Glucocorticoid sensitivity in multiple sclerosis; what makes the difference?

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Chapter 5

Discussion

Part one: Glucocorticoid sensitivity in MS

Decreased GC sensitivity in the light of MS pathogenesis.

Several pathological states, most of them chronic inflammatory diseases, have been associated with a (relative) reduced peripheral sensitivity for GCs. These are bronchial asthma, RA, osteoarthritis, systemic lupus erythrematosus, Crohn's disease, ulcerative colitis, septic shock/ acute respiratory distress syndrome, and familial/ sporadic GC resistance syndrome (172). In this thesis we provided additional evidence that also in MS, especially RRMS, there is a decreased GC sensitivity of blood cells (monocytes).

There is strong evidence that in MS patients there is an increased HPA axis activity, at least in a larger subgroup (46). We hypothesized that increased HPA axis activity, goes along with decreased peripheral GC sensitivity. One of the arguments is that despite high levels of cortisol found in MS patients, signs of hypercortisolism are unusual. Recently, increased HPA axis activity in 62% of the MS patients has been found, as measured by the DST (135). The same study showed decreased GC sensitivity and GR affinity of lymphocytes compared to HCs (135), while no differences were found regarding the number of GR binding sites. These findings support the hypothesis that there is a disturbance in the HPA axis feedback regulation as well as decreased GC sensitivity of the peripheral target tissue (immune cells), that plays a role in the pathological state of chronic inflammatory diseases, like MS.

The feedback loop of the HPA axis and the immune system makes it complicated to unravel whether decreased GC sensitivity of immune cells in MS is a cause, in which GCs are less able to restrain the immune system, leading to ongoing inflammation, or a consequence of chronic inflammation. It is possible that both mechanisms play a role, with an increasing effect at each other.

Chrousos (44) describes two aberrant responses of the HPA axis to inflammation. The excessive response: high levels of cortisol are being produced in reaction to inflammation. This results in a relative repression of the immune system and an increased susceptibility for infections.

The second, a defective response of the HPA axis to inflammation, a relative GC deficient state occurs. Endogenous GCs have less suppressive effect on the immune system, which can result in relative resistance to infections, but increases the risk at chronic inflammatory or autoimmune diseases. A subgroup of MS patients with HPA axis hypo-activity has been found. These were relatively young MS patients with severe MS, that came to autopsy. High inflammatory activity was associated with lower levels of CRH mRNA expression in the hypothalamus and shorter time to death (45).

In a larger group of MS patients, on the other hand, signs of increased HPA axis activity have been found. The mechanism behind increased HPA axis activity in MS is unknown, but it may be a result of a reduced negative feedback mechanism. Functional studies showed a decreased response to the DST (71), and increased cortisol levels after combined DST- CRH tests (48-52).

A decreased feedback mechanism of the HPA axis, together with decreased GC sensitivity of immune cells may lead to insufficient suppression of the immune system. This may increase the effect of a hypo responsive reaction of the HPA axis to inflammation, leading to severe uncontrollable inflammation. In persons with a primary normal reaction of the HPA axis to inflammation or even in persons with an excessive response, and this may also lead to less control of inflammation.

We did not observe differences in GC sensitivity between patients in a clinically active phase of the disease versus those in a stable phase. This may suggest that GC sensitivity is an, at least partly, genetically determined factor, possibly changing under influence of chronic inflammation, rather than during acute inflammation. Evidence has been found that inflammatory cytokines in acute inflammation may represent additional factors contributing to drive the HPA axis activity during relapses, but not being the major cause in HPA axis activity (44), supporting this hypothesis.

In the introduction we described several mechanisms for altered GC sensitivity. In this thesis we have studied the possibility of variation in the GR-gene as explanation for the individual determined baseline GC sensitivity which may of influence on disease course.

Decreased GC sensitivity in relation to polymorphisms of the GR-gene

We did not find a direct association between *in vitro* GC sensitivity of peripheral blood cells and polymorphisms of the GR gene, associated with altered GC sensitivity in MS patients. Although this was a relatively small study, this may suggest that also other factors play a role in GC sensitivity in MS patients. For example cytokines (111-113), IFNβ (173), or other unknown factors can influence a basically determined GC sensitivity.

