The stress-axis in multiple sclerosis: Clinical, cellular and molecular aspects
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General introduction and scope of the thesis
1. Multiple sclerosis

1.1 Epidemiology and etiology
Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), in which autoimmune demyelination and neuronal damage in the brain and spinal cord lead to severe and progressive neurologic decline, often resulting in premature death. Currently, MS affects 2.5 million people worldwide and is about two times more prevalent in women than in men. It may be considered the most common disabling neurological disease in young adults, as disease onset usually occurs between the age of 20 and 30. However, MS can also affect children and older people.

![Prevalence of multiple sclerosis per 100,000 individuals, worldwide.](image)

**Figure 1** Prevalence of multiple sclerosis per 100,000 individuals, worldwide. From Atlas multiple sclerosis resources in the world 2008. © World Health Organization 2008.

Although the ultimate etiology of MS has yet to be defined, it is becoming increasingly clear which factors may contribute to disease susceptibility. The fact that prevalence increases with distance to the equator has led to the hypothesis that lim-
ited exposure to sunlight, perhaps by way of decreased vitamin D synthesis, leads to an elevated risk for developing MS (figure 1).²,³ In support of this, patient and animal data do indicate that both exposure to sunlight and vitamin D status may have an effect on disease susceptibility and severity, also independently from each other.⁴–⁶ In addition, many different viruses have been implicated as a causative agent in MS.⁷ A notable candidate among these is the Epstein-Barr virus (EBV), although its role as a etiologic factor in MS is intensely debated.⁸–¹⁰ Indeed, viruses have been shown to be able to induce autoimmunity via several mechanisms.¹¹ One mechanism postulated to cause virus-induced autoimmunity in MS is molecular mimicry, which entails cross-reactivity of virus-specific T cells with self-antigens.¹² In addition to the environmental factors, susceptibility is clearly determined by genetic makeup, for which studies strongly point towards a role of immunologically relevant genes, in particular of those involved in regulation of T-cell immunity.¹³,¹⁴ Therefore, it is widely believed that susceptibility to MS is determined by a complex interaction between genetic and environmental factors.

1.2 Clinical and pathological heterogeneity of MS

The hallmark of MS neuropathology is white matter demyelination, though it is characterized by many other features, such as accumulation of activated microglia and macrophages within lesions, the presence of microglia nodules in the normal-appearing white matter (NAWM), neuronal damage, oligodendrocyte loss, perivascular cuffing of leukocytes and grey matter demyelination.¹⁵–¹⁸ Importantly, MS also features a process of myelin repair called remyelination. At neuropathological examination, this is evident in so-called shadow plaques, previously demyelinated areas where due to remyelination new and usually thinner myelin sheets have been formed around axons, thereby limiting further loss of neuronal functioning.

One of the most striking features of MS is its clinical and pathological heterogeneity. This is most clearly reflected by the variability in MS disease severity. Disease severity may be defined by the overall rate of MS progression and ensuing disability, the latter commonly measured by the expanded disability status scale (EDSS) of Kurtzke.¹⁹ The EDSS system scores a healthy state as 1, the most severe stage
as 9.5 and death due to MS as 10.\textsuperscript{19} Progression of MS, and thus severity, is highly variable and unpredictable: some MS patients may remain relatively asymptomatic for decades, whereas others may progress to a state of severe disability and death within years or in cases with fulminant disease even within months after onset.\textsuperscript{20} Patients with a low EDSS score (3 or less) after a period of at least 10 years are said to have benign or mild MS, which is seen in about 10\% of the cases. In addition, MS displays various patterns in its clinical course. Based on these patterns, three major subtypes of MS are designated, irrespective of disease severity (figure 2):

1. **Relapsing-remitting MS (RRMS)**
   Characterized by unpredictable attacks, which may or may not result in permanent neurologic deficits, followed by periods of remission.

