



UvA-DARE (Digital Academic Repository)

The stress-axis in multiple sclerosis: Clinical, cellular and molecular aspects

Melief, J.

Publication date
2014

[Link to publication](#)

Citation for published version (APA):

Melief, J. (2014). *The stress-axis in multiple sclerosis: Clinical, cellular and molecular aspects*. [Thesis, fully internal, Universiteit van Amsterdam]. Boxpress.

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

[7]

Summary and general discussion

SUMMARY AND GENERAL DISCUSSION

The studies included in this thesis aimed to provide a better understanding of the relation between activity and regulation of the hypothalamus-pituitary-adrenal (HPA)-axis and various aspects of multiple sclerosis (MS): clinical course, constituents of cerebrospinal fluid (CSF), lesion pathology, and molecular as well as cellular characteristics of normal-appearing white matter (NAWM). The heterogeneous nature of MS plays a central role in all chapters and the presented findings suggest that HPA-axis activity is a major determinant for this.

All research described here has been performed using post-mortem brain tissue, CSF as well as neuropathological evaluations and data from the extended medical records of donors enrolled into the programs of the Netherlands Brain Bank (NBB). Therefore, I will first discuss the potentials and pitfalls of this type of research, which will aid in interpreting the observations made in this thesis. In addition, much of the data has been generated by a newly developed method for immediate isolation of primary human microglia from post-mortem human tissue. Therefore, I will also dedicate a separate part of this discussion to the various aspects of this line of research. As each thesis chapter offers a detailed discussion of the presented results, I will focus more on the general conclusions that can be drawn from the respective studies. In doing so, I will address both the biological and clinical relevance of the main findings and provide suggestions for future research.

SUMMARY

MS is a chronic inflammatory disease of the central nervous system (CNS), in which autoimmune demyelination leads to severe and progressive neurologic decline. A hallmark of MS is its clinical and pathological heterogeneity. This is most evident in the highly variable and unpredictable disease course: 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.²⁰

Strongly implicated in the heterogeneity of MS are inter-individual differences in cortisol production by the hypothalamus-pituitary-adrenal (HPA)-axis. In most MS patients, the HPA-axis is strongly activated. However, we and others identified an association between low HPA-axis activity and more severe MS.^{25,89,95} Therefore, this thesis aimed to provide a better understanding of the relation between activity and regulation of the hypothalamus-pituitary-adrenal (HPA)-axis and various aspects of multiple sclerosis (MS): clinical course, neurodegeneration, lesion pathology, glucocorticoid receptor (GR) polymorphisms, and molecular as well as cellular characteristics of normal-appearing white matter (NAWM).

The introductory **chapter 1** provided an overview of the current knowledge on MS and described basic and pathology-related aspects of HPA-axis functioning. In addition, it introduced microglia as key players in CNS immunity and MS that may be strongly affected by cortisol secreted by the HPA-axis.

In **chapter 2** of this thesis, a comprehensive analysis was performed of the pathological and molecular underpinnings of the negative association between HPA-axis activity and disease severity of MS. The results showed that high HPA-axis activity in MS is associated with slower disease progression, especially in females with secondary progressive MS. Patients with low HPA-axis activity had greater numbers of active lesions and tended towards having less remyelinated plaques than patients with high HPA-axis activity levels. Moreover, normal-appearing white matter

(NAWM) of MS patients with high HPA-axis activity displayed gene expression profiles that were partially cortisol related and less inflammatory, more neuroprotective and permissive for remyelination. These data suggest that HPA-axis activation as generally observed in MS patients directly impacts on lesion activity and molecular mechanisms in NAWM, thereby reducing disease severity.

Chapter 3 studied the relation of MS severity, HPA-axis activity and soluble CD163 (sCD163) with several polymorphisms in the glucocorticoid receptor (GR) gene that are associated with altered GC sensitivity. The findings indicate that GR haplotypes conferring high GC sensitivity coincide with more aggressive MS but do not affect cortisol secretion by the HPA-axis or CD163 shedding.

