Microbial perturbations in Crohn's disease

10

IMPACT PARAGRAPH

The prevalence of inflammatory bowel diseases (IBD) is rising worldwide. Currently, about three in 1,000 people in Western countries and up to one in 1,000 people in Asian countries suffer from IBD.[1] This chronic disease is characterized by an alternating disease course with phases of active disease (exacerbations) and phases of remission. During exacerbations, patients can experience intestinal symptoms, including abdominal pain, diarrhea, and bloody stool. A subgroup (30-50 %) also suffers from extra-intestinal symptoms, such as, arthritis, and uveitis. IBD includes Crohn's disease (CD) and ulcerative colitis (UC).[2,3] While CD can occur in the entire gastrointestinal tract and is characterized by skip lesions affecting all layers of the intestinal wall, UC is manifesting as distal to proximal continuous inflammation, which is restricted to the mucosal layer of the large intestine.[2,3] The occurrence of exacerbations and complications vary largely between patients.[4] Since a cure is yet not possible, immunosuppressant drugs to ameliorate symptoms and inflammation are currently the most important treatment.[5,6] However, treatment response is highly individual and unpredictable, which makes the treatment choice very challenging for the attending gastroenterologist. Considering the disease impact for the individual patient, IBD can significantly decrease the quality of life and impair societal participation.[7] Besides, IBD leads to high direct and indirect health care costs. To help combatting the suffering of IBD patients as well as reduce the associated costs for society, scientific research is highly needed to improve the prediction of disease course as well as treatment success.

In-depth research has unequivocally demonstrated the crucial roles of the intestinal microbiota and the intestinal barrier function in the pathophysiology of IBD and the occurrence of exacerbations. Nevertheless, the current understanding on these contributors remains incomplete and insufficient to improve IBD care. Consequently, studies delving into the intricate workings of the intestinal microbiota and the functionality of the intestinal barrier hold immense potential to augment pertinent knowledge and, ultimately, alleviate patient distress by improving the management of IBD. The research presented in this thesis makes a valuable contribution towards bridging certain gaps in knowledge.

The intestinal microbiome is a very complex ecosystem, of which not only the composition but also its metabolites have large impact on health and disease. We employed a combination of observational and experimental research methods to gain a deeper understanding of the role of the commensal intestinal microbiota in Crohn's disease (CD). First, we focused on further understanding of a bacterial species that often has been found to be associated with CD, *i.e. Bacteroides fragilis*. Additionally, experimental studies were conducted to explore the interaction between different bacterial products and the intestinal barrier, as well as immune

cells. Moreover, we investigated the bidirectional effects of common IBD drugs in individual host-derived microbiota.

Scientific impact

The different studies presented in this thesis underscore the importance of focusing on functional parameters when studying the contribution of the intestinal microbiota to CD pathophysiology and treatment. Previous research predominantly focused on alterations in microbiota composition when investigating, for instance, exacerbation and remission, or the impact of medical drugs.[8–10] In **chapter 2**, we combined an observational cross-sectional study with an experimental study. Although we found a higher prevalence of *B. fragilis* during exacerbations, we did not detect a negative effect on *in vitro* barrier function, thereby questioning the pathophysiological relevance of the observed increase in *B. fragilis* prevalence. Moreover, our findings demonstrate that differences or alterations in prevalence and abundance of microbial taxa may not directly indicate positive or negative effects to the host. Still, further research is necessary to understand the functional role of potentially harmful intestinal species, including *B. fragilis*, in CD pathophysiology.

In **chapter 6**, we reviewed the evidence on drug-microbiota interaction on various IBD drugs. Here, we also mainly found observational studies investigating alterations in microbiota composition in response to a certain drug. Unfortunately, the study results were often incomparable or contradictory. In addition, observed alterations in the microbiota composition only allow speculations about their direct impact on host physiology, which may not reflect the actual situation. As we showed in **chapter 7**, IBD drugs are able to induce multiple functional alterations to the microbiota, while the composition was almost not affected. These functional alterations may subsequently interfere with the host immune system or metabolism and may directly impact the disease state or drug response in IBD. In line with studies on other drug classes,[11,12] our findings clearly demonstrate that microbial function matters. However, functional studies on drug-microbiota-host interactions are yet scarce. Therefore, experimental and translational studies are crucial to increase the knowledge on the functional role of the microbiota perturbations in IBD as well as their interactions with immunosuppressant drugs. Fortunately, the awareness on drug-microbiota interactions is rising. To support the scientific community in designing meaningful studies on drug-microbiota interactions, we formulated specific recommendations in **chapter 6**. These recommendations include the improved definition of patient groups by considering inter-individual microbial differences, the promotion of longitudinal clinical studies combined with experimental studies, and the combi-

nation of complementary read-outs to elucidate the functional interactions between the microbiota and the patient at once. Combining forces, the scientific community may be able to obtain meaningful insights, which can timely lead to specific clinical implementations, ultimately improving personalized treatment and supporting the attending gastroenterologists in their treatment choice.