2. **Secondary progressive MS (SPMS)**
   Characterized by initial relapsing-remitting pattern that converts to steady neurological decline without remission.

3. **Primary progressive MS (PPMS)**
   Steady neurologic decline starting directly at the onset of disease without relapses or periods of remission.

Combinations of the described MS courses may also be observed, for example a pattern of steady neurologic decline with superimposed relapses, which is sometimes referred to as progressive-relapsing MS subtype.

In some, but not all patients, cognitive symptoms are present, in particular in advanced disease. Moreover, in about 60\% of the patients depression is observed as a comorbidity at some point during the disease.\textsuperscript{21} The heterogeneity of MS is also apparent at the neuropathological level, as a great variability is seen between patients in for example the number and anatomical distribution of lesions, the extent of demyelination and remyelination, the degree of inflammation and the perivascular cuffing of leukocytes.\textsuperscript{17,18,22} The extent to which these neuropathological features occur and their location in the brain and spinal cord together determine the clinical presentation of MS in an individual patient. It has been proposed that distinct categories of demyelinating lesion pathology exist.\textsuperscript{17} However, it is still a matter of de-
bate whether pathological heterogeneity is a reflection of inter-individual differences in the mechanisms underlying demyelination, or of stages of lesion development over time that ultimately converge to the same mechanism. On the other hand, there is a strong notion that lesion pathology is homogeneous within MS patients. Related to this, there is a great interest in the mechanisms that determine the clinical and pathological heterogeneity of MS between patients. Genetic factors play a role, as for example glucocorticoid receptor (GR) haplotypes were found to be associated with fast progression of MS. In addition, polymorphisms leading to high glutamate levels in MS were shown to coincide with high degrees of neurodegeneration. Stress-axis responsiveness is another factor that has been strongly implicated in heterogeneity of MS and is therefore the subject of this thesis.

1.3 Pathogenesis of MS
Whereas the primary cause of MS lesion development is still unclear, much is known about the major events that lead to plaque pathology, such as blood-brain barrier (BBB) disruption, influx of peripheral leukocytes and activation of microglia. MS is classically seen as an autoimmune disease that is mediated by CD4⁺ T cells specific for CNS-associated self-antigens, in particular myelin components. However, a
central pathogenic role has also been ascribed to auto-antibodies directed against myelin or neuronal antigens, which form oligoclonal bands in the cerebrospinal fluid (CSF) that serve as diagnostic hallmark of MS. Nevertheless, there is still no coherent model of MS pathogenesis. At the same time, accumulating evidence indicates that microglia may be crucially involved in MS lesion development and expansion. In many cases, clusters of microglia are present in the NAWM even before MS lesions develop. Additionally, microglia activation precedes the onset of autoimmune demyelination in the most studied animal model for MS, experimental autoimmune encephalomyelitis (EAE). Within acute or chronic active MS lesions, activated microglia and macrophages are the predominant cell type and they are actively involved in phagocytosis of myelin. Moreover, their number correlates with the extent of axonal damage in MS lesions and with neuronal dysfunction in EAE. Therefore, microglia are a highly relevant therapeutic target, even more so as their strong plasticity implies that modulating microglial functioning in vivo may be a feasible therapeutic strategy.

1.4 Therapy of MS
As no single etiologic agent is known to cause MS, there are currently no options for developing preventative or curative treatments. Available MS therapies are slowing down disease progression by targeting the immune system in various ways. Glucocorticoids (GC) are among the earliest and most widely used therapeutic agents in MS, as they are well known for their broad anti-inflammatory properties. Short-term treatment with high-dose GC is frequently applied to reduce the duration and severity of acute MS relapses. However, the (chronic) use of high-dose GC has considerable negative side-effects, while there is no proven positive effect on long-term disease progression. It has been well-established that GCs profoundly affect all cellular compartments of the immune system, which is not surprising given the fact that all leukocytes display GR expression, including microglia and macrophages. The immunosuppressive effects of GC on adaptive immunity are primarily mediated by inhibiting T-cell immunity, whereas macrophages are polarized towards a regulatory phenotype. In addition to these immunomodulatory features, GCs are
though to enhance BBB integrity and reduce leukocyte migration. However, due to their lipophilic nature, GCs can pass the BBB freely and directly affect microglia, which also implies that they represent a clear cellular target for the endogenous GC, cortisol, produced by the HPA-axis.