Chapter 4 and 5 were dedicated to the ex vivo and in vitro characterization of primary microglia from NAWM of control subjects and MS patients, to identify basic and disease-specific alterations in microglia phenotypes and immune responsiveness. These data were generated by developing 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. By ex vivo flow cytometric analysis, the basic phenotype of isolated microglia was specified by comparison with autologous macrophages from the choroid plexus. Moreover, phenotypic alterations were found of microglia in NAWM that were associated with peripheral inflammation and MS. Furthermore, an 'alerted' phenotype was identified in microglia from MS NAWM, characterized by an activated morphology, upregulation of immunoregulatory molecules and unresponsiveness to lipopolysaccharide (LPS). In addition, glucocorticoid responsiveness of microglia from MS NAWM was altered.

Chapter 6 described a microarray study in NAWM of MS patients. By weighted gene co-expression network analysis (WGCNA) and additional approaches, genome-wide expression data were investigated for gene expression profiles NAWM of MS patients is associated with severity of MS and HPA-axis activity. The data indicated that HPA-axis activity strongly impacts on molecular mechanisms in NAWM of

MS patients, but for a large part independently of disease severity. The study yields various molecular targets that could be eligible for functional assessment of their therapeutic potential to prevent lesion formation in NAWM of MS.

GENERAL DISCUSSION

1. Research using human post-mortem brain tissue: benefits and limitations

1.1 The value of post-mortem brain research for understanding MS

Many animal models have the inherent drawback that they simulate only certain aspects of a disease, which raises the question to what extent observations can be extrapolated to human pathology. This particularly applies to MS, as it is characterized by a extensive range of clinical, neuropathological and immunological features that each are highly heterogeneous. There is indeed a plethora of MS animal models – foremost experimental autoimmune encephalomyelitis (EAE) – that differ in many respects, such as selected autoantigen and immune effector mechanism, method of induction and animal species. However, each of these models lacks one or more principle features of MS neuropathology, as they may for example fail to show actual demyelination, remyelination, or autoimmune inflammation.

Though invaluable for many reasons, there are also clear practical limitations to research in living MS patients. For example, clinical MS studies often make use of patient-derived blood and liquor to identify potential prognostic biomarkers and perform phenotypic analysis of peripheral leukocytes. However, various reports indicate that there is a poor correlation between phenotypic profiles of leukocytes in peripheral blood and those present in the CSF or the brain, in particular with respect to T lymphocytes.^{328,329} In addition, available imaging techniques are highly instrumental for diagnostic purposes and, in the case of magnetic resonance imaging (MRI), may strongly aid to identify patterns in the anatomical distribution and time-dynamics of MS neuropathology. However, none of these approaches allow for the study of

cellular and molecular mechanisms within the human central nervous system (CNS). Therefore, post-mortem brain research using molecular and cellular techniques to study the human CNS compartments specifically affected in neurologic diseases, is a crucial and indispensable source of information for understanding pathogenesis and identifying targets for therapy.

1.2 General aspects of post-mortem brain research

Post-mortem brain research crucially depends on the availability of high-quality donor tissue that is dissected with short post-mortem delay (PMD) and characterized for neuropathology according to standardized procedures, and complemented by well-documented medical histories. This type of research also entails specific guidelines for matching patient and control groups. For example, groups should not only be matched by sex and age, but also for PMD and pH of the CSF. Both these parameters may have a confounding effect on biological observations.^{330,331} Especially pH, which is a reflection of the agonal state and extent of hypoxia before dying, has been shown to strongly relate to overall tissue quality, including that of RNA.³³⁰ Not surprisingly, we found that very low pH values (pH <6.1) are often associated with low cell yield and viability in primary microglia isolates.

In comparison to patient-related research, there is almost always an age-bias towards elderly subjects in post-mortem cohorts. Inherently, observations in such cohorts are made at the end of the disease process. It is therefore often argued that post-mortem research has the major disadvantage that pathological mechanisms can not be studied in relation to certain disease phase and it is always uncertain to what extent findings are a cause or a consequence of the studied pathology. However, an advantage of post-mortem research is that the whole clinical course is known, and not just a part of it. For MS cases, this includes for example valuable information about overall disease progression and severity, time point of conversion from relapsing-remitting to progressive disease and use of medicines.

