Chapter 2.1
Reduced biological activity of recombinant IFN-β and treatment response

Introduction
1.1 General introduction: Multiple Sclerosis

Multiple Sclerosis (MS) is an immune mediated demyelinating disease of the central nervous system (CNS), characterised by bouts of neurological symptoms and increasing disability. MS was first described in 1868 by Charcot\(^1\), and the presumed aetiology of MS is a combination of a genetic predisposition and environmental factors such as a viral infection.

**Pathogenesis**

MS is generally considered to be an autoimmune disease involving inflammation, demyelination and axonal degeneration. The inflammation is assumed to be caused by the migration of auto reactive T lymphocytes across a disrupted blood-brain-barrier into the CNS. Interaction with activated antigen presenting cells (microglia, macrophages), locally produced pro-inflammatory cytokines and complement activation, leads to degradation and digestion of myelin by macrophages\(^2\). Demyelination can lead to clinical symptoms by means of conduction block. Exposed axons segments are more vulnerable to ongoing inflammatory injury and axonal degeneration may follow\(^3,4\). Evidence for intrathecal IgG synthesis in more than 95% of MS patients\(^5\), suggests an abnormal B cell response in the CNS. CSF oligoclonal antibodies have been studied for the presence of antibodies against viruses, bacteria and CNS components, but no specific antigen has yet been identified\(^6\). Axonal loss has been described early on in the disease course and seems to exist independent to inflammatory processes as neurodegeneration can progress in absence of inflammation. This is supported for example, by the finding that CNS changes in MS are not confined to MS lesions but involve both grey and white matter throughout the CNS\(^7\). A recent theory is formulated which proposes that MS should be seen as an ‘inside-out’ disease, which initiates in the brain, rather than with an aberrant response of the immune system\(^8\).

**Epidemiology**

There is epidemiological evidence for a predominance of MS in female (two third of MS patients is female)\(^9,10\) and MS prevalence is higher in Western countries and with higher latitude degrees (larger distance from the equator)\(^11,12\). In the Netherlands about 17.000 people have MS, an estimate of 100 per 100.000. In 1992 55% of these patients had severe disability or a handicap\(^13\). MS is most often diagnosed in young people between 20 and 50 years of age, 90% of disease onset is between 15 and 50 years\(^14\).

**Disease spectrum and clinical course**

A diagnosis of MS entails most likely a heterogeneous group of diseases, supported by the identification of different MS subtypes and a great inter- and intra-individual variability in
disease severity within these subtypes. Moreover, a spectrum of demyelinating diseases or MS-like diagnoses exist, such as Neuromyelitis Optica (NMO), transverse myelitis (MT), Acute Disseminated Encephalomyelitis (ADEM), Marburg’s disease and Balo’s concentric sclerosis. Most patients present with a monophasic episode of complaints, which completely or partly resolves in the course of days to weeks. This first episode is called a clinically isolated syndrome (CIS) and typical complaints are monocular visual loss as a result of an optic neuritis, sensory complaints, a feeling of an electric discharge over the spine on head flexion (l’Hermitte sign), diplopia, dizziness, bladder dysfunction or walking difficulties. In these patients more episodes of complaints (relapses or exacerbations) occur, which is typical for the relapsing-remitting (RR) MS subtype. Most of these patients progress after a variable period of time to the secondary progressive (SP) MS subtype, in which patients gradually accumulate disability with or without superimposed relapses. One third of MS patients show a progressive disease course from the start, the primary progressive (PP) subtype. PP-MS is more common in males and typically present with a gradual progressive spastic paraplegia and/or gait ataxia. MS relapses are usually defined as patient-reported or objectively observed signs, typical of an acute demyelinating event of the CNS, current or historical, with a duration of at least 24 hours in the absence of an infection or fever. The accumulation of lasting symptoms due to incomplete recovery of relapses or disability progression independent from relapses is measured with the Kurtzke Expanded Disability Status Scale (EDSS).