In HCs, the situation was different. We did find an association between the polymorphism N363S and decreased GC sensitivity *in vitro*. Both increased sensitivity in HCs *in vivo* as measured in the DST (94) as well as decreased *in vitro* GC sensitivity (62) have been reported. An important factor that may explain differences in assays between these studies, is that GC responsiveness is gene-, cell-, tissue-, and context-dependent (43, 103). Another potentially important factor that should be considered is the relative small proportion of carriers of the N363S polymorphism in these studies.

The mechanism by which the N363S polymorphism influences GC sensitivity has not exactly been elucidated yet. The N363S variant is located in the *trans-* activation domain. The change of asparagine to serine creates a potential phosphorylation site and may regulate DNA binding by the GR (105, 106). It has been found that the N363S polymorphism has a unique gene expression profile compared to the wild type GR gene (174).

Chapter 2.2, although based on a relatively small sample, supports the hypothesis that GC sensitivity is in part genetically determined, but also demonstrates that in chronic inflammatory disease, like MS, other factors may out weight this genetic impact.

Part Two Clinical implications

Polymorphisms of the GR-gene and disease course

There are several reasons to investigate a possible relation between variation in the GR-gene and disease course in MS. First, there is evidence for a degree of concordance within multiple families with respect to disease progression, either from onset or after a period of RRMS (175). Second, a genetic basis of GC sensitivity is supported by the finding that there is a significantly lower *in vitro* GC sensitivity in 30% of HC with small variation in 8 months (81, 110). Furthermore the response to the DST remained stable over years in healthy elderly (176).

The ER22/23EK polymorphism in the GR-gene has been associated with a decreased feedback response of the HPA axis, as measured by the DST (61). Furthermore it has an effect on the GR-A GR-B ratio, which may explain differences in peripheral GC sensitivity (62). This can be an unfavourable combination in patients with MS. Indeed, we found that carriers of the ER22/23EK polymorphism, had a more aggressive disease course as measured by the time to EDSS 6, a measure of disability progression. When haplotypes were inferred, the haplotype Tth*III*I, ER22/23EK, 9β-G, was, in a larger population, associated with a more aggressive disease course.

Drawbacks of using EDSS as measure for disease course

Disease severity in MS is difficult to measure, and several methods can be used when studying disease severity in the context of genes influencing disease course (177). When measuring disability there are some drawbacks in using the EDSS as an outcome scale. The EDSS as measure of disability does not include cognitive functions, fatigue, and hardly disability due to other than walking problems. Furthermore the EDSS score can fluctuate over time (170), especially in between a time interval of two years. The fact that the patients in our studies had long disease duration makes the EDSS score more robust. Using a survival analysis, both the proportion and time to disability can be studied simultaneously. In large natural history studies, time to reach irreversible EDSS 4, 6 or 7 (respectively 8, 20 and 30 years) seems to be a reliable measure for disease course (4). In our study, time to EDSS 6 in the noncarriers is comparable to these findings (time to EDSS 6 was 18 years (CI95% 16-20 years)).

Contrary to these findings, in heterozygous patients for the haplotype Tth*III*1, ER22/23EK, 9β, the mean time to EDSS 6 was 10 years. Another parameter for disease severity is the age at which patients reach disability milestones. This seems to occur independent from the number of relapses (4). When examining our patients we found that the median age at which patients reached EDSS 6, was 52 years. This is comparable with a median age of 54.7 for patients to reach EDSS 6 in the above named study. Carriers of the haplotype Tth*III*1, ER22/23EK, 9β reached EDSS 6 at the median age of 44 (CI95% 38 to 51 years), versus 55 years (CI95% 52 to 58 years) in noncarriers (Figure 1: p= 0.01, log rank test)).