1.3 Assessing clinical course and severity of MS

To be able to make statements about the clinical relevance of molecular and cellular

observations, it is essential to use parameters that accurately reflect aspects of the MS disease course, such as rate of disease progression. For this, information is necessary on the pattern of the clinical MS course, disease onset (i.e. appearance of the first symptoms), time points of reaching expanded disability status scale (EDSS) scores of 3, 6 and 9, as well as conversion to the progressive phase.

In the studies described in this thesis, the validity of disease duration as an indicator for severity of MS is confirmed by its strong correlation with time to EDSS 6, which is a widely used measure of MS severity.^{162,167} For MS patients with short disease duration, the clinical files of the NBB were used to verify whether individuals died due to causes directly related to MS, such as legal euthanasia and pneumonia. Notably, chapter 2 describes the finding that duration of MS and time to EDSS 6 both correlated to percentages of active lesions in the cerebrum, showing that these parameters are associated with pathological correlates of MS disease severity as well.

1.4 Studying stress-axis activity

Due to HPA-axis responsiveness to the physiological process of dying, post-mortem cortisol levels in CSF are about 20-fold higher than those assessed in living patients.^{101,166} Importantly, in all donor (sub)groups included in our studies we routinely found a significant positive correlation between cortisol levels and numbers of CRH positive neurons in the periventricular nucleus (PVN), which indicates that both parameters assessed post-mortem indeed reflect HPA-axis activity during life. This is further supported by the finding that post-mortem cortisol levels in CSF strongly correlate with ante-mortem levels in CSF and serum in control subjects and MS patients.^{101,166} Importantly, PMD and pH value did not correlate in any of our studies with cortisol levels in the CSF or with numbers of CRH positive neurons in the hypothalamus, pointing out that these parameters have no confounding effects on indicators for post-mortem assessment of HPA-axis activity.

1.5 Using post-mortem tissue to isolate, phenotype and culture microglia

Many observations in this thesis are made by immediate isolation of primary human microglia from fresh post-mortem brain tissue. Of key importance for this research

were the facilities of the NBB (www.brainbank.nl), as they facilitated dissection of brain tissue with a very short PMD of 6 h on average. This is particularly relevant for the study of MS brains, which already require a more extensive and thus time-consuming protocol that includes post-mortem MRI scanning and dissection of as many lesions as possible. The availability of such high-quality tissue ensures that primary cells could be isolated with good yield and viability. This new isolation method enabled us to apply highly sensitive techniques to define basal characteristics of microglia as well as features related to MS pathology in the NAWM. In comparison with microglia isolation procedure used before, a major improvement of the method described here is the fact that it excludes effects of culture and adherence as much as possible. The findings in chapter 4 of this thesis, as well as data from a previous study on human microglia isolated from operation material, indicate that microglial culture inevitably coincides with phenotype changes, including rapid upregulation of CD14 and instigation of responsiveness to LPS.²⁰⁶ This is likely due to the absence in culture of microenvironmental cues that are normally present in the CNS and cause microglia to maintain their specific phenotype, as is also well established for many types of tissue macrophages.^{211,332} Therefore, observations made in our setup likely more accurately reflect the *in vivo* biology of microglia. Moreover, it offers the opportunity to link *ex vivo* microglial phenotypes directly to data obtained by downstream applications.

2. Determinants of stress-axis responsiveness

2.1 Neurodegeneration versus inflammation

There is extensive evidence that both inflammation and neurodegeneration activate the HPA-axis in neurological diseases. Activation of the HPA-axis takes place in virtually all types of immune responses, and was shown to reflect the severity of inflammation.^{333,334} Indeed, many cytokines and other inflammatory mediators are well-known for their ability to stimulate CRH secretion by neurons in the PVN, such as interleukin (IL)-1 β , IL-6 and prostaglandins.^{74,75,335,336} Importantly, activation of

the HPA-axis by immune responses is part of a negative feedback mechanism that serves to restrain inflammation through the actions of glucocorticoids produced in the adrenal gland.³³⁷ On the other hand, progression of EAE is known to be strongly associated with a specific desensitization of the HPA-axis to pro-inflammatory cytokines.⁹⁴ This fits very well with the studies included in the present thesis, which indicate that HPA-axis activation in MS is of great importance for suppression of disease activity and that low cortisol production is associated with more severe lesion pathology and fast disease progression.