**MRI**

Magnetic resonance imaging (MRI) is used in diagnosis, prognosis and monitoring of efficacy of disease modifying treatments in MS. MS patients show typically formed lesions (ovoid) in characteristic areas of the CNS (periventricular, juxtacortical, infratentorial and spinal cord). A new MS lesion is the result of a sequence of events, which can be made visible on MRI. First a breakdown of the blood-brain barrier is seen in association with inflammation. Demyelination occurs early in the inflammation phase and is inferred from methyl and methylene groups on short time echo spectroscopy. Demyelination and inflammation lead to conduction block and the production of symptoms. Oedema develops and reaches a peak after about a month. It was proposed that with blood-brain barrier the oedema starts to be absorbed. A residual scar remains after 2 to 3 months, represented by the presence of non-enhancing T2 lesions or ‘black holes’ on T1 MRI sequence. Typical MRI T2 lesions in patients with a first event suspect for a demyelinating event predict conversion to CDMS.
Introduction

Diagnosis

The diagnosis of MS is based on the occurrence of one or more clinical episodes suggestive for demyelinating CNS disease and evidence of disease dissemination in time (DIT) and dissemination in space (DIS). However, no single diagnostic tests for the diagnosis of MS exists, therefore it remains a diagnosis of clinical probability for which diagnostic guidelines have been formulated. To fulfill the initial diagnostic criteria of Poser in 1983, a patient has to present with at least two clinical relapses suggestive of demyelinating disease, at least 1 month apart with each episode implicating disease activity in a different CNS localisation (clinically definite MS; CDMS). In 2001 MRI criteria were incorporated in the diagnostic guidelines (McDonald criteria). Accumulating evidence showed a high sensitivity for MRI to detect abnormalities in CDMS patients and MRI was useful to exclude alternative diagnoses, most commonly vascular CNS disease. MS diagnosis according to McDonald criteria is based on a clinical episode suggestive of MS combined with a certain minimum number of lesions, characteristic in form and CNS location, on Magnetic Resonance Imaging (MRI). In some cases a positive cerebral spinal fluid (CSF) result, i.e. the presence of oligoclonal bands (OCB) could substitute for radiological dissemination in space. With the McDonald criteria MS diagnosis could now be made in some patients after a single relapse. In 2005, the revised McDonald criteria were formulated. Dissemination in time could now be established with a new T2 lesion on MRI at least 1 month after disease onset and the criteria for the diagnosis of PP-MS were further clarified. In the latest revisions of the diagnostic criteria of 2010 (Table 1), the number of CNS lesion on MRI needed for diagnosis was reduced, possible leading to earlier diagnosis in some patients in the future. Dissemination in space was simplified by replacing the McDonald criteria for this with the Swanton criteria. The presence of ≥1 T2 lesion in two out of four typical CNS locations (periventricular, juxtacortical, infratentorial and spinal cord) establishes DIS. Dissemination in time is fulfilled with a new T2 lesion or a new gadolinium enhancing lesion on a follow-up MRI irrespective of the time interval between first and second MRI. Or with a single MRI on which an asymptomatic gadolinium enhancing lesion is seen in the presence of another non-enhancing lesion. Abandonment of the previous requirement of a 1 month interval between MRI’s did not compromise specificity.
Table 1. 2010 Revised McDonald MS diagnostic Criteria. Diagnosis of MS requires elimination of more likely diagnoses and demonstration of dissemination of lesions in space (DIS) and time (DIT)31.

