Chapter 4

General discussion and future perspectives
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The first research question was already developed in the introduction. In brief, we ask (1) what is the role of MxA mRNA as a biomarker for bioavailability of recombinant IFN-β and (2) what is its value as a marker for IFN-β treatment response? The study described in chapter 2.1 confirms a titer dependent correlation between MxA mRNA expression and the development of NAb. MxA mRNA and NAb were tested for after a mean treatment duration of 4.8 years at two time-points with a median interval of 3 months between measurements. A second measurement was performed only when MxA mRNA expression was negative or suboptimal at the first assessment. In contrast to most studies evaluating MxA mRNA as a biomarker for treatment response, we used a 4 hour interval instead of a 12h interval. The smaller time interval was chosen to allow for pre- and post-IFN-β injection samples to be taken on the same day. Although a peak response of MxA mRNA to IFN-β is shown to be around 12-13h, a significant response is already seen after 4h\(^1\). The importance of pre- and post-injection samples was confirmed in our study. Because we were able to evaluate not only absolute expression values, but also the rise in response directly after injection (MxA mRNA induction) at second visit, two false negative results for IFN-β bioactivity were avoided. For screening purposes however, a single post injection measurement was recommended. One patient showed good biological availability of IFN-β, i.e. high MxA mRNA expression after IFN-β injection, in the presence of high-titer NAb (defined as NAb titer >150TRU/mL). This is in contrast to studies showing complete abolishment of IFN-β bioactivity with high titers of NAb. However, cut-off values used for NAb positivity vary between laboratory and with test used. For example, titers determined by an MxA induction-based assay results in titers that are 4-5 times higher than titers found with the cytopathic effect assay (CPE) which was used in this study\(^2\). On the other hand, some patients showed a negative MxA mRNA response to IFN-β without the presence of NAb. Non-antibody-mediated absence of IFN-β bioactivity has been suggested in other studies to be the result of circulating soluble IFNAR receptors\(^3\), to non compliance, to ineffective IFN beta injections or to laboratory error\(^4,5\). A shortcoming of this study is that 10 of the 55 patients invited for second measurement were unavailable for MxA testing, most because of treatment cessation. In these patients, 9 were tested for NAb and 4 were NAb positive. It seems therefore unlikely that NAb status and associated treatment failure was the sole reason for treatment cessation.