In addition to inflammation, neurodegeneration has been identified as a major drive of HPA-axis activation in neurologic diseases. Indeed, increased activity of the HPA-axis has been well established not only in MS, but also in Alzheimer's disease and major and bipolar depression, as well as in aging.^{70,101,338–341} In one of the populations studied here, we found a particularly strong correlation between glutamate and HPA-axis activity in patients with primary-progressive MS (PPMS). It has been shown that glutamate, when present in the CSF or in glutamatergic projections, can activate the HPA-axis through direct binding to various glutamate receptors on CRH-producing neurons in the hypothalamus.^{155–158} Moreover, neurodegeneration in MS was reported to be associated with the presence of gene alleles that lead to high glutamate levels.²⁴ In accordance with this, blocking glutamate receptors in EAE led to substantial amelioration of disease, increased oligodendrocyte survival and reduced axonal damage.³⁴² We also found a prominent positive correlation between tau and glutamate in the CSF of PPMS patients, further supporting the idea that CSF glutamate levels reflect neurodegeneration associated with this type of MS.¹⁵⁹ Stimulation of CRH release from the hypothalamus due to neurodegeneration may also explain why high HPA-axis activity in MS patients was found to be predictive for fast disease progression and associated with cognitive and neurologic disability in certain studies.^{90,96}

2.2 Gender and subtype-specific differences

Gender differences in MS are well recognized.^{168,169} In addition, there is ample evidence that there are sex-differences in HPA-axis regulation that are relatively subtle

under basal conditions, but become pronounced following a stressor.^{343–345} Moreover, the age-related increase in HPA-axis activity was found to be present only in men and not in women.³⁴⁶ In general, HPA-axis responsiveness tends to be lower in women than in men of the same age after puberty, a difference that is observed till females reach their menopause.³⁴⁷ Perhaps this also contributes to the higher prevalence of MS in women than in men, as animals with low HPA-axis responsive were found to be more susceptible to induction of EAE.^{93,94} Some observations described in this thesis indicate that HPA-axis regulation in MS might also be gender-specific. This is based on the finding that strong correlations ($r > 0.5$) were present between cortisol levels and indicators of disease severity in females, whereas these were totally absent in the male patient groups (chapter 2). However, this may also be due to the fairly small sample size, especially of males. These observations should therefore be verified in larger populations.

2.3 Differences in glucocorticoid receptor genotype

The glucocorticoid receptor (GR) is another factor that is implicated in heterogeneity of MS by affecting HPA-axis regulation and induction of glucocorticoid (GC)-responsive molecular pathways. Therefore, we studied several GR polymorphisms associated with altered GC-sensitivity for their relation with MS disease course, cortisol secretion and levels of soluble CD163 (sCD163) in the CSF. Interestingly, GR haplotypes (*BclI* and N363S) that confer increased GC sensitivity were associated with more aggressive disease course, but not with alterations in levels of cortisol or sCD163 in CSF. These data were in contrast with previous reports that studied the same GR haplotypes and did not find an association of the *BclI* and N363S GR haplotypes with faster progression of MS.^{23,191} In fact, these studies indicated that a more aggressive disease course occurs in MS patients carrying the *Tth111*–ER22/23EK–9 β haplotype, which is associated with a reduced GC sensitivity.^{23,191} These discrepancies could be due to differences in the size of the studied population, which was much smaller in our post-mortem study. In addition, we grouped carriers of GR haplotypes according to their effect on GC-sensitivity, whereas previous studies on MS grouped carriers of each individual GR haplotype. Therefore, new

insights may be provided by combining data from the MS patient population used in our study and from the population used in those earlier studies, to re-analyze all data using the grouping applied in this thesis.