One mentioned hypothesis is that ongoing neurodegeneration causes a steady decline of

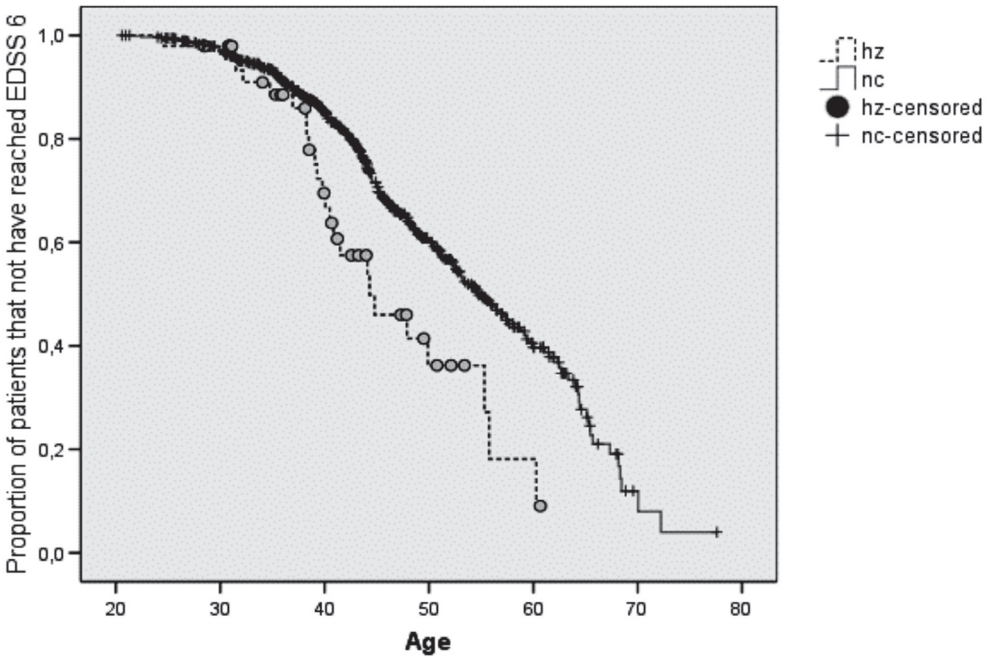

Figure 1. Kaplan Meier curve of age at which patients reach EDSS 6 in heterozygous (HZ) carriers (dashed line) and non carriers (NC) (solid line) of the haplotype TthIII, ER22/23EK, 9β. EDSS = expanded disability status scale.

the HPA axis feedback control (thus resulting in increased HPA responses). This could be due to the relative resistance of the GR at the CNS level, leading to decreased response in the hypothalamus, hippocampus and prefrontal regions, which are brain areas involved in the GC feedback control, leading to increased HPA axis activity (124, 178).

At some point, the HPA axis hyperactivity might seize control of the inflammation. Clinically, this point would be expected to coincide with the transition from RRMS to SPMS (46). It could be hypothesized that this point, is influenced by the genetically determined GC sensitivity based on variation in the GR-gene. The findings in figure 1, suggests that this haplotype influences the time at which degeneration occurs. In chapter 3.2 we found that patients having this haplotype, that had a larger T1 lesion volume on MRI, which can be considered as an indication of a more destructive disease process (127).

Assuming that decreased GC sensitivity, based on a change in the GR- gene, goes along with HPA axis hyperactivity. The above finding is in line with evidence from one study, suggesting a direct relation between HPA axis hyperactivity and neurodegeneration (129). Also, it has been shown that HPA axis hyperactivity in patients with progressive disease (SPMS and PPMS) correlated with disability (48, 49).

When taking a close clinical look at the patients with the haplotype TthIII1, ER22/23EK, 9β in our study by a neurologist, who did not know anything about this group of patients, it emerged

that patients who were having fast progressive disease, had high EDSS scores, myelopathy, PPMS or diffuse spinal cord problems were overrepresented in this group (approximately 75%). Response to IFNβ was in general not very good, or they had not been treated although-, or because of-, a fast disease progression took place. This strengthens the observation that these patients had a more aggressive disease course.

GC sensitivity and IFNβ treatment

An interesting observation was that the use of IFNβ was found to be associated with a lower GC sensitivity. This may point towards a direct effect of IFNβ on GC sensitivity or may be due to selection bias, i.e. patients with a more aggressive disease course being more frequently treated with IFNβ.