In chapter 2.2, we studied the association of biological non-responders to IFN-β, defined as patients with a negative or suboptimal MxA mRNA expression and an absent MxA mRNA expression and induction after 3 months, with the occurrence of clinical relapses in 126 MS patients treated with IFN-β for a median of 48 months. A higher annualized relapse rate and a lower likelihood to remain relapse free were found in biological non-responders to IFN-β. Possible false positive results of MxA mRNA expression because of a viral infection, again, cannot be ruled out. It would be interesting to see if NAb block the MxA mRNA response
not only in response to IFN-β injections but in response to a viral stimulant as well. This has not been studied in vivo to our knowledge. Analysis with MxA mRNA expression levels alone at two time-points 3 months apart, without combining them with induction results, resulted in a significant higher relapse rate in patients with an absent MxA mRNA response, but not in a significant lower proportion of relapse-free patients. In this study the combination of MxA mRNA expression and induction seems a more robust method to identify poor treatment response. A shortcoming of this study is that not all measurements were performed in all patients at both visits. As a consequence the analysis of ‘biological responder status’ and relapse rates was performed in a smaller group, leaving out the patients with initial good MxA mRNA response, as measurements at both visits were needed to determine response status. Furthermore, we looked at the number of relapses and corticosteroid use in the year before testing for MxA mRNA. This period selection for looking at clinical disease activity is arbitrarily chosen for purposes of clinical usefulness. Perhaps a longer period for clinical evaluation would result in a different outcome, although one would expect a better correlation with longer follow-up. We conclude that MxA mRNA is a useful biomarker for monitoring IFN-β treatment response, as MxA response to recombinant IFN-β is stable over time, can indicate the presence of NAbS, is -when absent- reflective for the total loss of IFN-β bioactivity and is correlated with MS disease activity. MxA mRNA measurement has some advantages over the use of NAbS as a treatment response marker, as NAbS measurements are difficult to standardize between laboratories, controversy exist over at which NAbS titer level IFN-β bioactivity is completely abolished and MxA mRNA addresses also non-antibody mediated reduction of IFN-β bioactivity. Also, for NAbS a phenomenon is described by some authors, where a certain level of antibodies against cytokines results in a longer lifetime of the cytokine-receptor complex by protecting it against metabolic degradation or that early neutralizing antibodies with low (still developing) affinity to IFN-β enhance the effect of an IFN-β injection in the first 6 months. MxA mRNA on the other hand, does not differentiate between poor compliance, ineffective IFN-β injection, circulating soluble IFNAR and NAbS. In addition, MxA mRNA does not provide information on the likelihood of NAbS persistence, which the titer levels of NAbS do. Furthermore, disease breakthrough during IFN-β treatment does not always reflect biological responsiveness to treatment, but can also reflect the spontaneously occurring variation in underlying disease activity. In chapter 2.3 we investigated the relationship between four IRF5 gene-associated polymorphisms and the induction pattern of IFN-regulated genes (the pharmacological response) to IFN-β treatment response in 30 RR-MS patients. A correlation was found for rs2004640 and rs4728142, but not for rs10954213 and 30-bp insertion-deletion gene variants. Patients who are homozygous for the rs2004640 T risk allele or the rs4728142 A risk allele showed a low or absent induction of 10 IFN-β-response genes, reflecting a poor pharmacological response to IFN-β. In 73 RR-MS patients from Cataluña and Amsterdam two
MRI scan during IFN-β at least 12 months apart were available. In this group homozygosity for the rs2004640 T allele was associated with the development of new T2 lesions during IFN-β treatment. In order to investigate whether these two IRF5 polymorphisms were related to clinical relapses, we determined in an independent validation cohort of 261 RR-MS patients from Harvard and Amsterdam the time to a first relapse during IFN-β treatment. In patients homozygous for rs2004640 T allele the time to a first relapse was shorter than in others, only a trend was observed for patients homozygous for rs4718421 A allele. Shortcomings of this study are considerable, as groups were analysed with different outcome measures and clinical follow-up varied between centres and groups. Furthermore, as NAb status was unknown in these patients, antibody-mediated reduction of treatment efficacy was not accounted for in the analysis. NAb status was unlikely to influence the MRI results, however, as selected MRI scans were performed mostly in the first or second year of IFN-β treatment and NAbS are developed relatively late (most between 12-18 months). IRF5 polymorphisms are associated with susceptibility to several auto-immune diseases, among which MS was confirmed in three independent cohorts. The rs4728142 SNP was found to have a strong association with MS, rs2004640 was found in one cohort, but not confirmed in the other two cohorts for the association with MS. A trend for IRF5 rs3807306, but not for rs4728142 was seen in predicting treatment response to IFN-β in a study of 106 responders and 112 non-responders to IFN-β treatment. Furthermore, a recent study demonstrated that a high expression of IRF5 on macrophages, resulted in switching to a more pro-inflammatory response in macrophages and facilitating a potent T helper cell 1 (TH1) and 17 (TH17) response. Experimental data suggest that IFN-β inhibits IL-17 in vitro and is beneficial in mice with TH1-induced experimental autoimmune encephalomyelitis (EAE), a mouse model for MS. But IFN-β seemed ineffective in TH17-induced EAE and even aggravated signs and symptoms. In this study, MS non-responders to IFN-β showed higher pre-treatment levels of IL-17F, a cytokine produced by TH17 cells. This suggests a biological pathway involving both IRF5, interferon type I and TH17 for IFN-β treatment response. Unfortunately, the potentially predictive value of pre-treatment levels of IL-17F on treatment response could not be confirmed in a large multi-center study, which included MRI in the definition for treatment response. Future, prospective studies using MRI, clinical relapses and disability progression as outcome measures combined with information on NAbS status may be warranted to confirm the role of IRF5 polymorphisms in predicting poor treatment response to IFN-β.