3. Pathological, cellular and molecular effects of stress-axis responsiveness

Of note, the important role of local GC signaling within plaques has been suggested by a study that found local activation of enzymes for cortisol breakdown and synthesis, which was dependent on the lesion stage.¹²⁶ Expression of the enzyme that increases local bio-availability of cortisol, 11 β -hydroxysteroid dehydrogenase type 1 (11 β HSD1), was found to accumulate in microglia/macrophages in the rim of in chronic-active plaques. In contrast, expression of 11 β -hydroxysteroid dehydrogenase type 2 (11 β HSD2), the cortisol-inactivating enzyme, was absent in the same cells. The opposite was seen in more acute lesions, where expression of 11 β HSD1 was decreased and that of 11 β HSD2 increased. Moreover, *in vitro* myelin phagocytosis by macrophages led to prolonged induction of 11 β HSD1 and decreased expression of 11 β HSD2. This study also found MS relapses to be associated with lowered cortisol levels in the CSF and not in serum. Together, these findings suggest that GC may initially be inactivated in MS lesions, facilitating the activation of microglia and infiltrating macrophages that upon myelin phagocytosis convert into foam cells that exert anti-inflammatory properties by producing 11 β HSD1 and chemokine (C-C motif) ligand 18 (CCL18).¹²⁷ Importantly, culture of primary human microglia with dexamethasone readily induced CCL18 on primary human microglia in our hands, which revealed a specific impairment of CCL18 induction in microglia from NAWM of MS patients. This may bear relevance for effectiveness of GC therapy, as CCL18 is one of the most abundant chemokines secreted by immature dendritic cells and preferentially attracts naive and memory T cells and induces in these cells a CD4⁺CD25⁺FoxP3⁺ regulatory T-cell (Treg) phenotype.^{254,256,348} As such, CCL18 could represent a GC-induced mechanism that is centrally involved in creating an immunotolerant CNS microenvironment and is possibly impaired in microglia from

NAWM of MS patients.

A substantial part of this thesis is dedicated to the question to what extent molecular mechanisms in the NAWM of MS patient are associated with HPA-axis activity and disease severity. This question is also related to the growing notion that MS pathogenesis may originate in NAWM, as it was found to contain various changes in inflammatory and neuroprotective gene expression profiles.^{15,120,193} There is a strong interest in unraveling these molecular profiles, as this may lead to identification of events that precede or determine permissiveness for MS lesion development.^{32,259}

Based on the data in this thesis, it can be concluded that there are clear molecular alterations in NAWM of all MS patients. We found that in total 778 genes were upregulated and 544 genes were downregulated in NAWM of MS when compared to that of control subjects. The top 10 most strongly upregulated genes in MS was dominated by antibody subunits, which is likely related to the synthesis of autoantibodies and the presence of oligoclonal bands in the CSF of MS patients.²⁶ This indicates that, independent of disease severity and in absence of lesion pathology, synthesis of antibodies is clearly enhanced the CNS of MS patients. Lassmann and co-workers previously suggested that the disease process of multiple sclerosis may die out in aged patients with long-standing disease.²² However, our results suggest that this is not the case in the NAWM. This also is apparent from data showing a high incidence (60%) of active lesions in the hypothalamus in post-mortem MS brain tissue.³⁴⁹

In addition, the studies in the present thesis indicate that HPA-axis activity clearly impacts on molecular mechanisms in NAWM of MS patient, though for a large part independently of disease severity. In general, molecular profiles associated with high HPA-axis activity and relatively mild MS were characterized by expression of genes that actively regulate inflammation, and by molecules involved in remyelination, anti-oxidative mechanism and neuroprotection.

Strikingly, figure 3 of chapter 6 shows that patients with mild MS, when compared to those with severe MS, are more dissimilar from control subjects regarding expression profiles of inflammatory genes in the NAWM. This may indicate that different pathological processes are at play in SPMS patients with severe disease.