Several studies have shown that IFNβ can influence the HPA axis. Higher levels of cortisol and ACTH suggesting hyperactivity of the HPA axis were found direct after injection with IFNβ in patients with cancer, chronic hepatitis as well as in HC, and recently in MS patients. In most MS patients this activity decreases after 3-6 months, and HPA- axis activity attenuates. Except in one patient with frequent relapses and most signs of inflammatory activity on MRI, the HPA- axis activity increased (173, 179). It was suggested that decrease in HPA axis activity, as assessed by DST-CRH tests, could be an indication for long-term benefit of IFNβ therapy.

Drawbacks of candidate studies

In this thesis we used candidate studies to investigate the role of SNP's on disease course in MS. In the past, several candidate studies have found polymorphisms that were associated with disease course (2). However these could not be confirmed in later studies. In candidate studies, one compares the genetic make-up of those with and without the disease and seeks to identify what is different. Association studies are known for several limitations. Important is that the study population is homogenous and well phenotyped. There is a relative large chance of being false positive if not corrected for multiple testing. On the other hand they are often underpowered because the tested SNP's are relatively rare. In our study, eight percent of the MS patients, as well as of the controls were heterozygote for the haplotype Tth*III*1, ER22/23EK, 9β (allele frequency 3.4%), which is comparable with other studies. We were able to confirm the results of the study in chapter 3.2 in an independent cohort of patients in chapter 3.3. Furthermore, from many of the studied polymorphisms it is uncertain whether they are really functional. The polymorphisms in this study have been associated with altered GC sensitivity, and especially for the ER22/23EK polymorphism there is strong evidence that this is a functional polymorphism. Taken together, the results from the studies in this thesis satisfy quite well to the requirements to assure quality of association studies (140).

Stress in MS

Since MS for the first time has been described by Charcot in 1877, psychological stress has been considered as a triggering factor for exacerbations. Although a possible relation between

relapses and stressful life events has been suggested in later studies, these do not permit causal inferences (180). Stressful situations result in aberrant activation of the HPA axis in a way that frequently results in increased cortisol levels. These would theoretically restrain the immune system and protect against or shorten an exacerbation, instead of provoking an exacerbation. Several studies have found a delay between the stressful event and an exacerbation, and that has led to the stress resolution hypothesis (181).This suggests that the dissipation of stress with the accompanied decrease in cortisol level facilitates the development of active inflammation and increases the risk for an exacerbation a few weeks after the onset of stress. Recently, evidence came from a study during war stress in which there was a significant raise in number of exacerbations (182). In that situation, the exacerbation occurred at the moment of the highest level of stress. One of the problems in studying stress responses is the definition of a stressful life event. A proposed definition is: an environmental triggering event (stressor), which overwhelms the individual's ability to cope or adapt, and results in a psychological or biological reaction. There is a lot of variability in these stressors, they may be chronic or acute, more or less severe, or differ concerning the source (work related, familial etc.). Furthermore coping strategies also differ between and within individual patients (47). The interaction between decreased GC sensitivity in MS patients, or between the haplotype Tth*III*1, ER22/23EK, 9β, and stressful live events, remains speculative but intriguing.

Several years ago doctors tended to advise MS patients to avoid stressful events, however there are no data that suggest that MS patients should refrain from controllable time limited activities associated with psychological stress or physical activities, particularly if they are meaningful to the individual (47). There are few studies on a positive effect of psychological interventions and stress management, and more data are needed (183).

Part three clinical outcome measures

Outcome measures to detect clinical response to IVMP

Due to poor responsiveness of the EDSS, detecting changes after IVMP treatment in MS is difficult. In recent years, new outcome scales, including patient orientated outcomes cales, which may be more clinical meaningful, have become more important. Chapter 4.1 shows that different outcome scales measure different aspects of treatment effect after IVMP. We compared responsiveness of the EDSS with the MSFC, a quantitative physician orientated outcome scale, and with the GNDS, a patients oriented outcome scale. It turned out that the GNDS seemed most sensitive to detect change when compared with the EDSS, as well as when compared to patient's perception. It was noted that the changes reported in the GNDS were concerning rather subjective measures, like cognition, mood, fatigue, and others (e.g. pain or spasm). This gives an important signal that patient's perception can differ from physician based measures. However the problem with the GNDS is that the exact cut off point to detect a significant change is not clear. The sensitivity to detect improvement was low for the MSFC.

