Role of intestinal microbiota in cardio-metabolic diseases

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SUMMARY

Chapter 1 serves as a general introduction illustrating the role of intestinal microbiota in cardiometabolic diseases. The main underlying cause of cardiometabolic diseases is atherosclerosis, which is mainly caused by dyslipidemia and inflammation. The role of intestinal microbiota on the local and systemic alternation of the immune system is discussed in this chapter. Furthermore, this chapter focusses on the technical aspects of cardiometabolic and microbiota research. Therefore, bone marrow transplantation (BMT), a widely used tool to study gene function in atherogenesis, and Next Generation Sequencing, a tool for profiling the intestinal microbiota, are discussed in detail.

Experimental bone marrow transplantation is a relatively easy and effective strategy for assessing the role of specific immune related genes in pathophysiological mechanisms in mice. Remarkedly, the application of BMT in obesity research is hampered by the significant reduction in high-fat diet (HFD)-induced obesity, which is caused by the BMT procedure. In **Chapter 2** we characterized the metabolic tissues that may be affected by the BMT procedure and that may impair the HFD-induced response. Taken together, we concluded that the BMT procedure has multilevel effects on the organism and affects metabolically important organs. These multi-level effects are associated with metabolic abnormalities that involve both white adipose tissue (WAT) and pancreas dysfunction in response to a HFD. Therefore, the metabolic effects of BMT cannot simply be neglected and should be seriously considered in study design and interpretation of the data.

In addition to the metabolic side effects of BMT as characterized in **Chapter 2,** conditioning regimens of BMT acutely induce systemic inflammation, intestinal damage and result in shifts in the gut microbial composition. As alterations in the intestinal microbiota community are associated with both obesity and immunity, this could explain the metabolic side effects of BMT. In **Chapter 3,** we aimed to investigate whether, post-BMT, the peripheral immune system is modulated as a direct consequence of BMT induced alterations in the gut microbiota. We investigated in mice the effect of BMT on the response patterns of splenocytes and peritoneal macrophages to various pathogenic stimuli as markers of the extra-intestinal immune system. Splenocytes are a mixed population of immune cells and thus represent responses from both the adaptive and innate immune system, whereas peritoneal macrophages represent the innate immune system. The potential role of microbiota in the response patterns of splenocytes and peritoneal macrophages was investigated by co-housing control mice with BMT-treated mice and by transfer of cecum content to germfree mice. We show that 24 weeks post-BMT, splenocytes but not peritoneal macrophages display increased cytokine response patterns upon ex-vivo stimulation with various pathogens as compared to untreated controls. The pattern of BMT-induced cytokine responses was transferred to splenocytes, and not to peritoneal macrophages, of healthy controls via co-housing and transferred to germfree mice via transplantation of cecum content. Thus, gut microbiota

increase the cytokine responses pattern of splenocytes after BMT. This phenotype can be transferred to splenocytes of healthy controls by co-housing or to germfree mice via transfer of cecum content, indicating that they are independent of BMT-induced intestinal damage and microbial leakage.

In **Chapter 4,** we aimed to investigated the effects of oral administration of a single bacterial species on metabolic and immunological markers of atherogenesis in mice. Previous mouse studies showed that oral administration of the intestinal commensal bacterium, *A. muciniphila*, protected against HFD-induces obesity and improved metabolic and immunological markers such as glucose tolerance, and hypercholesterolemia and cytokine secretion by different immune cells. However, the effects of *A. muciniphila* on atherogenesis were not investigated yet. We investigated the effects of *A. muciniphila* on lipid metabolism, immunity, and cuff-induced neointima formation in hyperlipidemic APOE*3-Leiden (E3L).CETP mice. We determined the effect of 4 weeks oral *A. muciniphila* administration in E3L.CETP mice on 1) plasma lipid levels, 2) the immune response by measuring portal vein lipopolysaccharide (LPS) levels, mesenteric lymph node (mLN) immune cell composition and ex vivo responses of circulating leukocytes to LPS, and 3) neointima formation and composition. We found that administration of *A. muciniphila* lowered hyperlipidemia in hypercholesterolemic E3L.CETP mice and had immunomodulatory properties. As both hyperlipidemia and immune responses are involved in the pathogenesis of atherosclerosis, these observations suggest that *A. muciniphila* has anti-atherogenic potential. However, in contrast to our hypothesis, *A. muciniphila* was unable to ameliorate atherosclerosis in our cuff-induced neointima formation model, suggesting that the anti-atherogenic effects of *A. muciniphila* were not sufficiently strong in this mouse model.

In several chapters of this thesis, monitoring the composition of intestinal microbiota and compositional shifts in intestinal microbial communities after an experimental intervention were important experimental read outs. In the field of microbiota research, sequencing a relative small part of the 16s rRNA gene, the so called V4 region, is a widely used method for monitoring the microbial composition. In **Chapter 5,** we aimed to assess whether sequencing the full-length 16S rRNA gene affected the results and interpretation of a dietary intervention compared to sequencing only the V4 region of this gene. To compare the effects of the dietary intervention measured on either the PacBio platform for sequencing the full-length 16S rRNA gene or Illumina MiSeq platform for sequencing the V4 region, we performed taxonomic analysis and diversity analysis. To test this, mice were fed a diet without and with the prebiotic inulin. From cecum samples, two primary data sets were generated: 1) a 16S rRNA full-length data set generated by the PacBio platform; 2) a 16S rRNA V4 region data set generated by the Illumina MiSeq platform. A third derived dataset was generated by in silico extracting the 16S rRNA V4 region data from the 16S rRNA full-length PacBio data set. Analyses of the primary and derived 16S rRNA V4 region data indicated similar bacterial abundances, and α- and β-diversity. However, comparison of the 16S rRNA full-length data with the primary and derived 16S rRNA V4 region data, revealed differences in relative bacterial abundances, and α- and β-diversity. We conclude that sequencing the full-length 16S rRNA gene provides a different view regarding bacterial relative abundance, in-sample diversity and in in-between-sample diversity, as compared to V4 sequencing regardless of sequence

analysis platform. This clearly has implications for interpretation of biological data after a dietary intervention.

In **Chapter 6,** we describe the causal role of intestinal microbiota in host metabolism, immunity and pathophysiology of atherosclerosis. The studies described in this thesis showed that oral administration of the bacterial species *A. muciniphila* affected host lipid metabolism. In addition, our cohousing experiments after BMT indicated that intestinal bacteria play a crucial role in systemic immune responses. Furthermore, we provided critical insight in two generally accepted and widely applied research tools in cardiometabolic research and microbiome research, BMT and 16s rRNA sequencing, which should be taken into account when designing and interpreting results from experiments using these tools.