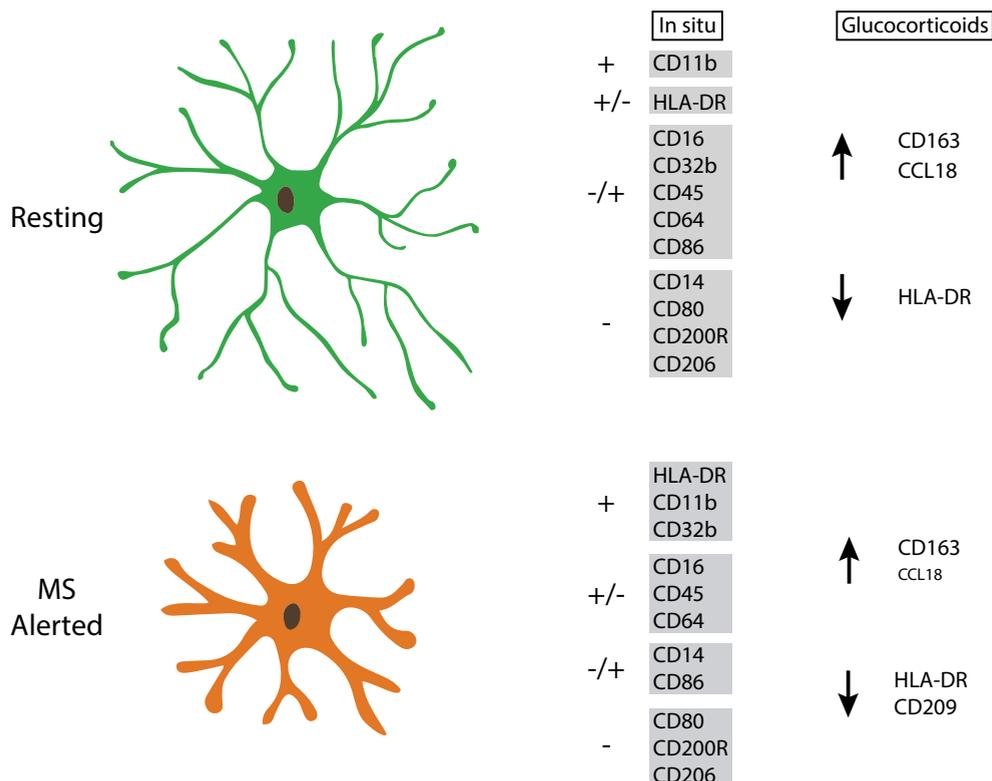


Figure 1 Microglial phenotypes and immune responsiveness. Hypothetical model of the phenotypic properties and glucocorticoid-responsiveness of resting microglia in NAWM of control subjects and alerted microglia in MS NAWM, as described in chapter 4 and 5. Indicated in the first column are the phenotypic markers of microglia in situ, based on analysis performed by ex vivo flow cytometry. The second column depicts up- and downregulation of differentiation markers in response to glucocorticoids, as determined by culture with dexamethasone.

Perhaps, severe MS coincides with a lack of induction of genes that actively regulate inflammation. On the other hand, it could also be that there is a distinct molecular pathology of MS in patients with severe disease, which leads to rapid clinical progression. Generally, GCs are seen as immunosuppressing agents. Though endogenous and exogenous GC do play crucial roles in controlling inflammation, especially in chronic situations, they were recently shown to also exert pro-inflammatory effects.³²⁶ For example, a microarray study on dexamethasone-treated PBMC indicated not only anti-inflammatory, but also several pro-inflammatory molecules were upregulated on monocytes, including IL-7 receptor (IL-7R). In line with this, the data

in chapter 6 identified IL-7R as one of the molecules most strongly correlated to cortisol levels in MS patients. Thus, the favorable effect of exogenous and endogenous GCs on MS disease activity are probably not only due to broad immunosuppression. GCs actively skew the innate immune system in a beneficial direction, as also proposed by others.³⁵⁰ Still, at least a part of the therapeutic effects of GC are mediated by repression of adaptive immunity, as they were shown to reduce inflammation in EAE by inducing T-cell apoptosis.^{351,352}