Since long the EDSS is being used as the clinical outcome measure to determine patient's

clinical status, or to measure treatment effect. However it has a low responsiveness to change, and the correlation with patient's perspective of a clinically meaningful change is low. Recently, it has been shown in primary progressive MS, that a 20% worsening in the T25FW (24), a quantitative test of ambulatory function, has a higher event rate than the EDSS. Combining EDSS and T25FW resulted in a further increase (158).

In chapter 4.2 we used two components of the MSFC, the T25FW and the nine hole-PEG test (9-HPT), instead of the 'complete' MSFC. We compared the T25FW, the 9- HPT and the EDSS, as well combined tests, with patient's perception, using a transition question. The patient was asked whether he/she has experienced a change since the start of the therapy. The combination of T25FW and 9-HPT, using a cut-off of 20% as significant change turned out to be the optimal combination of measures to predict patient's perceived improvement. Determining clinically significant change on the basis of a change in one out of two scales allows us to detect a higher number of patients who show a clinically significant change, without leading to a concomitant loss of accuracy in terms of being relevant to the patient.

Although it has been shown that the scores on outcome measures for deterioration and improvement are not necessarily equal (168, 169), it is interesting to elaborate on the results of chapter 4.2 in the light of MS clinical trials on DMT. Especially in patients with an EDSS below 4.5, who are by far the largest population in the pivotal trials for the currently approved DMT, the accuracy of the EDSS change is disappointing.

So far, there has been strong persistence among scientists, clinical trial designers and regulatory authorities in adhering to the EDSS to document disability progression in MS clinical trials and reluctance to accept alternative outcome measures, which is partially due to concerns about the clinical relevance of changes on these alternative outcome measures. The study in chapter 4.2 shows that this is not justified.

It is challenging to find outcome scales that are responsive to clinical relevant changes in patients with a relatively low disability status. For all measurements included in the study in chapter 4.2, a significant change in the higher disability range was more often perceived as clinically relevant than in the lower disability range. Patients in the low disability range, the positive predictive value (PVV), which points to the likelihood that the significant change is relevant to the patient, was lowest for the EDSS score (56%) and highest for the 9-HPT (70%). The optimal and most accurate combination of tests in this group was T25FW and 9-HPT with a PPV of 70% and a negative predictive value (NPV) of 63%, and a positive likelihood ratio of 2.84.

Future perspectives

As always in scientific research, there are several remaining issues. These need to be addressed in future studies. Several questions have been raised from this thesis.

GC sensitivity and pathogenesis

An important question that remains is whether decreased GC sensitivity in MS is a cause or a consequence of chronic inflammation. In this thesis we have found evidence for a genetic

influence on GC sensitivity, but other factors may influence GC sensitivity during life, for example the disease itself, (ongoing inflammation) and IFNβ therapy. The regulation of GCs is under the control of the HPA axis, which is a feedback mechanism. Further insight in these mechanisms may help to find prognostic factors for disease progression or treatment response and may be helpful in finding therapeutic strategies for MS.

A longitudinal study of MS patients early in the disease, in which GC sensitivity as well as feedback of the HPA axis will be monitored, for example in every season each year, is recommended. It may reveal information about HPA- axis activity in relation to peripheral GC sensitivity. Do MS patients have a hypo responsive HPA axis activity to inflammation in the early phase changing to hyperresponsivity? Does dysregulation of the HPA axis activity occurs first, or does the peripheral GC sensitivity changes first. To what extend are these changes related to the transition from the RRMS to the progressive phase of MS? We hypothesize that GC sensitivity plays a role in the timing of the neurodegeneration process getting the upper hand on the inflammation process, i.e. how fast a patient will reach the progressive phase or the age in which a patient reaches the progressive phase. The latter being a factor indicating disease progression, which occurs independent of previous relapses.

New MRI measures have been developed, focussing on the normal appearing brain tissue and brain atrophy, that seem to correlate better with clinical disability (184, 185). They may turn out to be better parameters for disease severity.

Clinical outcome scales, which have been shown to be clinical meaningful to patients, including patient orientated outcome scales should be used to monitor the clinical effects of changes in HPA axis activity and GC sensitivity.