<table>
<thead>
<tr>
<th>Clinical attacks</th>
<th>Lesions</th>
<th>Additional criteria to make diagnosis</th>
</tr>
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<tbody>
<tr>
<td>2 or more</td>
<td>Objective clinical evidence of ≥ 2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack</td>
<td>None. Clinical evidence alone will suffice; additional evidence desirable but must be consistent with MS</td>
</tr>
<tr>
<td>2 or more</td>
<td>Objective clinical evidence of 1 lesion</td>
<td>DIS; OR await further clinical attack implicating a different CNS site</td>
</tr>
<tr>
<td>1</td>
<td>Objective clinical evidence of ≥ 2 lesions</td>
<td>DIT; OR await a second clinical attack</td>
</tr>
<tr>
<td>1</td>
<td>Objective clinical evidence of 1 lesion</td>
<td>DIS; OR await further clinical attack implicating a different CNS site AND DIT; OR await a second clinical attack</td>
</tr>
<tr>
<td>0 (progression from onset)</td>
<td></td>
<td>One year of disease progression (retrospective or prospective) AND at least two of: DIS in the brain based on ≥1 T2 lesion in periventricular, juxtacortical or infratentorial regions; DIS in the spinal cord based on ≥2 T2 lesions or positive CSF</td>
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Table 1. 2010 Revised McDonald MS diagnostic Criteria. Diagnosis of MS requires elimination of more likely diagnoses and demonstration of dissemination of lesions in space (DIS) and time (DIT)31.

<table>
<thead>
<tr>
<th>Evidence for Dissemination of lesions in space (DIS)33</th>
<th>Evidence for Dissemination of lesions in time (DIT)34</th>
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<tbody>
<tr>
<td>1. ≥1 T2 lesion in at least two out of four areas of the CNS: periventricular, juxtacortical, infratentorial or spinal cord</td>
<td>1. A new T2 and/or gadolinium –enhancing lesion(s) on follow-up MRI, with reference to baseline MRI, irrespective of the timing of the baseline MRI</td>
</tr>
<tr>
<td>2. Gadolinium enhancement of lesions is not required for DIS</td>
<td>OR 2. Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time</td>
</tr>
<tr>
<td>3. If a subject has a brainstem or spinal cord syndrome, the symptomatic lesions are excluded and do not contribute to lesion count</td>
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Evidence for Positive CSF
Oligoclonal IgG bands in CSF (and not serum) or elevated IgG index

