Microbes in the inflamed gut

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Chapter eight

Discussion and perspectives

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Inflammatory bowel disease is a collective term for chronic inflammatory conditions in the gastro-intestinal tract affecting millions of people worldwide and these numbers are only going up in association with the adoption of a Western lifestyle and urbanization [1–3]. The need for new therapeutic strategies is high as many patients insufficiently respond well to treatment so stay undertreated increasing occurrence of side effects or complications of the disease such as cancer, extra-intestinal manifestations or fistulae [4]. The multifactorial nature of the disease asks for a comprehensive, holistic view of the disease in which the microbiome, immune response and metabolism of the patient are taken into consideration [5]. To contribute to this, the aim of this thesis was to study the various aspects of the gut microbiome in inflammatory bowel disease.

Studying the microbiome in disease is complex and a clear definition of a 'healthy' or normal 'microbiome' is lacking [6,7]. There is significant interpersonal variation in microbial communities and diversity. Especially in diseased states there seems to be more variation in microbial community composition than in healthy individuals, the so-called 'Anna Karenina' principle referring to Leo Tolstoy's dictum that "all happy families look alike; each unhappy family is unhappy in its own way" [8,9]. Instead of merely describing the microbiome based on taxonomy, we need to delve deeper into the mechanistic interactions between microbes and host. This involves linking microbial functioning to the immune and metabolic functioning of the host. Integration of various -omics methods allows researchers to capture the spectrum of interactions between microbiota and their host, as well as the effects of these interactions [10]. Recent efforts in this direction have illuminated the differences in microbiome composition and its functioning between patients and healthy controls [11,12].

In this thesis we showed that also over the course of the disease the microbiome-host interaction is affected. In **part I** of this thesis, entitled **"Interactions between microbiota and host in inflammatory bowel disease"**, we focused on the microbiome-host interaction in pediatric patients with inflammatory bowel disease. We performed multi-omics analysis, in pediatric Crohn's disease (**chapter 2**) and ulcerative colitis (**chapter 3**) to

elucidate possible differences between patients with active disease and those in remission.

In chapter 2 we used a unique approach integrating different omics datasets in a machine learning model. To the best of our knowledge, we are the first to combine and integrate datasets from the fecal microbiome (both bacteriome and mycobiome), proteome and metabolome and the metabolomes of urine and plasma. We corroborated previously observed differences between active disease and remission, validating our approach. By integrating the different datasets in a machine learning model, we revealed intercompartmental interactions between microbes, proteins and metabolites that would otherwise not have been detected. *Ruminococcaceae* and *Faecalibacterium* were identified as microbial key players in the alterations between active disease and remission. Pathway analysis showed altered purine metabolism between active disease and remission, which was independent of thiopurine use. Changes in purine metabolism were confirmed in a public single cell sequencing dataset obtained from intestinal biopsies of pediatric Crohn's disease patients and controls.

The ulcerative colitis cohort described in chapter 3 was smaller than the Crohn's disease cohort, here we only included microbiomics and metabolomics datasets. Initially we aimed to include a proteomics dataset in this study as well. However, due to the small sample size we gained many missing values which could not correctly be imputed. This reduced robustness made us decide to exclude this dataset from our study.

In ulcerative colitis, the purine metabolism was also altered between patients experiencing active disease and those in remission. Other papers also showed the importance of a bacterial-host purine axis for immune system function and wound healing [13–15]. Future research is needed to follow up on the purine metabolism in inflammatory bowel disease and elucidate on the exact mechanisms of action. Additionally, we found differences in glutamate metabolism, arginine and proline metabolism, glutathione metabolism, pantothenate and Coenzyme A metabolism, the urea cycle, and the TCA cycle between active disease and remission.