Other important therapeutic agents for MS are interferon-beta (IFN-β), glatiramer acetate and Natalizumab. IFN-β is a type I interferon that is used to treat RRMS and is able to reduce the frequency clinical exacerbations. However, a large part of the RRMS patients does not respond to the therapy due to development of antibodies against IFN-β. The therapeutic effect of IFN-β is thought to be primarily brought about via modulation of T cells, by causing a shift from a Th1 to a Th2 phenotype. The identification of glatiramer acetate treatment was based on serendipity, as it was originally developed as a synthetic derivate of myelin basic protein and was intended to be used for induction of EAE. Various clinical trials have demonstrated a positive effect of glatiramer acetate on relapse rate, though the there was only a small overall effect on disease progression. Like IFN-β, glatiramer acetate has the capacity to induce a Th2 phenotype in T lymphocytes, and is thought to enhance in these cells the production of brain-derived neurotrophic factor (BDNF). One of the most promising therapies for MS that is currently being used is administration of an antibody against the very-late-antigen-4 (VLA-4, CD49d), which goes by the commercial name Natalizumab. The integrin VLA-4 is expressed on most leukocytes and facilitates their extravasation and passing of the BBB by binding the vascular cell adhesion molecule (VCAM)-1 on vascular endothelial cells. By inhibiting migration of leukocytes to the brain parenchyma, this antibody successfully decreased relapse rates and disease progression in MS patients.
2. The stress-axis

2.1 Basic functioning

As the stress-axis involves the hypothalamus, as well as the pituitary and adrenal gland, it is commonly referred to as the hypothalamus-pituitary-adrenal (HPA-) axis. The hypothalamus forms the starting point of the stress-axis and is located at the base of the cerebrum. Phylogenetically, it is one of the oldest parts of the CNS and by interacting with the endocrine, immune and autonomic nervous system it is crucial for the regulation of functions, such as body-temperature, water and food-intake, blood pressure, circadian rhythm and sexual behavior. Importantly, the hypothalamus is also strongly involved in responsiveness to psychological and physiological stress as part of the HPA-axis.\textsuperscript{53–60}

At the anatomical level, the hypothalamus is composed of several nuclei, clusters of neurons with specific neurobiological and/or neuroendocrine functions. The nucleus responsible for regulation of the stress-axis is situated adjacent to the third ventricle and is referred to as the paraventricular nucleus (PVN). The neurons in the PVN involved in stress-axis functioning are those that produce corticotropin-releasing hormone (CRH), which is secreted into the bloodstream and is thereby transported to the anterior lobe of the pituitary gland where it induces release of adreno-corticotropic hormone (ACTH) from corticotropic cells. While CRH is the primary factor controlling ACTH release, additional other factors secreted by hypothalamic neurons are capable of modulating this. A particular important example is arginine-vasopressin (AVP), which is able to potentiate the effects of CRH up to 30 times.\textsuperscript{61–66} Especially in conditions of chronic stress leading to elevated stress-axis activity, CRH-producing neurons in the PVN may also start secreting AVP. Co-expression of CRH and AVP is therefore an important indicator of stress-axis hyperactivity.\textsuperscript{67–70} Upon induction by hypothalamic factors, ACTH is secreted from the anterior pituitary into the general bloodstream, by which it is transported to the adrenal gland. Subsequently, cells in the zona fasciculata of the adrenal cortex are stimulated by ACTH to secrete cortisol, the main GC in humans.