Chapter 2.4 focuses on the relevance of IL7R genotype and mRNA expression in a prospective Dutch cohort of MS patients. Also, the possible influence of IL7R splice variants, and the subsequent change in soluble/membrane-bound IL7R ratio, on MS disease activity and IFN-β treatment response is evaluated. The homozygote C (risk allele) genotype of the exon 6 SNP rs6897932 was confirmed as a risk factor for the development of MS in a Dutch population.
of 687 MS patients with an odds ratio of 1.65, when compared to 174 unrelated Dutch Caucasian healthy controls. Carrier-ship of the C risk allele was predominantly present in the secondary progressive MS subtype, but no association was found between genotype of rs6897932 and disease severity as measured with relapse rate, EDSS, MSFC and MRI during two years of follow-up. Other large studies found no correlation as well between IL7R SNP and markers of disease severity\textsuperscript{29,30}. Genes involved in MS susceptibility may not be the same genes that are involved in disease severity\textsuperscript{31}, although some genes, predominantly in the HLA region, seem to be involved in both\textsuperscript{32,33,34,35,36}. In a smaller subgroup of 95 RR-MS patients with a similar genotype distribution of rs6897932 as the original cohort and selected on the basis of availability of mRNA samples, functional analysis of the IL7R SNP rs6897932 analysis was performed. In contrast to a previous study\textsuperscript{29}, the relative expression of membrane-bound to total IL7R mRNA expression did not correlate with disease severity, which was confirmed in a more recent paper\textsuperscript{37}. A subgroup of these patients was treated with IFN-β during follow-up. No correlation between IL7R expression and treatment response was observed. This negative finding could be the result of the small number of patients and thus a limited power to detect differences. Alternatively, it could be explained by differences in mRNA expression of different cell types used (whole blood versus peripheral blood mono-nuclear cells (PBMC)) or variations in treatment regimes at follow-up. Also, the effects of relative IL7R mRNA expression could change with disease duration and may be more apparent in the secondary progressive phase. Surprisingly, no association was found between genotypes of SNP rs6897932 and relative membrane-bound to total IL7R mRNA expression. This supports however, previously found contradicting results in studies that compare genotypes with mRNA expression\textsuperscript{29,38}. Recent work shows that IL7R mRNA expression is regulated not only by splicing, but by other post-transcriptional mechanisms as well, such as alternative polyadenylation. In this context, cleavage and polyadenylation specificity factor 1 (CPSF1) has been shown to promote skipping of exon 6, leading to less full-length cell membrane bound IL7R\textsuperscript{39}.

Chapter 3.1 focuses on the role of endogenous IFN-β, as measured with spontaneous MxA mRNA expression, as a disease modulator in MS. In a prospective cohort of 116 untreated patients with early MS, spontaneous MxA mRNA expression was found to be related to clinical exacerbations, the number of contrast enhancing lesions (CELs) on MRI and to be indicative for the time to a subsequent relapse. Thus, supporting the value of spontaneous MxA mRNA expression as a biomarker for disease activity. A lower MxA mRNA expression was seen in MS patients compared to healthy controls, suggesting a different activity level of endogenous IFN-β pathways. In contrast, other studies found MxA mRNA levels to be comparable between healthy controls and treatment naïve MS patients\textsuperscript{24} or even higher in untreated MS patients compared to healthy controls\textsuperscript{35}. MxA mRNA expression at baseline was expressed significantly higher during clinical remission, compared to patients in a
clinical exacerbation. This is unlikely to be the result of a viral induced MxA mRNA response as no clinical symptoms of a viral infection were reported and leukocyte counts were normal at that time in all patients. Although, sub-clinical viral infections can not be completely ruled out. The variation in baseline untreated MxA mRNA expression in MS patients is a remarkable finding as MxA mRNA levels have been shown to be stable over time in humans and are constant during IFN-β treatment over time. These findings could suggest a ‘protective’ role for a higher level of type I IFN activity in MS. A cross-sectional association of baseline MxA mRNA expression with CELs on baseline MRI was found, but not with CELs on follow-up MRI at year 1 and 2. This was not an unexpected finding, as a temporary breakdown of blood-brain barrier in pro-inflammatory conditions, shown by gadolinium leakage, is a well known finding and is less likely to be preserved over time. Contrast enhancement of active lesions on MRI is only visible for about 3 weeks, and could easily be missed with yearly scans. For the use of CELs on MRI for disease activity for example in treatment monitoring, monthly MRI are recommended. MxA mRNA at baseline showed no correlation to the development of new T2 lesions on MRI in two years of follow-up, although it did predict the time to a subsequent relapse. This dissociation between clinical relapses and MRI was unexpected and may be explained by the use of fixed time points for MRI (at year 1 and 2). Furthermore there was no predictive value of the number of T2 lesion on the baseline MRI for future relapses. This is in contradiction to several studies that show a predictive value of MRI lesions for clinical relapses and the time to conversion to clinically definite MS (CDMS) in CIS patients. A possible confounder in this study is the initiation of disease modifying treatments in some but not all patients during follow-up. Although the portion of patients started on treatment during follow-up was comparable between patients with a high MxA mRNA expression and a low MxA mRNA expression at baseline, treatment regimes could have unevenly influenced the radiological and clinical measures of disease activity. There is no evidence that MxA mRNA directly mediates disease activity in MS. Although in this study only MxA mRNA was measured, most studies evaluating IFN-β activity measure MxA together with other IFN-regulated genes and find similar results in all responses.