4. Characteristics of microglia alertness in NAWM of MS

A large part of this thesis focused on studying the phenotype and immune responsiveness of microglia isolated from in NAWM of control subjects and MS patients. Many studies have reported phenotypic alterations of microglia in NAWM of MS patients.^{15,117,119,120} Considering the role of microglia as immune sentinels that are strong determinants of the inflammatory milieu within the brain parenchyma, it is thought that modulating microglia functioning in NAWM could be a highly interesting therapeutic strategy in of MS. Moreover, as microglia are highly sensitive to microenvironmental changes, their phenotypic features in the NAWM may provide clues on MS pathogenesis, specifically regarding events that precede or determine permissiveness for MS lesion formation. One of the main findings of the studies performed in this thesis is that CD45 is a highly sensitive indicator of microglia activations status, as it shows low expression on resting microglia and is progressively upregulated with increasing inflammation. Indeed, elevated CD45 expression on microglia 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.³²

By assessing basal phenotypic characteristics of human microglia in control NAWM, we were able to define microglia phenotype changes in different pathological conditions. First, we could establish that the presence of peripheral inflammation in subjects without neurologic disease goes along with an enhanced microglia activation status in the NAWM. Secondly, we revealed an 'alerted' activation status

in microglia from NAWM of MS, defined by increases in size, granularity and expression of CD45 and CD32b. Notably, LPS-responsiveness in primary microglia was absent, even in those isolated from NAWM of subjects with peripheral inflammatory conditions or MS. In contrast, microglia from NAWM showed distinct responses to M2 stimuli. GC responsiveness was altered in microglia from MS NAWM, as dexamethasone led to a decreased induction of the anti-inflammatory chemokine CCL18. Figure 1 displays the phenotypic characteristics of primary microglia from the NAWM of control subjects and MS patients, assessed *ex vivo*, and the effects of GC, as observed *in vitro*.

It remains unclear to what extent our observations fit the concept of microglial priming.^{239,353} According to this concept, primed microglia are characterized by an activated morphological appearance and expression of the anti-inflammatory cytokine transforming growth factor-beta (TGF β) under basal conditions that is followed by excessive production of pro-inflammatory cytokines, most notably IL-1 β , upon induction of systemic inflammation. Though phenotypes of microglia from NAWM of MS patients were also characterized by morphological changes and upregulation of immunoregulatory molecules, we did not find any differences in TGF β mRNA levels in microglia from NAWM of control subject and those of MS patients cultured under basal conditions for 72 h. Importantly, we observed in these cells an absence of *in vitro* LPS responsiveness at 18 h, which in strong contrast with the observations done on primed microglia *in vivo*. The mechanisms underlying this LPS-responsiveness is still unclear, though various types of tissue macrophages have been reported to display similar characteristics.²⁵²

5. Future directions and concluding remarks

5.1 Assessing and modulating HPA-axis activity in MS patients

Studies of HPA-axis regulation are commonly conducted by application of the combined dexamethasone-CRH test, which does not give information on responsiveness of CRH producing neurons to inflammatory mediators. Based on the data in

this thesis and other studies, we may conclude that patients that fail to produce adequate cortisol levels in response to the inflammatory component of MS might be at particular high risk for rapid neurological decline.²² However, there are clearly ethical and practical limitations to assessment of HPA-axis responsiveness to inflammation in MS patients. Therefore, finding appropriate ways to evaluate MS patients for HPA-axis responsiveness to inflammation might be of considerable prognostic relevance.

Thus far, most studies on HPA-axis activity in MS patients are based on measurement of cortisol levels in the blood. The study by Heidbrink et al. suggests that cortisol levels in the CSF are a probably more reliable indicator of GC signaling within the brain parenchyma and are clearly related to disease activity of MS.¹²⁶ Though lumbar punctures confer significant physical discomfort, monitoring CSF cortisol levels at a restricted number of time-points during stable disease and acute relapses may provide valuable insight into the HPA-axis responsiveness towards the inflammatory component of MS pathology. Therefore, one recommendation for the clinic would be to measure/monitor cortisol levels in the CSF (and not the serum). In combination with testing HPA-axis responsiveness to inflammatory stimuli, measuring CSF cortisol levels could give a more accurate insight into immunomodulation that may be expected from endogenous GCs. This approach may serve as a prognostic tool and could help to design customized supplementation strategies for GC treatment of MS patients.