1.2 Treatment in MS

MS remains an incurable disease of unknown aetiology. Inflammatory demyelination and axonal injury are the pathological hallmarks of MS. Acute exacerbations which lead to significant complaints are usually treated with corticosteroids intravenously. However, there is no consensus about the optimal dose, route or duration of corticosteroid therapy17. Also, the impact of steroids on recovery of clinical symptoms is limited36. Since 1993 several drugs that modify MS disease course have been approved for the treatment of RR-MS and in some cases for CIS and SP-MS patients. These disease modifying treatments (DMT) target primarily the inflammatory component of MS and cause a reduction of the number of relapses and the number of new T2 and/or gadolinium enhancing lesions on MRI. In studies with a longer follow-up time, a beneficial effect on disability progression is found, especially
with the newer drugs (natalizumab, fingolimod). Axonal loss that occurs independent of inflammatory processes is unlikely to be influenced by DMT. Interferon beta (IFN-β) and glatiramer acetate (Copaxone®) are first-line agents, with well known long-term safety profiles. However, they are injectables which give rise to compliance issues. IFN-β belongs to the family of cytokines and is a glycosylated protein of 166 amino acids, produced with recombinant DNA technology in animal cells and will be discussed in more detail later. Glatiramer acetate is a polymer of four amino acids found in myelin basic protein (MBP), an important protein for the myelination of nerves in the CNS. Although the exact beneficial mechanisms in MS are unknown, it may function as a decoy for the immune system because of its resemblance to MBP. Mitoxantrone (Novantrone®), a cytotoxic agent, was introduced in 2000 for patients with highly active disease or a poor response to first-line treatment. Potential serious side-effects, such as cardiotoxicity, myelosuppression and leukaemia, related to the cumulative lifetime dose administered, restrict treatment duration and warrant regular monitoring. Natalizumab (Tysabri®), a monoclonal antibody that binds to alpha-4 integrin on mononuclear leukocytes and blocks their entry into the CNS, received approval for relapsing MS in 2004. It was temporarily withdrawn from the market after three reports of progressive multifocal leukoencephalopathy (PML), an often fatal viral disease of the CNS. These patients were treated with more than one immunosuppressive drug and because no other cases were reported at that time, it was reintroduced in 2006 as monotherapy only. Natalizumab is administrated once a month intravenously and shows a two third reduction in relapse rate, but also lowers disability progression rates compared to placebo. Since the reintroduction, over 375 cases of PML in natalizumab treated patients are reported worldwide, with a 23% mortality rate. The estimated overall risk of PML is 2.1/1000 patients. The risk of PML is increased in patients with previous exposure to immunosuppressants and with treatment duration of natalizumab of more than 12 months. More recently, the presence of anti-JC virus antibodies was established as a risk factor for the PML, although fluctuations in serostatus were found in a longitudinal study. The first oral treatment for relapsing MS, fingolimod (Gilenya®), was introduced in 2010 and was shown to be superior to Avonex. Fingolimod is a sphingosine-1-phosphate (S1P) receptor modulator. It binds to S1P receptors on lymphocytes, thereby reversible trapping them in the secondary lymphoid organs and preventing migration of auto reactive lymphocytes to the circulation and the CNS. Long-term safety data are unavailable, but side-effects include infections, macular oedema, hypertension and elevated liver enzymes. Cardiac side-effects, i.e. bradycardia, atrioventricular block, tend to be transient but may warrant electrocardiogram (ECG) tests, especially when patients are on anti-arrhythmic drugs or have a history of conduction block or syncope. A newer oral drug, teriflunomide, has been approved for RR-MS. Teriflunomide inhibits dihydroorotate dehydrogenase, a mitochondrial enzyme involved in de novo pyrimidine synthesis which may result in a reduction in the
number of activated lymphocytes in CNS. Dimethyl fumarate (BG12) is an oral formulation of fumaric acid, which has been used to treat psoriasis and work as an immunomodulator, inhibiting proinflammatory cytokines and chemokines and promoting anti-inflammatory activity and was FDA approved for RR-MS in March of 2013. Alemtuzumab, or Campath-1H, was shown to reduced relapse rate and reduces the accumulation of disability in early RR-MS. In a head to head comparison study it was found to be more effective than IFN-β-1a. It is a monoclonal antibody directed against CD52, which is a protein with a unknown function on the cell surface of lymphocytes. Alemtuzumab treatment is associated with the development of secondary autoimmunity, 30% of patients develop autoimmune thyroid disease, both Graves’ disease and hypothyroidism. Despite the lymphopenia induced by alemtuzumab, only increases in mild-to-moderate infections are seen, such as upper respiratory tract infections and urinary tract infections.

1.3 Interferon-β treatment in MS

Clinical studies in the 1970s showed a reduced interferon (IFN) response to viral induction of cells of MS patients compared to controls. In combination with the known antiviral properties of IFN and the presumed role of viral infection in the aetiology of MS, treatment trials with IFN were initiated. Efficacy of IFN-β in RR-MS patients was first established with IFN-β-1b (Betaferon®). A reduction in relapse rate of approximately one third, a reduction of new T2 lesions on MRI and no or only a modest effect on disability progression was found. IFN-β-1a (Rebif® and Avonex®) followed with similar efficacy and side effects profile. In CIS patients IFN-β delays conversion to CDMS. The limited effect seen on clinical deterioration may be the result of the relatively short duration of the pivotal trials (2-3 years). The beneficial effects of IFN-β in MS may be due to a combination of anti-inflammatory, anti-proliferative and pro-apoptotic responses, although the exact mechanism is unknown. IFN-β is currently one of the main first-line disease modifying drugs in MS. A significant portion of MS patients show breakthrough disease activity under IFN-β treatment or experience side-effects. With the development of alternative treatments, showing different efficacy rates and side-effect profiles, careful patient selection for each treatment regime is required. Attempts have been made to optimize the treatment with IFN-β by PEGylation. PEGylation of an IFN means that at least one molecule of polyethylene glycol (PEG) is covalently added, a modification that increases the stability, solubility, half-life, and possible the efficacy of IFN-β. A phase I study demonstrated that subcutaneous PEG-IFN beta-1a at a dose of 125 µg every 2 or 4 weeks might be at least as efficient and safe as the current standard therapy with IFN beta-1a.
1.4 Responders and non-responders to IFN-β treatment