Chapter 3.2 discusses if NAbs against IFN-β can persist after treatment cessation and what the possible effects of these long-term persisting NAbs are. In our study, we confirmed the existence of NAbs persisting long after IFN-β treatment discontinuation in 17 of 71 (24%) MS patients, with a median follow-up interval of 25 months, but up to 61 months. Persisting NAbs were reported in two previous smaller studies as well. NAbs against IFN-β-1a S.C. were more likely to persist after treatment cessation, and persisting NAbs were often high-tighter NAbs (65%). Thus, confirming the higher likelihood for patients on IFN-β-1a to develop high-tighter NAbs during treatment and confirming the predictive value of the level of NAbs titers for persistence over time. The reason for the maintenance of NAbs after cessation of IFN-β therapy is speculative. One explanation could be long-lived plasma
Another possible explanation is that recombinant IFN-β cross-react with wild-type IFN-β, and it is therefore conceivable that NAbs-producing B-lymphocytes are activated by intermittently produced natural IFN-β, for example during viral infections. Persisting NAbs positive patients in our study, demonstrate a more severe disease course. NAbs negative patients showed a reduction in relapse rate after treatment cessation, compared to pre-treatment phase. This was not found in persisting NAbs positive patients; some even showed an increase in relapse rate. Persisting NAbs positive patients progress faster to an EDSS of 6 and were more often switched to second-line treatment, i.e. mitoxantrone or natalizumab. The mechanisms through which persisting NAbs negatively influence MS disease course are unknown and remain speculative. The tendency to develop and sustain NAbs could reflect a more active immune system and as a result show a more severe disease course. This was somewhat supported by our finding that pre-treatment relapse rates were slightly higher, although not significantly higher, in patients that would develop persisting NAbs later on. On the other hand, patients that develop anti-IFN-β NAbs do not have a higher tendency to develop NAbs against natalizumab. Another possible explanation is that persisting NAbs change the activity of IFN-β regulated pathways by blocking ‘natural’ or endogenous IFN-β and in this way negatively influence disease course.

Conclusions and future work

The impact of antibody-mediated reduction of IFN-β treatment efficacy is considerable. The best way of testing for NAbs, is formulated in recent transatlantic recommendations (Table 1). Testing for NAbs is routinely advised during IFN-β treatment, biological activity with MxA mRNA can provide additional information when NAb titers are low and intermediate (Table 4 of the article). If in the presence of persisting low or intermediate NAb titers MxA bioactivity is absent, treatment switch should be considered and is even recommended when the patient is doing poorly. In patients who are doing poorly, high-titer NAbs at one time point is sufficient to recommend therapy switch. In stable patients, NAbs measurement should be repeated and when high-titer NAbs persist, therapy switch considered. One could argue that the last recommendation can be stricter, as high-titer NAbs block all biologically active treatment and are likely to persist for years. With persisting high-titer NAbs it may not be correct to let the clinical course be the deciding factor, as MS course is variable and even untreated patients may do well for long periods of time. These formulated recommendations may be a long way from the clinical practise, as has been indicated by a questionnaire study initiated in the UK in 2007, reporting that most MS neurologists do not use NAb tests in a standardised way for clinical decision making. In the Netherlands, the frequency of testing for NAbs has increased (no published data), but no information is available on the treatment decisions that may have followed. Despite newly available drugs, most MS patients are still treated with IFN-β. In addition to MS patients treated with IFN-β as a first-line treatment,
there will also be patients who are switched to IFN-β after discontinuation of natalizumab as a de-escalating strategy to prevent clinical worsening because of immune reconstitution inflammatory syndrome. Monitoring for anti-IFN-β NAb will remain an important theme for the treating neurologist.