The role of genetic variability in the GR-gene has to be further addressed. What is the relation with the haplotype Tth*III*1, ER22/23EK, 9β and peripheral GC sensitivity and HPA axis activity in a longitudinal study? It could be hypothesized that persons have a genetically determined baseline GC sensitivity of immune cells, which decreases due to chronic inflammation during the disease. It could be hypothesized that GC senoitivity of immune cells is genetically determined, however, chronic inflammation during the disease course may decrease this GC sensitivity.

GC sensitivity and therapy response

We found evidence for a predictive value of *in vitro* GC sensitivity and response to IVMP. *In vitro* response before IVMP could be used to predict the treatment effect of IVMP in an individual patient in the situation at that moment. This can possibly influence the dose of IVMP, leading to an individual best dose, with a balance between best effect and lowest side-effects. Further research on this hypothesis is warranted.

Chapter 2.1 provided additional clues for an association between IFNβ treatment and GC sensitivity. There is evidence that IFNβ influences both GC sensitivity and HPA axis activity. Whether this is part of the mechanism in which IFNβ exerts its treatment effect, is not known. On the other hand GC sensitivity may influence response to IFNβ. Further research about the prognostic value of GC sensitivity as well as HPA axis activity, in response to IFNβ treatment,

can be valuable in making the decision which patient to treat with IFNβ or with another type of DMT.

The haplotype Tth*III*1, ER22/23EK, 9β may play a role in predicting treatment effect IFNβ. We did not direct this question, but when we had a closer look at the patients having this haplotype, patients who did not respond to IFNβ seemed to be overrepresented. This, however, is just an observation; further study about this subject is worthwhile.

Several studies have investigated the role of the polymorphism ER22/23EK, whether or not in combination with other GR-gene polymorphisms, in chronic inflammatory diseases (Crohn's disease (64), Graves opthalmoplegia (65), psoriasis (66) and RA (58, 67)). These studies included relative small groups of patients and inconsistent results were found. So far, only one study has addressed the question whether polymorphisms in the GR-gene have an effect on treatment with GCs in patients with asthma. No association was found, perhaps because of the small number of patients included (186).

GC sensitivity and prognosis

So far it is not possible to predict the disease course in an individual MS patient. This insecurity about the disease course probably has a negative influence on the quality of life. Furthermore, since there are several treatment options, ranging from having a mild to an intensive influence on the immune system, one should be able to balance the risk of a severe form of MS against side effects of an intensive therapy.

There are some known risk factors for a more severe disease course, but these have been shown only on group levels. Characteristics of the relapses in the first years of the disease and the occurrence of a progressive phase seemed to be most reliable (4). However, recent studies have shown that age at which progression occurs (time reached irreversible EDSS 6) is independent from previous relapses (4).

The development of a large number and volume of lesions during the initial years of MS has been strongly associated with a greater risk of disability occurring in the later years (7).

GC sensitivity may play a role as a prognostic factor for disease course. One longitudinal study showed that Dex-CRH hyperactivity significantly predicted disease progression over a three year follow up (178).

Several candidate studies have found polymorphisms which were associated with disease course (187) (177). However, most of them could not be confirmed in later studies or await confirmation. A proposal has been done to develop a predictive tool, based on a clinical score, however this has to be validated (188).

Ideally, it would be possible to make an individual profile based on several factors, (e.g. clinical and MRI parameters, MRI, genetic factors, and biomarkers (189)), which will help to identify MS patients with a high risk for an aggressive disease course. MRI techniques are being developed that will be more able to differentiate between several pathogenetic processes, and may be helpful in predicting disease course. They seem to be more sensitive to detect

pathology, than the current MRI techniques, for example atrophy measures and measuring the normal appearing brain tissue (NABT).

The Tth*III*1, ER22/23EK, 9β haplotype has a low allele frequency; heterozygotes could be found in 8% of our studied population. Furthermore, one has to be careful being too enthusiastic about results from candidate studies, because of the mentioned drawbacks. However, we did found a hazard ratio of 2.3 which is more than the LOD score of the recently found genetic factors for susceptibility. Therefore the TthIII1, ER22/23EK, 9β haplotype could be, together with other factors, a factor that can be part of such an individual profile to identify patients who will develop an aggressive disease course in MS (and perhaps in other chronic inflammatory diseases). Patients who are homozygous for this haplotype, which is very rare, may have an even more aggressive disease.