The reported efficacy of IFN-β in MS in randomized placebo-controlled trials is obligatory presented on group level. Clinical experience is that treatment response varies greatly across individuals. One study reports a variation in reduction of active T2 lesions on MRI between 0% in some and ≥ 60% in most patients treated with IFN-β-1b. Why some patients respond better to IFN-β treatment than others is largely unknown, but is most likely a combination of genetic as well as disease heterogeneity. Identifying non-responders to IFN-β treatment is currently a clinical decision usually made after 1 or 2 years of treatment, based on the persistence of relapses, progression of disability, the development of new T2 or gadolinium enhancing lesions on MRI and the opinion of the individual neurologist. Intra-individual variability of disease activity in MS patients makes it difficult to compare disease activity from one period to another. In other words, it is difficult to conclude if patients are experiencing a clinical remission or are responding well to any given treatment. A considerable fraction of patients is therefore on ineffective treatment at high cost, potentially accumulating further disability and often suffering treatment related side-effects. To date there is no validated biomarker to (early) identify treatment failure or to predict treatment response to IFN-β in individual patients. MRI is used as a paraclinical measure for disease activity, and is considered more sensitive than clinical relapses or disability progression on EDSS. MRI is able to detect new disease activity 5-10 times more frequent than clinical relapses in patients with RR-MS and SP-MS. Monitoring treatment response with the development of active lesions with gadolinium enhanced images is recommended with monthly MRI, as gadolinium enhancement is temporary, which is however not feasible for long-term follow-up. A different and promising strategy for predicting IFN-β treatment response for individual patients at an early stage, is to look at the so-called ‘IFN type I signature’. This is the magnitude and pattern of IFN-regulated gene activity, before initiation of IFN-β treatment and the response or induction of these genes (up and down regulation) during treatment. Also, some gene polymorphisms involved in IFN type I pathways have been suggested to play a role in IFN-β treatment response. For example, the interferon regulatory factor 5 (IRF5) gene, encoding for a transcription factor that plays an important role in the innate as well as the cell-mediated immune response, has been suggested to be involved in IFN-β treatment response. Genetic polymorphisms in human IRF5 that lead to the expression of various isoforms or a higher expression of IRF5 mRNA, have been associated with several autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren’s syndrome, inflammatory bowel disease and MS. IRF5 has diverse activities, such as the activation of IFN type I genes, for inflammatory cytokines and for tumour suppressors. Human IRF5 is expressed in many splice variants with distinct cell type-specific expression and differences in regulation and functions. Furthermore, IRF5 activation leads to a shift in macrophage population.
to a macrophage lineage that produces pro-inflammatory cytokines, chemokines and co-stimulatory molecules, which in turn leads to a potent Th1 and Th17 response\(^8\).