The cause of low HPA-axis responsiveness in some MS patients is not immediately clear. However, there are data that suggest that a high incidence of hypothalamic lesions may lead to desensitization of CRH-producing neurons for inflammatory cytokines. For example, close proximity of activated macrophages to CRH-expressing neurons was associated with impaired activity of the HPA-axis, suggesting that an excess of macrophage-derived cytokines abrogates sensitivity of these neurons for inflammatory mediators.²⁵ In line with this, progression of chronic EAE in rats was found to be related to a reduced HPA-axis drive by inflammation.⁹⁴

In summary, a better understanding of HPA-axis regulation in MS and the molecular mechanisms of GC employ to suppress disease activity may be of benefit for

MS patient in several ways:

- Assessment of basal HPA-axis activity and responsiveness to inflammation could be used as a prognostic tool and may identify patients that are at particularly high risk for fast progression.
- It may improve the use of GC for treatment of MS, as therapeutic regimes could be tailored to the specific needs of the patient.
- Selection of GC-induced molecular pathways associated with slow progression of MS promotes translational research and development of new therapeutic approaches.

5.2 Molecular targets identified by microarray analysis

In this thesis, I specifically focused on identifying molecular pathways in the NAWM associated with HPA-axis activity and/or disease severity, as these could represent highly eligible therapeutic targets for prevention of MS lesion development. A good example of a molecular pathway that is associated with high cortisol levels and may be of therapeutic benefit in MS is the upregulation of annexin 1 (ANXA1), also known as lipocortin 1. The upregulation of this molecule strongly suggest GC-induced molecular effects in the NAWM of MS patients, as annexin 1 is known to be an essential mediator of GC-induced immunomodulatory effects.³⁵⁴ Moreover, intracerebroventricular administration of annexin 1 protein reduced the severity of EAE in rats.³⁵⁵ Another approach for MS therapy could be based on induction of gap junction alpha-4 protein (GJA4) on endothelial cells, which was shown to be protective against recruitment of monocytes and is strongly associated with high HPA-axis activity and mild MS.³²²

5.3 Microglia in NAWM of MS patients

The availability of the newly developed microglia isolation procedure paves the way for various studies that could considerably advance the understanding of MS pathogenesis. A first evident step would be to perform transcriptome analyses on microglia isolated from different anatomical areas of post-mortem brains from control sub-

jects and MS patients. Ideally, these data should be compared to data obtained from autologous choroid plexus macrophages or monocyte-derived macrophages. In this way, microglial gene signatures may be identified that are region-, cell type- and MS-specific. Similar analyses could also be performed to identify (changes in) microRNA expression profiles and DNA-methylation patterns. Another line of research could be based on identifying the ability of microglia phenotypes to induce adaptive immune responses by performing co-cultures with HLA-mismatched T lymphocytes, in a mixed lymphocyte reaction (MLR). These MLRs could be performed directly *ex vivo* or after stimulation with inflammatory mediators and/or phagocytosis of myelin. Of relevance for all aforementioned experiments is a well-designed antibody panel to determine the expression of crucial phenotypic markers that reliably indicate the activation status of microglia directly after isolation and perhaps also after downstream experiments. Good candidate markers for this would be CD45 and CD14, as they were shown in this thesis to serve as sensitive indicators of microglial activation status. The ultimate goal of these lines of research would be to find pathways that could be targeted to modulate microglia in such a way that MS lesion formation is prevented by inhibiting microglia activation or even by reverting microglia from an alerted to a resting state. Alternatively, microglia may be specifically skewed towards a phenotype with enhanced immunosuppressive and neurotrophic properties.

5.4 Concluding remarks

On the whole, this thesis supports the conclusion that high stress-axis activity substantially contributes to suppression of MS disease activity, as the presented evidence strongly suggests that it impacts on clinical course, lesion pathology as well as cellular and molecular mechanisms in the NAWM. Finding ways to evaluate and possibly modify stress-axis responsiveness to inflammation might therefore improve the clinical care of MS patients. In addition, identifying mechanisms to modulate microglia and GC-related molecular pathways in the NAWM may represent a valuable therapeutic strategy for preventing lesion formation and disease progression in MS.