Although in both chapter 2 and 3, we only included patients with relatively mild forms of disease and could not capture the wide clinical spectrum of inflammatory bowel disease by distinguishing between subtypes and locations of inflammation, we were able to show that there are major differences in microbiome, metabolic and immune functioning, on both a local and systemic level, between patients with active disease and those in remission. In addition to pointing out the differences between active disease and

remission, our studies also elucidate on the differences and similarities between ulcerative colitis and Crohn's disease. For example, in both disease forms purine metabolism was affected. A major difference in metabolic manifestation between ulcerative colitis and Crohn's disease is that the fecal metabolome is more profoundly altered in active Crohn's disease, while more differences in the urinary metabolome where observed for active ulcerative colitis. Further research is needed to elucidate the complex pathways in both CD and UC and the role of the gut microbiota.

It is important to note that omics studies remain observational and descriptive. To move beyond associations, mechanistic studies are needed to unravel the exact functioning of certain pathway and interactions. For example, when observing a positive correlation between a metabolite and a bacterial species, it remains unclear if the alterations in metabolite production are the cause or the consequence of alterations in microbial abundances. A positive association between a metabolite and a species could indicate that the metabolite promotes the growth of that species, but also that the species produces that metabolite. Alternatively, this correlation might be the consequence of a third factor involved. *In vitro* experiments are necessary to study direct interactions from metabolite to microbe and *vice versa*. In **chapter 2,** we studied to that end a specific microbe-metabolite interaction: the effect of the inflammation associated protein lactoferrin on the growth of the *Collinsella aerofaciens* (phylum Actinobacteria) in an *in vitro* culture. Although we observed a negative correlation between lactoferrin and *Collinsella* in our patient study, adding lactoferrin to the growth medium seemed to have a positive effect.

Host-microbe interactions are part of complex networks and are seldom unidirectional, highlighting the need for more complex models resembling the gut environment as well [17]. Human disease models including organoids and gut-on-a-chip systems such as HuMIX and GUMI, can be employed for this purpose [18–20]. To reduce complexity, well-characterized synthetic communities can be included to study the perturbations of another species [21]. However, not all microbial species are culturable yet, and the complexity of the gut environment cannot be fully captured in an *in vitro* model. Therefore, cohort studies remain of importance too. Future longitudinal studies in patients and in the general population are needed to elucidate whether the observed altered pathways and interactions are underlying the causes or consequences of the disease. Advanced sequencing techniques and bioinformatics pipelines will allow for a more

complete picture of the genetic diversity of a microbiota community and its functioning, and thus shed light on the variability within strains of the same species [22].

In **chapter 4,** we reviewed the role of serotonin in the gut. Serotonin is an example of a compound that connects the microbiome with the host, playing a role in both health and disease, including inflammatory bowel disease. Serotonin can be synthesized by both the host and bacterial species inhabiting the gut. Additionally, bacteria can modulate the availability of tryptophan from which serotonin is made, and *vice versa,* host serotonin metabolism affects microbiota composition and functioning. In the gut, serotonin functions as neurotransmitter regulating the enteric nervous system, as signaling molecule activating the immune system, and promoting epithelial integrity.

Serotonin's multifaceted role in gut health makes it an interesting target for treatment. Animal and clinical studies have been performed investigating the potential of altering serotonin metabolism. In animal models, the inhibition of serotonin production in enterochromaffin cells (but not in neuronal cells) improved disease state and reduced inflammatory responses [23–25]. Direct blockage of serotonin synthesis was also evaluated in humans. The compound *para*-chlorophenylalanine was shown to be effective in treating carcinoid syndrome and emesis induced by chemotherapy [26–28]. However, treatment with *para*-chlorophenylalanine has also been linked to depression and alterations of the central nervous system. Therefore, the development of this compound for therapeutic use has been aborted and studies in inflammatory bowel disease have not been performed [25,27]. Selective serotonin reuptake inhibitors (SSRIs), prescribed for depression, exhibit anti-inflammatory properties and affect the gut microbiota [29]. However, randomized trails evaluating the efficacy of SSRIs in patients with inflammatory bowel disease are lacking. Many compounds are described targeting serotonin signaling by modulating the serotonin receptors, but evaluating animal studies on the success of these compounds is complicated due to the high diversity in serotonin receptors, the differences in experimental design, and clinical studies in patients with inflammatory bowel disease are yet to be performed.