### 1.5 Neutralizing antibodies to IFN-β treatment

One of the reasons patients do not respond to IFN-β treatment is the development of anti-IFN-β antibodies that reduce or block the biological activity of recombinant IFN-β. They are called neutralizing antibodies (NAbs). Like other chronically used recombinant proteins, such as insulin, recombinant IFN-β preparations are immunogenic and may elicit an antibody response, primarily of the IgG isotype\(^9\). In the pivotal IFN-β trials NAbs were routinely measured and were shown to develop between 6 and 18 months of treatment and are unlikely to be (newly) formed after two years of continued treatment\(^10\). Several risk factors are identified for the formation of NAbs, such as administration regime, i.e. dose, route (intramuscular or subcutaneous) and frequency\(^11\). Also, a genetic predisposition for the development of NAbs to IFN-β products has been recognized\(^12\). The most important risk factor determining immunogenicity is the product formula itself, which differs in production method, glycosylation status or the tendency to form of aggregates\(^13,14,15\). IFN-β-1b s.c. (Betaferon\(^\text{®}\)) was most frequently associated with the development of NAbs, IFN-β-1a s.c. (Rebif\(^\text{®}\)) and IFN-β-1a i.m. (Avonex\(^\text{®}\)) to a lesser extend. With continued treatment, patients treated with IFN-β-1b were more likely to reverted back to a NAb negative status compared to IFN-β-1a, regaining the full effect of IFN-β treatment\(^16,17,18\). The presence of high-titer NAbs is a predictor for the persistence of NAbs over time, and is more common with IFN-β-1a treatment compared to IFN-β-1b\(^19,20,21\). NAbs are generally cross-reactive between IFN-β products\(^22\). An international discussion started if NAbs are clinically meaningful and what is the best way to measure them\(^23\). Authors from North America and Europe both recognize the potential effect of NAbs on IFN-β efficacy, but differ in their interpretations of the consequences of NAbs for clinical practise. The European guidelines recommend testing for NAbs in the first 24 months of treatment, repeating the test in NAbs positive patients and to discontinue treatment in patients with high-titer sustained at repeated measurements with 3-6 months interval\(^24\). North American guidelines state that IFN-β treatment is associated with the development of NAbs, that it is likely that NAbs influence radiographic and to a lesser extent clinical effectiveness of IFN-β, that although sustained high-titer NAbs are associated with a reduction in the therapeutic effects of IFN-β on clinical and radiographic measures of MS disease activity, there is insufficient information on the utilization of NAbs testing to provide specific recommendations regarding when to test, which test to use, how many tests are necessary, or which cut-off titer to apply\(^25\).

Some authors argue that the impact of NAbs on IFN-β therapeutic efficacy remains uncertain, as NAbs are formed temporary in most patients and do not often persist after years of continued treatment. Furthermore, data concerning NAbs were acquired from trials using different measurement techniques for NAbs and were not designed to evaluate the effect of
NAbs on treatment efficacy\textsuperscript{105}. To measure the clinical effect of a phenomenon (NAbs) that is sometimes temporary, with titer levels that vary, on a treatment which is only partially effective is difficult and may lead to conflicting results. Others say that several studies were underpowered to measure the effect of NAbs on clinical measures of treatment efficacy, because of the relatively late appearance of NAbs (6-18m) in combination with a fairly short duration of follow-up in these studies (2-3 years)\textsuperscript{103}. NAbs positive patients showed a higher relapse rate in the Interferon beta-1b relapsing-remitting MS study and the PRISMS (IFN-\(\beta\)-1a s.c.) study in year 3 and 4 of follow-up, but not in the first two years\textsuperscript{65,106}. The impact of NAbs is earlier and more consistently shown on MRI. High-titer NAbs positive patients showed a higher number of T1 gadolinium enhanced lesions and more new or enlarging T2 lesions on MRI during the pivotal phase 3 trials and extended follow-up for all three IFN-\(\beta\) products\textsuperscript{106,107,108,109,110}. Furthermore, accumulating evidence with studies measuring IFN-regulated gene products showed complete abolishment of IFN-\(\beta\) bioactivity in the presence of high-titer NAbs\textsuperscript{111,112,113}, although the effect of low- or intermediate-titer NAbs was less predictable\textsuperscript{114}.