Once secreted, the majority of circulating cortisol (±90\%) is bound to cortico-
steroid-binding globulin (CBG). Cortisol is able to enter the CNS via the blood circulation and by way of the CSF. However, in its unbound form, the hydrophilic nature of unbound cortisol enables it to easily cross the BBB and cellular membranes. The local bioavailability of cortisol is further regulated by 11β-hydroxysteroid dehydrogenase (11βHSD) 1 and 2, which catabolize and metabolize cortisol, respectively. Importantly, cortisol controls stress-axis activity through a negative feedback mechanism, as it suppresses secretion of CRH and AVP in the hypothalamus and ACTH production in the pituitary (figure 3).

2.2 Neuroendocrine-immune interactions

Interaction between the stress-axis, the nervous system and the various compartments of innate and adaptive immunity play a key role in regulation of inflammation.\textsuperscript{71–73} Various factors released during systemic or localized inflammation are capable of modulating stress-axis activity. Inflammatory cytokines, including tumor necrosis factor-a (TNFa), interleukin (IL)-1\textbeta and IL-6 are capable of either directly or indirectly activating the stress-axis. Probably the most potent compound in this respect is IL-1\textbeta, which strongly activates the stress-axis by stimulating neurons in the PVN to release CRH.\textsuperscript{74} In addition, IL-6 is able to activate the stress-axis by direct interaction at the level of the pituitary or adrenal gland (figure 3).\textsuperscript{75}

A cytokine related to IL-6, leukemia inhibitory factor (LIF), is especially important for stress-axis responsiveness towards inflammation, as it has been shown to strongly stimulate production of pro-opiomelanocortin, the precursor of ACTH, in the pituitary. Moreover, LIF was shown to reduce GR expression in the PVN, thereby limiting central suppression of stress-axis activity by cortisol. Thus, LIF greatly influences the setpoint of the stress-axis and its production during inflammation likely acts to maintain cortisol secretion at an adequate level. Another well-known class of inflammatory mediators that activate the stress-axis by inducing CRH release from hypothalamic neurons are prostaglandins, in particular prostaglandin E\textsubscript{2}. Production of prostaglandins is strongly induced by all innate cytokines in various cell types in the CNS and serves for amplification and transmission of signals associated with acute inflammation.\textsuperscript{72} As prostaglandins are strongly induced in the brain microvas-
culature by circulating innate cytokines, they are also centrally involved in relaying effects of peripheral inflammation across the BBB into the CNS. Aberrant functioning of the stress-axis is strongly associated with immune dysregulation. Hyperactivity of the stress-axis in the absence of inflammation, for example in Cushing’s syndrome, results in immunosuppression and increased susceptibility to infection. Conversely, hypoactivity of the stress-axis is associated with increased susceptibility to and severity of inflammation. For example, subjects with a constitutively hypoactive stress-axis, as seen in Addison’s disease, require GC supplementation during infection and inflammation to prevent cytokine-induced toxicity.

The effects of stress-axis activity on the immune system are ultimately mediated by GC produced by the adrenal gland, primarily cortisol in humans. The cellular effects of cortisol are induced mainly by ligation to the GR within the cytoplasm. After this, the cortisol-GR complex may affect cellular functioning through two distinct mechanisms. The first one, called transrepression, involves suppression of other transcription factors by the cortisol-GR complex through direct protein-protein interactions. Alternatively, binding of the cortisol-GR complex to DNA sequences called glucocorticoid responsive elements (GRE) in specific genes may lead to their transcriptional inhibition or enhancement, the latter being referred to as transactivation. Non-genomic effects of GR ligation by cortisol are rapidly induced through interaction with membrane-associated receptors and were shown to exert anti-inflammatory effects by inhibiting synthesis of secondary messengers at play during inflammation, including prostaglandins. In conclusion, all these mechanisms are thought to play a role in immunosuppression, though they are certainly also involved in mediating the pleiotropic effects of cortisol within the human body.