Besides emphasizing the importance of microbial function alongside composition, we concluded that the physiological context matters. Many previous experimental studies focused on specific factors when exploring the role of microbes and barrier function in IBD. For instance, a few studies investigated the isolated or recombinant *B. fragilis* toxin on its impact on the epithelial barrier using immortalized intestinal cell lines. They found a strong barrier disrupting effect with a clear underlying mechanism.[13,14] However, when we applied complete *B. fragilis* culture supernatant from toxin-carrying strains to epithelial cell lines and CD patient-derived organoids, we did not observe any disrupting effect. On the contrary, we measured increased epithelial resistance in colonic cell line monolayers. Similarly, in the field of pharmacomicrobiomics, large screening studies focused on interactions between individual cultured bacterial strains and different medical drugs, discovering numerous bi-directional interactions.[15,16] However, when we subjected different IBD drugs to complete fecal cultures of CD patients and healthy individuals in **chapter 7 and 8**, we observed only minor effects on microbial composition. By combining these observations from our experimental studies, we can emphasize the importance of investigating microbial function within a relevant physiological context. Providing a physiological environment as much as possible is vital for obtaining meaningful results. Besides these insights, we contributed with discussing current and innovative study methods and providing specific recommendations for future study designs.

Finally, the work in this thesis has generated several relevant model systems and insights that can now be used by researchers in the field of IBD and beyond. First of all, an important foundation to the new research line on pharmacomicrobiomics within Maastricht University Medical Center+ was laid. Meanwhile research on the bi-directional interactions between the microbiome and drugs within our organization has been expanded to different types of diseases, including colorectal and breast cancer and initiated the collaboration of researchers from the fields of medical microbiology, pharmacology, gastroenterology, and surgery. Within this research line, we established an anaerobic high-throughput fecal culture assay. This assay allows to examine the interaction of different medical drugs, but also with other compounds, with individual patient-derived fecal samples by analyzing alterations in microbiota composition in combination with function, as described in **chapter 7**. In the near future, analyses of drug metabolism can be added as read-out to obtain

insights into microbiome-mediated drug metabolism. Furthermore, this model can now be applied by fellow researchers in other medical fields and easily be adapted to patient microbiota and drugs from other diseases thereby paving the way for precision medicine.

Furthermore, we established a 3D colonic organoid model using mucosal biopsies from IBD patients and embedded the work-up into the clinical infrastructure. In order to study epithelial barrier function, we established a fluorescent assay using confocal microscopy in combination with downstream analyses, including protein localization, quantification, and expression. Together with collaborating research groups within our organization, this disease-specific model can further be modified to monolayer organoids growing on a villus-shaped membrane and in co-culture with additional patient-derived specimens, including intestinal microbes, and immune cells.

The projects on pharmacomicrobiomics as well as on intestinal barrier function included patients from the comprehensive IBD South Limburg biobank cohort. Before, this biobank was used for observational studies. The work of this thesis introduced experimental research and functional read-outs to this cohort, which provides new opportunities to biobank research in our department and facilitates more translational insights. To offer more extensive experimental research opportunities, biobank sampling should be equipped with innovative sampling methods, which allows the conservation of relevant biological materials, such as viable microbes and stable metabolites, for future research questions and analyses.

Societal impact

All studies presented in this thesis were conducted with the overarching goal of expanding the knowledge to improve clinical care for IBD patients and alleviate their suffering. We contributed to a better understanding of the role of the intestinal microbiota in IBD disease course, intestinal mucosal barrier function, interaction with the tissue immune system, and medical drug treatment success. By emphasizing the importance of functional microbial characteristics and the physiological context, we guide the research field towards a promising direction which may timely lead to implementable tools for optimizing diagnostics and treatment selection. Using the new knowledge, diagnostic tools can be developed to examine specific functional microbial characteristics. Herein, the pharmaceutical industry can benefit from the new research direction and develop tradable clinical tools for functional microbial screening. For instance, screening tools to determine the individual microbial interaction with recommended IBD drugs can evaluate the personal suitability and may predict treatment response and tolerance. Subsequently, these tools can facilitate

the work of attending gastroenterologists by simplifying treatment choice using evident indicators. Furthermore, by the implementation of functional microbial diagnostic tools, the role of clinical laboratories will be expanded and will require closer collaboration with bioinformaticians, who maintain artificial intelligence-based algorithms that interpret the diagnostic results and provide clear recommendations to the attending gastroenterologists. For IBD patients, comparable tools offer non-invasive diagnostic techniques, since the intestinal microbiota can be evaluated from a stool sample. Ideally, this may avoid or minimize the need for multiple venipunctures to assess biochemical biomarkers or endoscopic examinations for IBD diagnosis and disease monitoring. Apart from the convenience it provides to patients, an improved personalized approach to drug treatment selection increases the likelihood of timely treatment success. Thereby, stool-based diagnostics can save time and costs, resulting in positive financial effects for the health care system by reduced direct and indirect health care expenses. Besides the anticipated screening tools, future insights into the functional effect of microbes on disease activity or treatment response may promote the biopharmaceutical industry to design microbial therapies to complement or improve current drug treatment. For instance, targeted adjustments of the microbial function by prebiotics, (genetically engineered) probiotics, bacteriophages, or enzyme modulators may contribute to limit intestinal inflammation and/or improve treatment response. Together, the anticipated scientific and industrial innovations will improve patient's quality of life more rapidly and reduce the risk to develop complications due to prolonged inflammatory activity.

Conclusion

This thesis contributes to scientific knowledge and improvement of IBD care by providing insightful functional results, valuable suggestions for future research directions, and a call to focus on functional microbiota studies to enhance the understanding and treatment of IBD. By communicating the findings of this thesis in renowned international journals with open access, the international research community can continue to expand knowledge and its clinical applications. Subsequent *ex vivo* and clinical studies will likely facilitate the development of non-invasive clinical tools that support clinicians in diagnosing and treating IBD.

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