Table 1. Recommendations on the use of NAb measurements.

<table>
<thead>
<tr>
<th>NAb negative</th>
<th>Diagnostic recommendation</th>
<th>Treatment recommendation</th>
</tr>
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<tbody>
<tr>
<td>Doing well</td>
<td>Repeat at 12 months</td>
<td>No change</td>
</tr>
<tr>
<td>Intermediate disease activity</td>
<td>Repeat at 12 months</td>
<td>Consider continuation of therapy*</td>
</tr>
<tr>
<td>Doing poorly</td>
<td>Do not repeat</td>
<td>Switch therapy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Low NAb titer</th>
<th>Diagnostic recommendation</th>
<th>Treatment recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doing well</td>
<td>Repeat at 3-6 months</td>
<td>If low titer is persistent, consider MxA assay</td>
</tr>
<tr>
<td>Intermediate disease activity</td>
<td>Repeat at 3-6 months</td>
<td>If no MxA bioactivity, consider switch to non-IFN-β therapy</td>
</tr>
<tr>
<td>Doing poorly</td>
<td>Do not repeat. May consider MxA assay for additional information</td>
<td>Switch therapy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High NAb titer</th>
<th>Diagnostic recommendation</th>
<th>Treatment recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doing well</td>
<td>Repeat at 3-6 months</td>
<td>If high titer is persistent, consider switch to non-IFN-β therapy</td>
</tr>
<tr>
<td>Intermediate disease activity</td>
<td>Repeat at 3-6 months</td>
<td>If high titer is persistent, consider switch to non-IFN-β therapy</td>
</tr>
<tr>
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<td>Do not repeat</td>
<td>Switch therapy</td>
</tr>
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The Interferon type I pathway can be important for predicting individual response to IFN-β response early on. DNA micro-array studies looking at transcriptional profiles of IRGs in PBMCs before and during treatment, suggest that an activated IFN type I signature and a low or absent induction of IRGs after treatment initiation is associated with a poor response to treatment. Furthermore, single nucleotide polymorphisms studies looking at genes involved in IFN type I pathways show variations in treatment response. In this respect not only IRF5 is suggested but also glypican 5 (GPC5), adenosine deaminase RNA-specific (ADAR) and IFNAR2. Interestingly, most pharmacogenomics studies did not systematically consider the presence NAbs in their analysis, which could be a considerable confounder.

Better understanding of the association of the IL7R *CC individuals with autoimmune disease is provided by the discovery that an enhanced amount of soluble IL7R, which occurs in these patients, is associated with enhanced IL-7 bioactivity by securing IL-7 levels over time. The IL-7/IL7R signalling pathway has also been implicated in variation in treatment
response. IL-7 drives the proliferation of naive T cells to Th1 cells and a Th1 driven form of MS is suggested to respond better to INF-β treatment\textsuperscript{61}. Furthermore, serum levels of IL-7 are inversely correlated to serum IL-17, which is produced by Th17 cells. Studies that measure cytokine levels, such as IL-17 and IFN-β\textsuperscript{22} underline that IFN-β can have opposite effects in different context: in Th1 conditions it may be beneficial, but in Th17 conditions harmful\textsuperscript{62}. This is supported by the observation that IFN-β treatment in NMO patients, a Th17-mediated disease, causes disease exacerbation\textsuperscript{63}. A decrease in IL-17A levels during IFN-β is described in several studies and lower levels of IL-17A were associated with treatment response\textsuperscript{64,65,66}. After non-myeloablative autologous haemotopoietic stem cell transplantation, a experimental treatment strategy for MS patients with aggressive MS that is refractory to other immunothearies, a persistent depletion of a proinflammatory CD161\textsuperscript{high} CD8+ T cell subset that produces IFN-γ and IL-17 was shown and associated with the absence of disease activity. This subset originates in the gut mucosa and expresses a CNS homing receptor CCR6, and is involved in the disease pathology as it was detected in white matter active lesions\textsuperscript{63}. Moreover, IFN type I signature is shown to be high in NMO patients\textsuperscript{63,68}.