Cells of both innate and adaptive immunity express the GR, and are therefore sensitive to GC-mediated immunomodulation. The impact of GC on the adaptive immune system is largely brought about by their effects on myeloid cells. In macrophages and dendritic cells, GC strongly reduce the capacity for antigen presentation, but also suppress production of IL-12, a cytokine that is essential for induction of a T-helper (Th)-1 phenotype in CD4+ T cells. The lack of IL-12 causes CD4+ T cells to
shift from a Th1 to a Th2 phenotype, coinciding with a switch from interferon-γ (INFγ) to IL-4 production.

These GC-induced changes in CD4⁺ T cells greatly affect the phenotypic properties of macrophages and other myeloid cells, as IFNγ (Th1) and IL-4 (Th2) are
associated with respectively classical (M1) and alternative (M2) macrophage activation. M1 activation refers to pro-inflammatory immune responses of macrophages induced by IFNγ or lipopolysaccharide (LPS) and results in production of various effector molecules that are known to confer neurotoxicity, such as tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), and reactive oxygen species (ROS). Conversely, M2 activation encompasses the induction of various tissue repair and immunoregulatory pathways in macrophages by compounds such as IL-4, IL-10 and GC, and is associated with expression of immune mediators that suppress inflammation and promote neuroprotection, such as IL-10 and transforming growth factor-beta (TGFβ). Through all these mechanisms, GC have the capability to strongly impact on the inflammatory milieu within the CNS.

2.3 The stress-axis in MS

Impairments in stress-axis activity have been linked to enhanced susceptibility to autoimmunity. For example, several studies suggest that suboptimal production of cortisol is involved in the onset and/or progression of rheumatoid arthritis (RA). Moreover, aberrant functioning of the stress-axis has been reported in inflammatory bowel disease.

Several lines of evidence implicate the stress-axis in MS pathogenesis, disease progression and even occurrence of comorbid mood disorders. Cortisol is immunosuppressive and routinely used in its synthetic form to treat MS relapses. In the EAE model for MS, rats with low stress-axis responsiveness indeed show higher disease susceptibility and reduced recovery rates. Clinical studies in MS patients demonstrated elevated basal cortisol plasma levels as well as marked stress-axis hyperactivity in the combined dexamethasone-CRH test. Post-mortem studies also showed chronic activation of the stress-axis in MS, as indicated by enlarged adrenal glands, higher cortisol levels in the cerebrospinal fluid (CSF) and increased CRH-producing neurons co-expressing vasopressin (VP) in the hypothalamus. Apparently, hyperactivity of the stress-axis in MS is not sufficient to prevent disease. Interesting in this respect is that we and others identified MS patients with a hypoactive stress-axis and a very severe clinical course of MS. In addition,
we found an inverse correlation between the number of CRH neurons and the number of active MS lesions in the hypothalamus. In line with this, cortisol release in the combined dexamethasone-CRH test was reported to be negatively correlated to the presence and number of gadolinium-enhancing lesions in MS patients. Thus, the stress-axis is generally activated in MS, but patients with a hypoactive stress-axis have particularly severe MS and more active lesions.

3. Microglia

3.1 Microglia: key players in CNS immunity and MS

Microglia are highly versatile immune cells of the myeloid lineage with many different cellular functions that may be either beneficial or detrimental and play a crucial role in innate and adaptive immunity. Also called the brain-resident macrophages,

