D

2013:

6th Seeon Conference (Seeon)

Antibiotic-induced alteration of the gut microbiota protects TNFdeltaARE mice from Crohn's disease-like ileitis

Weiher M, Clavel T, Schmid M, Walker A, Martinez I, Walter J, Schmitt-Kopplin P, Kollias G and Haller D

2012:

Kongress der Deutschen Gesellschaft für Ernährung (Weihenstephan)

Gut microbiota influences ileitis in TNFdeltaARE mice

Weiher M, Clavel T, Martinez I, Walter J and Haller D

6th Seeon Conference (Seeon)

Antibiotic treatment changes gut bacterial diversity and protects TNFdeltaARE mice from Crohn's disease-like ileitis

Weiher M, Clavel T, Walker A, Martinez I, Walter J, Schmitt-Kopplin P, Kollias G and Haller D

POSTER PRESENTATIONS

2015:

"The human microbiome" (EMBL) Heidelberg

Dysbiotic gut microbiota causes transmissive Crohn´s disease like ileitis independent of failure in antimicrobial defense

Schaubeck M, Clavel T, Lagkouvardos I, Calasan J, Haange SB , Jehmlich N, von Bergen M, Dupont A, Hornef M, Basic M, Bleich A, Haller D

"Gut Microbiota Modulation of Host Physiology: The Search for Mechanism" (Keystone Symposia) Keystone (Colorado)

Causative role of a dysbiotic intestinal microbiota in Crohn´s disease-like ileitis

Schaubeck M, Clavel T, Lagkouvardos I, Calasan J, Kollias G, Haller D

2014:

"Beyond the intestinal microbiome" (Herrenhauser Conference) Hannover

Causative role of the intestinal microbiota in Crohn´s disease-like ileitis

Schaubeck M, Clavel T, Lagkouvardos I, Waldschmitt N, Wehkamp J, Roulis M, Kollias G and Haller D

Digestive Disease week (Chicago)

Functional evidence for a causative role of the intestinal microbiota in Crohn´s disease-like ileitis using antibiotic-treated and germ-free TNF$^{deltaARE/+}$ mice

Schaubeck M, Clavel T, Waldschmitt N, Wehkamp J, Martinez I, Walter J, Kollias G and Haller D

2013:

United European Gastroenterology Week (Berlin)

Alteration of the gut microbiota protects TNFΔARE/+ mice from Crohn's disease-like ileitis

Schaubeck M, Clavel T, Walker A, Martinez I, Walter J, Schmitt-Kopplin P, Kollias G and Haller D

International Congress of Mucosal Immunology (Vancouver)

Antibiotic-induced alteration of the gut microbiota protects TNFdeltaARE mice from Crohn's disease-like ileitis

Weiher M, Clavel T, Walker A, Martinez I, Walter J, Schmitt-Kopplin P, Kollias G and Haller D

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from the European Molecular Biology Labor (2015)

Oral free paper price

from the United European Gastroenterology organization (2014)

Travel Grant

from the European Molecular Biology Labor to the “The human microbiome” conference in Heidelberg (June 2015)

Travel Grant

from Glaxo Smith Kline to the International Congress of Mucosal Immunology in Vancouver (July 2013)

Travel Grant

from the Volkswagenstiftung to the Herrenhauser Conference in Hannover (November 2014)

Travel Grant

from the UEG to the United European Gastroenterology Week in Vienna (October 2015

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Johann Wolfgang von Goethe

Faust - der Tragödie zweiter Teil

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____Aiglsbach 12.04.2016_____

CURRICULUM VITAE

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Seit Oktober 2015	Wissenschaftliche Angestellte am Max-Planck Institut für Neurobiolgie (Neuroimmunologie bei Prof. Hartmut Wekerle)
2010-2015	Doktorandin am Lehrstuhl für Ernährung und Immunologie von Prof. Dirk Haller
2007-2010	Masterstudium Ernährungswissenschaften (TU München)
	Masterarbeit: "Isolation and characterization of Lactobacilli of the human and mouse intestine"
2004-2007	Bachelorstudium Ernährungswissenschaften (TU München)
	Bachelorarbeit: "Enantioselective enzymatic esterification of secondary alcohols"
1995-2004	Gabelsberger Gymnasium Mainburg

Praktika:

2009	Dreimonatiges Auslandspraktikum an der University of Reading, UK
	„Probiotic beta-galactosidases and their effect on cell growth and adhesion"
2008	Forschungspraktikum am Lehrstuhl für Tierhygiene (TU München)
	Forschungspraktikum am Lehrstuhl für molekulare Lebensmittel- und Biotechnologie (TU München)
2006	Sechswöchiges Industriepraktikum im Qualitätsmanagement bei der Pharma-Firma Daiichi-Sankyo (Pfaffenhofen a.d. Ilm)
2005	Sechswöchiges Praktikum im Bereich der Diätassistenz im Krankenhaus Landshut-Achdorf

Stipendien:

2010-2012	Graduiertenstipendium der Konrad-Adenauer Stiftung
2005-2008	Stipendium der Begabtenförderung der Konrad-Adenauer Stiftung

Fortbildungen:

TUM Graduate School:"Statistik 1 und 2", "*Basics in Business & Administration for Scientists"*

Konrad-Adenauer-Stiftung: „Rhetorik im Vortrag", „Rhetorik im Gespräch"

Ehrenamtliches Engagement:

2000-2013 Jugendleiterin, -referentin beim deutschen Alpenverein (Sektion Mainburg)

2007-2008 Stipendiatengruppensprecherin der Lokalgruppe der Konrad-Adenauer-Stiftung (Gruppe München 6)

2005-2006 Tutorin des Tutorenprogramms Weihenstephan