The Interferon type I pathway is likely involved in MS disease severity, independent from treatment response. It may be difficult to determine the exact role of IFN-β in disease severity as IFN type I possesses both pro- and anti-inflammatory functions depending on the context of different simultaneous occurring immunological processes\textsuperscript{69}. Our work suggest a protective role of endogenous IFN-β for RR-MS disease activity, but the relationship of a short-acting cytokine on a clinical phenotype is likely to be more complex. There are for example, consistent findings that IFN-β contributes to the pathogenesis in other autoimmune diseases including SLE, NMO and RA\textsuperscript{68,70,71}. In MS, a recent study, described an increased level of expression of IFN-regulated genes in treatment naïve patients. Here, an exaggerated response of IFN-regulated genes to IFN-β treatment was associated with more enhancing lesions at baseline MRI. The authors postulated that pathogenetically distinct subtypes of MS, associated with differences in disease severity, were linked to variations in functional type I IFN pathways\textsuperscript{72}. Our finding that patients with NAbs that persist after treatment cessation seem to have a more aggressive disease course is interesting in this respect. As NAbs have been shown to block endogenous IFN-β but not endogenous IFN-α\textsuperscript{50}, the deterioration of disease course must be related to the IFN-β pathway and not the complete IFN type I pathway. To confirm this, spontaneous MxA mRNA could be measured in these patients. Our findings could also be a reflection of an altogether different and aggressive MS disease subtype that is more likely to develop and maintain NAbs.

Future work should be directed to develop a more individual tailoring of drug regimes, as more alternatives to IFN-β for MS treatment are on the market, with often better efficacy rates on group level than IFN-β. It is likely that IFN-β will remain an important first-line choice.
of treatment in MS. It has a well known side-effects profile and long term follow-up data are available. Also, it is possible that a subgroup of patients exist that have a near to 100% response to treatment. Testing for NAbs is important for the clinical neurologist, as it has direct therapeutic consequences and is currently widely available. Patients with persisting high-titer NAbs or persisting absent biological activity of IFN-β effectively do not receive any treatment and should be identified with routine testing for NAbs. Alternative strategies to manage NAbs are the further development of less immunogenic drugs, the prevention of NAbs development or the treatment of NAbs when they have appeared. Concomitant corticosteroid use has been shown to delay NAbs development and reduce NAbs titers in some studies, but results are inconsistent in others. High dose intravenously administered IFN-β has been shown to transiently decrease NAbs titers. Reported prevalence rates of NAbs against IFN-β-1a (not against IFN-β-1b) and especially of high-titer NAbs, seem to have decreased since testing for NAbs is more routinely performed. Authors speculate that the stricter monitoring of patients might have lead to the switching of NAbs positive patients to an alternative treatment or/and the preferential use of less immunogenic drugs or alteration of drug formulations. Rebif New Formulation and Pegylated IFN-β are introduced as less immunogenic alternatives to IFN-β. These are examples of biosimilars, which are competing versions of biopharmaceuticals that are approved based on an abbreviated approval procedure. They are relatively new products and have raised safety concerns. Biosimilars are therapeutically equivalent, however different production cell lines and purification methods lead to post-translational modifications with possible consequences to a product’s immunogenic profile. Additional safety surveillance should be directed to the incidence of anti-drug antibodies, the risks associated with antibody development, such as changes in disease severity, hypersensitivity reactions or neutralization of endogenous proteins.