Figure 4  The spectrum of microglia morphologies. On the far left are depicted ramified microglia under resting conditions that via several transitional stages, shown in the middle, may adopt an activated phenotype with an amoeboid macrophage-like morphology, as illustrated on the far right. Drawing by Pio del Rio-Hortega.
microglia were long though to originate from the same hematopoietic progenitor as other myeloid immune cells. However, fate mapping studies in mice demonstrated that microglia derive from primitive hematopoietic progenitors during embryogenesis.\textsuperscript{106} This means that microglia, at least in mice, represent an ontologically distinct myeloid cell population within the immune system. Morphologically, microglia are characterized by a small soma with extensive radial ramifications that are used to actively survey the microenvironment under physiological conditions.\textsuperscript{107} However, upon activation, microglia may proliferate and, via several transitional stages, adopt an amoeboid, macrophage-like morphology (figure 4).\textsuperscript{108,109}

As surveyor of parenchymal homeostasis in the brain, microglia are highly sensitive to microenvironmental alterations associated with damage, inflammation, or infection. Another central feature of microglia is their sensitivity to inflammation outside the CNS.\textsuperscript{110} As such, peripheral or systemic inflammation can alter microglia activation status or may even lead to detrimental microglial immune responses, which are though to be involved in exacerbation of neurodegenerative disorders and the occurrence of delirium.\textsuperscript{110,111} Depending on the specific circumstances, microglia are able to exert various effector functions that may be either neurotoxic or neuroprotective.\textsuperscript{112} Microglial effector functions include phagocytosis, antigen presentation and production of inflammatory cytokines, neurotrophic factors and reactive oxygen species.\textsuperscript{112}

The functional versatility and central role of microglia in neurodegeneration and inflammation has led to a strong interest in the mechanisms determining their phenotypic switches. A major question of interest is to what extent microglia display features of M1 and M2 activation in various pathologies. However, most of the data on macrophage polarization have been generated by \textit{in vitro} experiments. Importantly, the circumstances in these culture models are quite different from the physiological conditions under which innate immune responses occur \textit{in vivo}. In particular, it is now becoming clear that the tissue microenvironment profoundly affects the immune responsiveness of resident immune cells.\textsuperscript{113} Indeed, it has been well-established that effector functions exerted by microglia are the net result of the interaction between signals in the brain microenvironment and many different molecules for
recognition and regulation on the microglial cell surface (figure 5). Thus, microglia may be considered a product of their microenvironment, due to their high sensitivity to local aberrations. On the other hand, owing to their functional versatility,
microglia determine the surrounding inflammatory milieu to a very large degree.

Considering the key role of microglia in CNS (auto)immunity and the many unanswered question regarding their tissue-specific and pathology-related phenotype and functioning, a procedure for rapid isolation of these primary cells from post-mortem human brain tissue for in vitro studies is highly warranted. This is even more so, as many existing microglia isolation procedures were time consuming, since they included sequential enrichment steps based on differential adherent properties of glial cells types, in some cases preceded by several Percoll density gradient separations. These procedures required prolonged culture and adherence for enrichment of microglia, which is inevitably accompanied by changes in microglial phenotype before the start of the actual experiment. Therefore, observations made in an approach that excludes effects of culture and adherence as much as possible, will likely more accurately reflect the tissue-specific biology of microglia in vivo. In addition, it offers the opportunity to directly relate ex vivo microglial phenotypes to data obtained by downstream applications, such as in vitro assays on various effector functions, transcriptomic and proteomic analyses.