Influencing GC sensitivity as a therapy in MS

Once GC sensitivity is better understood, normalisation of GC sensitivity could be considered as a way to influence disease course. However it is important to realize, when influencing GC sensitivity or HPA- axis activity, that part is genetically determined. So GC sensitivity probably can only partly be influenced. For this purpose, identifying other factors that influence GC sensitivity is important.

There is some evidence that IFNβ influences HPA axis hyperactivity, possibly via increasing numbers of GR 's (190). It is not clear, however, if this is one of the mechanisms by which IFNβ exerts its effect.

There are some parallel findings between MS and major depression. Hyperactivity of the HPA axis, as well as decreased GC sensitivity have been found in patients with major depression (191). Furthermore the polymorphism ER22/23EK (as well as the *Bcl*I 1) was associated with susceptibility to develop major depression (63). In depressive patients, it has been shown that antidepressants are able to normalize HPA axis activity (191) .For this reason, this effect of antidepressants has also been studied in MS patients. In one small placebo-controlled double blind randomized trial in RRMS patients, normalisation of the HPA axis activity (52) was found after treatment with meclobemide, in combination with GCs. It was hypothesized that normalisation of the HPA axis improved response to therapy with GCs. From daily practice, we have never noticed that patients on antidepressants do better on IVMP treatment, or do have a less aggressive disease course. It can be hypothesized that MS patients who also develop a depression, do have a more pronounced HPA axis dysregulation, or may have a more decreased GC sensitivity, and that reducing HPA axis hyperactivity via antidepressants has less impact in this group. For that reason it is worthwhile to investigate these hypotheses in a prospective study.

Future perspective with regard to more fundamental research

In the recent years the use of micro arrays offers the opportunity to investigate up and down regulation of larger numbers of genes. This is a hypothesis- generating way of research, because most of the time there is no a priori hypothesis.

GCs are involved in an amazing array of functions, including every aspect of the resting and stress related homeostasis. It was found that 20% of the expressed leukocyte genome was positively or negatively affected by GCs (192). Several other studies have also been examining GR directed gene expression. Although these studies differ with respect to cell type, ligand microchip, and p-value for analysis, it is evident that the GR is actively involved in the transcription, whether up regulation or down regulation, of many genes (43).

Investigating the effect of *in vivo* GCs after IVMP by comparing gene profiles before and after IVMP could learn us more about the way GCs exert their function in MS patients. Responders versus non responders may have different gene profiles. Knowing these profiles may be helpful in determine indivual dose of GC, and may help to predict treatment effect. One has to realize that treatment effect is not so easy to measure, and it is important to use outcome scales that are among others, clinical meaningful to the patient.

To better understand the effect of the haplotype TthIII1, ER22/23EK, 9β, micro array studies could be done investigating whether different gene profiles are becoming activated in carriers and non carriers, and what implications this haplotype has in relation to response to treatment with IVMP as well as with IFNβ.

Conclusion

Blood cells of MS patients are less sensitive for GC compared tablood cells of healty controles. We hypothesize that in general GC sensitivity is at least partly genetically determined factor, which is additionally influenced by environmental factors such as inflammation, IFNβ treatment and other, yet unknown, factors.

The polymorphism ER22/23EK decreases GC sensitivity and has been associated with increased HPA axis activity, which is thought to reflect a disturbance in the negative feedback of the HPA axis. This combination results in an unfavourable situation in patients with chronic inflammation, like in MS. We found that the haplotype Tth*III*1, ER22/23EK, 9β, is associated with a more aggressive disease course in MS. Further studies are warranted to investigate whether this haplotype can be used as prognostic factor identifying patients who will develop an aggressive disease course.

Second, in this thesis we evaluated outcome scales to measure treatment response to IVMP. We found additional evidence that the EDSS seems less responsive to changes that are clinical meaningful from the patients' perspective. We showed that combined measures, like the T25FW and the 9-HPT, allows us to detect a higher number of patients who show a clinically significant change, without leading to a concomitant loss of accuracy in terms of being relevant to the patient.

This may help to define more optimal outcome measures since, outcome scales that have to detect not only improvement after IVMP, but also outcome scales that have to detect disease changes in disease progression as in trials, have to be clinical relevant to the patients.