A poor response to IFN-β is not solely neutralizing antibody-mediated. Patients with a pre-treatment active type I IFN pathway or a more Th17 driven form of MS are suggested to be likely suboptimal responders to IFN-β, independent of NAbs development. Natural killer cell phenotype and receptor changes have been associated with IFN-β treatment response as well. A recent discovery puts a new autoantigen in MS on the map: the potassium channel KIR4.1, located on the end feet of astrocytes in the CNS. Seropositivity for antibodies directed to KIR 4.1 is discribed in approximately half of MS patients and may signify a subgroup of MS patients with a different response to treatment regimes such as IFN-β but also to depletion of B cells by rituximab. At time of writing, clinical neurologists have to rely on the timely recognition of poor treatment response during treatment. Early predictors suggested include: IFN-regulated gene expression profiles before and during treatment, microRNA expression changes during treatment, single nucleotide polymorphisms in genes coding for IFNAR receptor subunits, cytokine serum levels of IL-8 or IL-10. These potential response markers still have to be consistently confirmed and are not yet
feasible for clinical practise. Several studies show a correlation between new T2 lesion and/or gadolinium enhancing T1 lesions on MRI with treatment outcome on a group level. Timing of the MRI scan performed, the definition of an ‘active’ scan and what constitutes a poor clinical treatment outcome varies between studies, however results are consistently confirming the predictive value of early (at 6 to 12 months of treatment) radiological markers on clinical disease activity. Careful monitoring of clinical symptoms in IFN-β treated patients with or without the help of MRI scans is at present the most reliable way to identify non-responders. A generally accepted definition of a poor response would be helpful for the establishment of more comparable future studies in this context.

The role of endogenous type I IFN pathway in disease activity is, although not a new concept in other autoimmune diseases, a topic that is interesting to further explore in MS. Could it distinguish between different MS subtypes, with an associated different disease course and appropriate treatment regime? Can it lead to the development of targeted novel immunomodulatory drugs? Does the role of endogenous type I IFN pathway differ in earlier compared to later MS phases, when neurodegeneration is more prominent than inflammation?

Finally, our results suggest that once developed, persisting NAb have a negative effect on disease course. From our data we are unable to tell whether or not this is a direct effect of NAb on disease course, or if patients with a more vigorous immune response are those who also suffer a more severe disease course. Their impact on inflammatory processes and on neurodegeneration remains unknown. Other possible consequences of long-term blocking of endogenous IFN-β because of persisting NAb, such as an increased incidence of infections or malignancies, should be examined in future studies.

In summary, the observation of a low expression of interferon in cells of MS patients after viral stimulation, which took place in the 70ties, initiated the interest in IFN as a possible treatment drug for MS. This occurred at a time were there was no known effective treatment for this debilitating disease effecting mostly young individuals. Clinical trials confirmed a partial effect on inflammation and IFN-β became a widely used - and for a long time the only- treatment for relapsing MS. Clinical experience revealed a remarkable inter-individual variation in how well people did on IFN-β treatment. This was partly attributed to patient related factors which cause variation in clinical disease course and partly attributed to the blocking of the biological activity of the drug by anti-IFN-β NAb. Absent MxA mRNA expression in IFN-β treated patients indicates the presence of NAb. Our data demonstrates a role for MxA mRNA expression as a treatment response marker and underlines the value of measurement of both MxA mRNA expression and induction in this respect. Recent studies provided more and more evidence that the potential of IFN-β to modulate MS disease course is dependent on the pre-treatment activity of type I IFN pathways and type I IFN activity variation is possibly related to MS clinical phenotype.
These pathways are difficult to unravel as many genes, gene expression profiles and post-translational modifications of gene products are likely involved. In this thesis, we contribute to the suggestion that endogenous IFN-β activity affects disease severity, at least in some MS patients. We were unable to confirm the proposed role of IL7R on disease activity or on IFN-β treatment response, but our data did support a role for IRF5 on disease course and treatment response modulation. Future work on type I IFN pathway differences in MS hold promise for an improved selection of patients eligible for IFN-β treatment and patients that will more likely benefit from glatiramer acetate or second-line treatment.
Chapter 4

References


General discussion and future perspectives


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