3.2 Microglia in MS

Microglia activation has since long been implicated as a early hallmark of pathology in MS and EAE. Activated microglia clusters, so-called nodules or pre-active lesions, are found in the NAWM of MS patients. However, other studies in post-mortem human tissue indicated that microglia throughout the NAWM of MS show subtle changes in inflammatory and neuroprotective pathways and alterations in their activation status. Interestingly, microglia activation, as determined by CD45 upregulation, was found to precede the onset of autoimmune demyelination in EAE, supporting the idea that changes in microglial activation status represent a crucial step in the initiation of MS lesion pathology. In line with this, mice depleted of microglia showed a delayed onset and decreased severity of EAE. The important role of microglia activation in EAE pathology was further confirmed by administration of the microglial suppressors macrophage migration inhibitory factor (MIF) or minocycline, which both attenuated EAE symptoms as well.
The mentioned EAE studies suggest that microglia mostly play a detrimental role in MS pathogenesis. Indeed, it could be easily imagined that microglia adopt a phenotype with features of classical macrophage activation that promotes the induction of pathogenic CD4$^+$ Th1 or Th17 responses, both of which are strongly implicated in MS.\textsuperscript{124,125} However, because of their versatile nature, microglia may also be able to take on a phenotype that is anti-inflammatory and neuroprotective. For example, foamy macrophages may, at least partly, originate from microglia and display clear anti-inflammatory effector functions, for example by producing high levels of the inflammation-limiting chemokine CCL18 and the cortisol-synthesizing enzyme 11$\beta$HSD1.\textsuperscript{126,127} Therefore, detailed study of the phenotypic and functional properties of microglia in NAWM of MS patients may give insights into the events that lead to lesion development and lead to strategies to modulate microglial functioning for MS therapy.

4. Aim and scope of the thesis

The principal aim of this thesis was to elucidate the role of HPA-axis activity in the clinical and pathological heterogeneity of MS. Specifically, we focused on investigating indicators of HPA-axis activity and clinical MS progression for their association with neuropathology, molecular mechanisms in the NAWM and genetic polymorphisms of the GR. In addition, we aimed to characterize microglia, as these cells represent a key constituent of CNS immunity that is strongly implicated in MS pathogenesis and may very well be affected by HPA-axis functioning.

In chapter 2, we investigated to what extent stress-axis regulation in MS patients impacts on disease severity, lesion type and gene expression profiles in the NAWM. This was done by studying indicators of stress-axis activity and diverse aspects of MS pathology, such as lesion pathology and extent of neurodegeneration. HPA-axis activity was determined by measuring cortisol in CSF and counting CRH-expressing neurons in the PVN of the hypothalamus, whereas neurodegeneration was based
on CSF levels of neurofilament heavy-chain (NfH), tau and glutamate. These parameters were used to perform an extensive correlative analysis to unravel associations of HPA-axis activity with progression and neurodegeneration in MS patients. In addition, patients with high and low stress-axis activity were compared for the frequency of active, inactive and remyelinated lesions in the cerebrum, and expression of GC-responsive and inflammatory genes in the NAWM. Specific attention was paid to potential gender and subtype-related differences in these analyses.

In chapter 3, we studied the relation of severity of MS, stress-axis responsiveness and soluble CD163 (sCD163) with several polymorphisms in the GR gene that are associated with altered GC sensitivity.

In chapter 4, we developed a new procedure for isolation of human microglia from post-mortem white and grey matter brain tissue, which omitted the need for adherence and thereby allowed for immediate phenotyping and in vitro testing. We studied phenotypic characteristics of human microglia under non-pathologic conditions and in the presence of peripheral inflammation. Furthermore, the responsiveness of primary microglia towards M1 and M2 stimuli was assessed, with specific interest for the impact of GC on microglia.

Subsequently, chapter 5 is dedicated to the ex vivo and in vitro characterization of primary microglia in NAWM of MS patients, to identify disease-specific alterations in microglia phenotypes and immune responsiveness.

Chapter 6 presents a microarray study to find out whether gene expression in NAWM of MS patients is associated with severity of MS and HPA-axis activity. By weighted gene co-expression network analysis (WGCNA) and additional approaches, gene expression profiles were investigated for molecular pathways that may be targeted for MS therapy.

Chapter 7 discusses the principal findings described in this thesis and states the main conclusions that can be drawn from them. In addition, it provides suggestions for future research.
On the whole, the data presented in this thesis indicate that high stress-axis activity substantially contributes to suppression of MS disease activity, as reflected by slower disease progression, less destructive lesion pathology and induction of beneficial GC–responsive cellular and molecular mechanisms in the NAWM.