In **part II** of the thesis titled **"Sporobiota in the gut in health and disease",** we focused on bacteria with specific developmental characteristic, i.e. the ability to form endospores, and what their role is in inflammatory bowel disease. In **Chapter 5** we reviewed the literature and gave an overview of what is known about spore formers and their functioning in the gut. By giving an overview of the sporulation processes, spore properties, and germination processes in the context of the gut, we contributed to a

better understanding of this specific group within the gut microbiota. Insight into the sporobiota will contribute to more knowledge about the microbiome in general and to the development of new therapies. Spores already show therapeutic potential as probiotics, vaccine vehicles and drug delivery systems. Recently, the first spore products extracted from fecal matter have been approved by the United States Food and Drug Administration (FDA), and more are currently under investigation [30]. Until now, these purified spore products have been primarily used in the treatment and prevention of recurrent *Clostridium difficile* infection, showing high efficacy and safety [30–34]. The use of this type of spore products in inflammatory bowel disease needs further investigation.

In **Chapter 6,** we proposed and validated the sporulation potential as a measure of the potential of an individual's microbiota to sporulate based on the presence of sporobiota-associated genes in their fecal metagenome. We illustrated the use of the sporulation potential in two public datasets and showed that there was no difference between healthy controls and inflammatory bowel disease patients, but that Crohn's disease patients have a higher sporulation potential than ulcerative colitis patients. Larger metagenomic datasets are needed to be assessed to confirm this. The use of sporulation potential might provide an opportunity to identify clinical conditions in which low abundance of spore formers and spores play role. These patients might benefit from sporobiota restoring therapies such as fecal microbiota transplantation (FMT) or recently developed spore products. FMT in patients with ulcerative colitis showed that sustained remission could be associated with shifts in microbiota composition and increased butyrate production, while relapse was associated with Proteobacteria, Bacteroidetes and *Ruminococcus gnavus* [35]. To identify these clinical conditions new studies are not necessary required since existing whole genome sequencing datasets can be reused. In addition, the sporulation potential might be a useful measure in the selection of successful donors for FMT studies or for preparation of spore products from fecal matter.

In **Chapter 7,** we investigated a widely cited story on the origins of fecal transplantation of which the therapeutic effect was attributed to *Bacillus subtilis* spores. We showed that it is unlikely that this story is true, which would mean that dromedary feces is of any therapeutical benefit due to the presence of *B. subtilis* spores, as the amount of *B. subtilis* spores in dromedary feces is too low. This chapter highlights the need for us, scientists, to remain critical at all times regarding literature data even if these, seemingly, have been generally accepted and are widely cited.

It is important to note that this thesis only touches upon a fraction of the complexity of the gut microbiome. The microbiome in our intestines is more diverse than the bacteriome and mycobiome studied in this thesis and also includes viruses, phages, protozoa and archaea. In addition, not only do the gut microbiota deserve attention in the context of inflammatory bowel disease, also the oral microbiome is being revealed as important in inflammatory bowel disease [36].

Conclusion and outlook

In conclusion, this thesis contributes to the understanding of the microbiome, and in particular the sporobiome, in inflammatory bowel disease. The findings presented here and in previous research underscore the significant role that microbes play in inflammatory bowel disease. This makes the microbiota an interesting target for treatment. Insight in the metabolites and microbial molecules produced by inflammatory bowel disease associated microbes is crucial for developing microbiome-based therapeutic. These therapies aim to impact the downstream signaling pathways relevant to disease pathogenesis by administering or inhibiting bioactive microbiome-modulated molecules [37]. The above mentioned FMT, treatments interfering with serotonin metabolism in the gut, and recently developed spore products are all examples of targeting the microbiota-host interaction in the gut. Additionally, numerous other strategies are available or under investigation, including dietary interventions [38,39] and pre-, pro and synbiotic treatments [40,41]. Given the patient specific nature of inflammatory bowel disease manifestations, a personalized approach guiding treatment choice would potentially enhance the effectiveness of both existing and to be developed therapeutic strategies [37,42,43].

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