**English Summary**

**Multiple sclerosis**

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system characterized by damage to the myelin, which is the isolating layer of neurons. It affects mainly young adults and is three times more predominant in women than in men. MS can be divided into four sub-types: relapsing-remitting MS (RRMS) which is characterized by attacks which can last from a few days to weeks, followed by a period of partial or full recovery from the symptoms. Secondary-progressive MS (SPMS) is characterized by initial attacks followed by a slow deterioration of brain function. Primary-progressive MS (PPMS) is characterized by the lack of attacks, with increased brain deterioration from the start of the disease. Finally, primary-relapsing MS (PRMS) is characterized by a continuous deterioration of brain function from the start of the disease, but in later stages patients suffer attacks, hence these two last forms cannot be distinguished in early stages of the disease. MS is usually more common in areas around the northern hemisphere and less around the equator which suggests a strong influence of the environment. However, the exact cause of the disease is currently unknown. Studies performed using brain tissue from MS patients have shown the presence of cells from the immune system in the brain. It is believed that these immune cells attack the myelin sheet, consequently reducing the transmission of signals through neurons. This immune cell entry initiates inflammation in the brain, also known as MS lesions, leading to cell and tissue damage. Therefore, this autoimmune response against myelin accounts for the neurological symptoms associated with the disease. Symptoms of MS can include visual disturbance, muscle weakness, difficulties in coordination and balance, numbness or tingling, memory problems, or changes in bowel and bladder function. Other symptoms include cognitive changes, fatigue and mood alterations.

**Blood-brain barrier function in health and disease**

The study of the brain vasculature emerged in the 19th century with the studies of the German scientist Paul Ehrlich. He observed that after injection of dyes into the body of animals, all organs of the body became stained, except the brain. However, Edwin Goldmann, one of Ehrlich students, observed that when he injected the dye directly into the brain, only the brain was stained but the remaining organs of the animal were not. Goldmann could, therefore, illustrate the separation between the brain and the rest of
the body. This separation is possible due to the presence of specific blood vessels in the brain. These blood vessels from the brain maintain a barrier between the brain and the immune system. Therefore, they are known as the blood-brain barrier, acting as a gatekeeper of the brain. The blood-brain barrier is composed by specialized cells that communicate with each other via specific proteins. This close interaction between cells provides a tight barrier, avoiding the entrance of molecules and immune cells from the blood to the brain. Therefore, the BBB guarantees proper neuronal and brain function.

In MS, inflammatory processes lead to an altered barrier function of the blood-brain barrier. This altered function of the brain vessels has a negative effect in MS patients. Therefore, the aim of this thesis was to understand how inflammation alters the function of the blood-brain barrier and how this affects the entrance of immune cells from the blood to the brain.

The function of blood vessels is determined by specific mechanisms that take place during the development of the central nervous system. One of these mechanisms is the Notch signaling. This signaling pathway occurs between neighboring cells and it starts when Notch receptors contact Notch ligands. This pathway has been shown essential for proper development of the blood vessels. However, the role of Notch signaling in blood-brain barrier function, in health and disease has not been studied. We show in chapter 2 that Notch signaling is affected by inflammation of the blood-brain barrier. This phenomenon has a negative effect in the barrier function of the blood vessels of the brain. This study highlights the importance of understanding how blood-brain barrier function is regulated in health and disease.

**Infiltration of immune cells into the brain**

In healthy conditions, the blood vessels in the brain form a barrier and no immune cells can access the brain. However, during diseases such as multiple sclerosis, the cells of the immune system enter the brain because the blood-brain barrier does not function properly. This dysfunction of the blood vessels is mainly due to the inflammation caused by these immune cells that entered the brain, leading to tissue damage.

Importantly, for successful entry into the brain, the cells from the immune system contact the blood-brain barrier via specific proteins that are present in the surface of the blood vessels. These proteins provide the first point of contact for the immune cells, allowing them to attach to and subsequently cross the blood vessels making their way into the brain. Therefore, understanding how this important process occurs in MS is of extreme importance for the development of new therapeutics that reduce the entry of immune cells into the brain of MS patients.
We show in chapter 3 that Notch ligands also play a role during the entry of immune cells into the brain. In healthy conditions, the blood-brain barrier shows a low amount of these ligands in its surface. However, in inflammatory conditions, like seen in MS, the blood vessels increase these surface proteins, helping the immune cells entry into the brain.

In MS, it is still not known why immune cells enter the brain. Some researchers believe that in MS, only the immune cells that recognize brain proteins, like myelin, can enter the brain. However, what regulates the entry of these specific immune cells to the inflamed areas of the brain is still unclear. Therefore, we wanted to investigate if the blood-brain barrier can show these specific proteins to the cells of the immune system. In chapter 4 we show that the blood vessels of the brain can present brain-specific proteins to immune cells. When these immune cells recognize these proteins, they enter the brain more easily than immune cells that do not recognize these brain proteins. This study highlights the important immune regulatory function of the brain vessels in disease initiation.

In MS, not only proteins are involved in the progression of the disease but also lipids. It has been shown that the balance of lipids is also deregulated in MS due to the inflammation in the brain. In chapter 5, we show that some lipids play an important role in the gate-keeping function of the blood-brain barrier. We show that some enzymes, that create lipids in the blood vessels, are produced in high quantities by inflamed blood vessels. As a result, the cells from the immune system enter the brain more easily, since the barrier function of the blood vessels is lost. Interestingly, in inflammatory conditions the blood-brain barrier can secrete these enzymes. In chapter 6 we investigated the potential use of these enzymes as a biomarker for MS. Our preliminary results suggest that the levels of these enzymes are increased in the serum of MS patients with blood-brain barrier dysfunction and decreased in patients that were taking medicines against MS. The development of better biomarkers in MS is essential for better diagnosis, prognosis and treatment options. However, due to the different types of symptoms and different disease progression between patients, biomarker discovery in MS still represents a great challenge.

Future perspectives and concluding remarks
In this thesis, we provide new evidence on how inflammation alters the function of the blood-brain barrier and its consequences for the entry of immune cells into the brain in MS. Our results further demonstrate that understanding the barrier function of brain vessels is still evolving. We demonstrate that different proteins and mechanisms are
important and possibly interconnected in regulating immune cell entry across the brain vessels. This might shed new light in understanding MS. Although current therapies in MS mainly target the entry of immune cells into the brain, patients often suffer from side-effects associated with decreased function of the immune system. Therefore, understanding the complex mechanisms that regulate the entry of immune cells into the brain in MS might lead to the discovery and development of better therapies to reduce barrier dysfunction and the negative immune cell entry to the brain.