English summary

Multiple sclerosis

Multiple sclerosis (MS) is an autoimmune, demyelinating and neurodegenerative disease of the central nervous system (CNS) characterized by chronic inflammation and blood-brain barrier (BBB) dysfunction. During disease pathogenesis, autoreactive immune cells cross the inflamed BBB and enter the CNS where they recognize and attack myelin antigens. The typical age of disease onset is between 20 and 50 years and MS is three times more predominant in women than in men. Three main clinical forms of MS exist: relapsing-remitting MS (RR-MS), affecting approximately 85% of MS patients, secondary progressive MS (SP-MS) and primary progressive MS (PP-MS). MS is clinically diagnosed by combining neurological and cognitive evaluation, clinical history of the patients, magnetic resonance imaging (MRI) and/or on the presence of biomarkers within the cerebral spinal fluid (CSF).

It is believed that MS arises from a combination of genetic, environmental and lifestyle factors. However, the underlying causal factors for MS still remain unknown. To date, there is no cure for MS but a number of treatments are available to reduce inflammatory relapse frequency and accumulated disability in RR-MS. Despite being moderately effective in RR-MS, this disease-modifying therapies (DMTs) approach has relatively little benefit in progressive MS and may lead to serious side effects. Therefore, there remains a high and unmet need for the discovery of more specific and safer drugs, potentially aimed at modulating disease progression.

In this thesis we investigated novel mechanisms underlying MS pathology focusing also on the potential therapeutic application of our findings.

Blood-brain barrier in health and disease

The BBB is a selective barrier composed by specialized brain endothelial cells (BECs) tightly connected through specific proteins. The major function of the BBB is to maintain brain homeostasis via supplying nutrients and excluding waste products from the CNS. The BBB also limits the passage of cells and molecules from the systemic circulation into the CNS and vice versa. Importantly, BBB dysfunction is a major hallmark of MS pathology and it is an early event during the course of disease. During an inflammatory attack, activated immune cells and CNS-resident cells secrete pro-inflammatory molecules which alter the structural architecture and function of the BBB, thereby allowing the traversal of lymphocytes and monocytes into the CNS.

In this thesis, we have investigated inflammatory-driven molecular mechanisms and signaling pathways involved in BBB dysfunction. Importantly, during development, the function of blood vessels is determined by specific signaling mechanisms, like the Notch signaling pathway which is a highly conserved pathway involved in cell-cell communication and vascular development. Nevertheless, whether the Notch signaling is involved in the specialized barrier function of BECs during health and neuro-inflammation has not been
studied before. In chapter 2, we aimed to address this question and show that inflammation alters the Notch pathway and promotes BEC dysfunction, thereby highlighting the functional importance of the conserved Notch signaling pathway in control of the brain endothelial barrier in health and disease.

In order to fulfill the needs of the CNS tissue, the BECs forming the BBB need to maintain their specialized phenotype and function. For a long time, scientists have believed that adult vascular endothelial cells were unable to de-differentiate but this hypothesis has been proven wrong. Indeed, like embryonic endothelial cells, also adult endothelial cells have the capacity to de-differentiate into mesenchymal cells through a process called endothelial-to-mesenchymal transition (EndoMT). EndoMT is characterized by the loss of endothelial-specific markers and by the acquisition of mesenchymal and stem cells-like properties. Nevertheless, in contrast to its well-established role in other tissues and diseases, the first reports of EndoMT in the CNS vasculature during a particular brain disorder dates back to 2013. Since then other reports indicated EndoMT in other brain diseases such as bacterial meningitis and brain tumors. Yet, the role of EndoMT of the BBB upon neuro-inflammation, as seen in MS, remains poorly understood. In chapter 3 we showed that chronic inflammation of human BECs promotes EndoMT and BBB dysfunction. Moreover, we observed for the first time EndoMT features in MS brain vasculature, suggesting that EndoMT might represent a novel pathological mechanism underlying BBB dysfunction during MS pathogenesis. Taken together, our results provide a better understanding of the molecular mechanisms underlying BBB dysfunction during neuro-inflammation and highlight the potential beneficial impact of novel and promising MS therapeutic strategies targeting the BBB.

**Inflammation and resolution of inflammation**

Importantly, a proper inflammatory reaction plays a beneficial role in the body’s intrinsic response against damaged cells and pathogens. In a healthy situation, upon the onset of inflammation, the process known as resolution of inflammation is immediately activated in order to successfully return to homeostasis and prevent chronic inflammation. The resolution phase of inflammation is mediated by newly discovered metabolites called specialized pro-resolving lipid mediators (SPMs). Currently, four different families have been classified: lipoxins (LXs), resolvins (RVs), protectins (PDs) and maresins (MaRs). Generally, SPMs exert their functions by decreasing the secretion of pro-inflammatory mediators, reducing leukocyte recruitment and transmigration to the inflammatory site and enhancing the phagocytosis of apoptotic cells and tissue debris.

Despite accumulating data suggesting an altered resolution process in several chronic inflammatory diseases and the protective role of SPMs in different experimental animal models of such diseases, the question remains whether MS is associated with an impaired resolution response, which is of high interest in view of potential future clinical applications. On the basis of this scenario, in chapter 4 we provided first evidence of peripheral defects in the resolution pathway in MS patients. Particularly, we showed that each disease subtype
was associated with distinct lipid mediator profiles that significantly correlated with disease severity. Moreover, we described the potential role of SPMs in reducing the activation of monocytes derived from MS patient as well as to counteract inflammation-induced BBB dysfunction and subsequent monocyte transendothelial migration. Based on our findings we propose that specific SPM signatures may be used as novel biomarkers for MS diagnosis and monitoring of disease progression.

Finally, in chapter 5 we evaluated whether LXA₄ treatment represents a novel therapeutic approach to limit neuro-inflammation in experimental autoimmune encephalomyelitis (EAE), a well-established animal model for MS. We showed that LXA₄ administration was able to reduce the severity of the disease in the LXA₄-treated mice, strongly inhibits leukocyte infiltration as well as the inflammatory profile of human activated cytotoxic T cells and finally normalizes the EAE-induced spinal cord lipidome. Our results reveal that SPMs treatment might serve as a novel tool to fight neuro-inflammation and inhibit T cell effector functions.

**Future perspectives and concluding remarks**

During MS, chronic inflammation and BBB dysfunction play a pivotal role in the disease development by promoting immune cell activation and migration into the CNS. In the first part of the thesis we provided evidence for different molecular mechanisms and signaling pathways involved in BBB function during neuro-inflammation. In the second part, we demonstrated impairment in the resolution process in MS patients and highlight the potential use of SPMs as novel biomarkers for MS diagnosis and progression. Furthermore, we investigated and provided evidence on the use of SPMs as new therapeutic tools to reduce neuro-inflammation in the animal model for MS, limit BBB dysfunction and potentially halt MS progression. In conclusion, this thesis reflects that our understanding of BBB dysfunction and resolution process in MS is still evolving. Our work has contributed to broaden the knowledge in these two fields of research, where inflammation represents the common denominator. Finally, it illustrates how interfering with such processes might serve as novel therapeutic avenue in the treatment of MS.