Microbial perturbations in Crohn's disease

Patients with CD suffer from a chronic inflammatory disease with an alternating disease course. Several gastro-intestinal and extra-intestinal symptoms can lead to a severe disease burden with a substantial negative impact on the quality of life.[1] Consequently, CD can lead to reduced societal participation, accompanied by mental disorders and a high economic burden.[2] CD is an immune-mediated inflammatory disease with a multifactorial predisposition and pathophysiology.[1] The multifactorial nature makes the disease phenotype highly variable, difficult to predict, and challenging to treat. Scientific research may improve clinical care by further understanding of factors and mechanisms impacting disease course and treatment response.

The past years, it became increasingly clear that microbial perturbations play a substantial role in CD onset and disease course.[3–5] However, the exact role in disease pathophysiology and the potential interaction with common inflammatory bowel disease (IBD) drugs is not clear. Therefore, this thesis aimed to expand the functional knowledge on interactions between the microbiota, IBD drugs, and the intestinal mucosal barrier in CD.

Main findings of this thesis

Part I
- Microbiota-barrier interactions -

Previous research on the intestinal microbiota in IBD found several bacterial taxa that are associated with IBD. One species that has frequently been linked with CD and exacerbations is *Bacteroides fragilis*.[4,6,7] In addition, the *B. fragilis* toxin (Bft), produced by toxigenic strains, has been shown to disrupt the epithelial barrier via cleavage of the adherens junction E-cadherin.[8–10] Still, the role of *B. fragilis* and its virulence factors in CD activity is not clear. In **chapter 2**, we investigated the prevalence and abundance of *B. fragilis* and its virulence factors Bft and Ubiquitin comparing fecal samples from patients with active CD and remission. We further aimed to examine the impact of *B. fragilis* culture supernatant on epithelial barrier function.

First, we found a 16 % higher prevalence of *B. fragilis* in fecal samples of CD patients experiencing an exacerbation (n = 88) as compared to fecal samples collected during remission (n = 93). However, the virulence factors Bft and ubiquitin were only present in a minority of samples and equally distributed between groups. The higher prevalence of *B. fragilis* indicates a potential role in CD exacerbations, but the underlying mechanisms remains to be elucidated. Next, we conducted an *in vitro* study, investigating the complete culture supernatant of six different *B. fragilis* strains. Unexpectedly, we found an increase and not a decrease in epithelial resistance of Caco-2 cell monolayers after 24 hours incubation, which was only observed with supernatants from different *bft*-positive strains. Since the only common differ-

ence between the *bft*-positive and *bft*-negative strains was the *bft* pathogenicity island, we hypothesize that the resistance increasing effect may be induced by the genomically *bft*-neighboring metalloprotease II. In our opinion, the unexpected resistance-increasing results stress the importance to study microbial contributions to disease pathophysiology in context. In line with this conclusion, we aimed to study the impact of all intestinal microbial products on barrier function in **chapter 3**. Therefore, we investigated the impact of the complete fecal microbial secretome in fecal water of twelve well-phenotyped CD patients and six healthy controls on epithelial barrier resistance in Caco-2 monolayers and on mucin degradation. Comparing endoscopy-classified exacerbation and remission samples, the epithelial resistance was significantly decreased by three out of six remissive samples, without disrupting the paracellular barrier as measured by FITC-D4 permeation. In addition, the secretomes of CD patients led to significantly higher *in vitro* mucin degradation as compared to those from healthy controls. Based on our findings, the fecal microbial secretome of CD patients might have a larger negative impact on the intestinal mucus layer than on the epithelial barrier. However, the observed mucus degradation might also demonstrate potential microbial adaptations to the CD-specific mucus composition and increased mucin production. Further research is needed to clarify these effects. In **chapter 4**, we described the development of IBD patient derived colonic organoids as a more physiological model to evaluate epithelial barrier function. Intestinal organoids are superior to conventional cell line monolayers, since they comprise multiple cell types, better recapitulate the human physiology and architecture, and when based on adult stem cells, can preserve disease and patient specific characteristics. Finally, we explored the immunoregulatory effects of feces-derived bacterial membrane vesicles in **chapter 5**. To this end, we isolated membrane vesicles from the same fecal samples as in chapter 3. Interestingly, membrane vesicles from CD patients induced less release of the pro-inflammatory cytokine TNF-α from THP-1 macrophages, whereas vesicles from healthy controls and remissive CD patients induced a higher release of the anti-inflammatory cytokine IL-10. Furthermore, vesicles from CD patients were less coated by IgA antibodies, which makes the recognition by the host immune system more difficult. Together, these findings suggest an immunomodulatory potential of fecal bacterial vesicles, which seems to differ between CD and healthy individuals as well as between different disease activity states.

Part II
- Microbiota-drug interactions -

Exploring the rising research field of pharmacomicrobiomics within IBD, **chapter 6** provides a comprehensive review about the current knowledge and pitfalls. In general, bi-directional drug-microbe interactions seem relevant for the treatment of IBD.

However, the overall evidence varies largely between common IBD drugs. Heterogenous study designs and different patient populations or disease models make it challenging to compare the study outcomes and draw firm conclusions for clinical translation. Further research and more consistent findings are needed to ultimately implement knowledge on pharmacomicrobiomics in IBD clinical practice to improve treatment success and quality of life. To add to the current knowledge, we investigated the bi-directional drug-microbiota interactions of the commonly used thiopurine drug 6-mercaptopurine (6-MP) and a promising new drug, tofacitinib, in **chapter 7**. To this end, we cultured fresh fecal samples from five CD patients and five healthy controls in the large mechanical *in vitro* colon model TIM-2[11] and exposed the fecal cultures to the test drugs for 72 hours. No drug-induced alterations on the microbiota composition were detected. In contrast, both drugs disappeared rapidly from the cultures, indicating drug interactions with the microbiota and subsequent potential effects on drug availability for the host. However, we noted a substantial interaction between the drugs and the model system, which emphasizes the critical evaluation of the suitability of the chosen model system for the respective experiment. Therefore, no firm conclusions could be drawn. In **chapter 8**, we aimed to include functional microbial outputs in another study on drug-microbiota interactions. Therefore, we established a simplified *ex vivo* model system, which includes fecal cultures in a 96-deep well plate in a well-controlled anaerobic environment. We examined the effect of budesonide, 6-MP, and tofacitinib on both, the microbiota composition and proteome using fecal cultures from five CD patients. Although all study drugs did only lead to minimal alterations in the microbiota composition, 6-MP and tofacitinib did significantly alter the microbial proteome. The study clearly showed that IBD drugs likely have a larger impact on microbial function than on microbial composition. Yet, the tools to analyze microbial proteomics need further development.