### 1.6 Measuring biological activity of recombinant IFN-\(\beta\)

Interferon-beta up-regulates and down-regulates several hundred genes by binding to its class I IFN receptor (IFNAR) and activating a cascade of intracellular events (Figure 1). It is unclear which of these gene products are responsible for the beneficial effects of IFN-\(\beta\) in MS, but stimulation of the IFNAR is a necessary first step for its therapeutic efficacy\textsuperscript{115}. Myxovirus resistance protein A messenger RNA (MxA mRNA), encoded by the Mx1 gene, shows a robust and specific response to IFNAR stimulation\textsuperscript{91,112,116,117} and this response is shown to sustain over time\textsuperscript{112}. Therefore, MxA mRNA is often used to measure biological availability of IFN-\(\beta\)\textsuperscript{91,118}. MxA mRNA response to recombinant IFN-\(\beta\) can be measured in peripheral blood mono-nuclear cells by real-time polymerase chain reaction (PCR). This method requires a strict interval between IFN-\(\beta\) administration and sampling of blood, to minimize false positive results\textsuperscript{119}, as MxA mRNA rises in response to IFN-\(\beta\) with a peak around 12-13h and reverts back to normal after 24-48h\textsuperscript{112,117}. Anti-IFN-\(\beta\) NAbs are related to a loss of biological activity of recombinant IFN-\(\beta\), by preventing a normal interaction of IFN-\(\beta\) with IFNAR. The virus-induced cytopathic effect assay (CPE) is recommended by the world health organisation (WHO) for measurement and quantification of NAbs\textsuperscript{104}, although this assay has some disadvantages. Inter-laboratory comparison is difficult, as different cell lines and viruses are used. The sensitivity of the assay varies with different sera dilutions and concentrations of IFN-\(\beta\) added to the assay. Furthermore, cut-off values for a NAbs positive result were arbitrarily chosen and controversy exists about at which level of NAbs titers IFN-\(\beta\) bioactivity is completely abolished. MxA-based assays can indicate the presence of NAbs in IFN-\(\beta\) treated patients by showing an antibody-mediated decrease of IFN-\(\beta\) biological activity. MxA based assays are unable to differentiate between antibody-mediated
reduction of biological activity, poor compliance, ineffective IFN-β injections, the presence of high levels of IFNAR soluble receptor or laboratory error\textsuperscript{120}.

**Figure 1.** The IFNAR receptor and signaling cascade of Interferon beta (IFN-β) (Modification of figure published in Nature Rev Immunol 2005\textsuperscript{121}). IFN-β is produced in response to viruses, bacteria and their products. For its action, IFN-β binds with its cell surface receptor (IFNAR). Janus kinases (JAKs) associated with the IFNAR receptor phosphorylate signal transducer and activator of transcription (STAT) complexes. As a result, an IFN-stimulated gene factor 3 (ISGF3) complex forms, containing STAT1, STAT2 and a third transcription factor called interferon regulatory factor 9 (IRF9) which moves into the cell nucleus. There, it binds to the promotor sequences (IFN-stimulated response elements) of IFN stimulated genes and thereby induces the transcription of these genes.

### 1.7 Immunogenicity of other current drugs

Protein-based drugs for the treatment of MS can be recognized by the human immune system as foreign and may consequently lead to an immunological response against the therapeutic agent. Breaking of immunological tolerance occurs because therapeutic proteins are not exact copies of the endogenous human proteins and because of other factors, such as contaminants and impurities in product formulation, treatment duration, dose, route of administration and patient related factors\textsuperscript{122}. Most patients treated with glatiramer acetate (GA) were reported to develop anti-GA-antibodies, with peak levels at 3-4 months\textsuperscript{123}. Three studies found no evidence of reduced efficacy\textsuperscript{123,124,125} but one study showed that purified anti-GA-antibodies of RR-MS patients reduced the \textit{in vitro} biological effects of GA and a trend for reduced efficacy for high titers\textsuperscript{126}. Natalizumab pivotal trials showed antibody formation around 3 months of treatment in 9% of patients, of which 6% were persistently positive\textsuperscript{47,127}. They may induce increased clearance of natalizumab or block natalizumab from binding to its target. Both high antibody titers and low concentration levels of natalizumab are associated with the occurrence of relapses and the development of gadolinium enhancing lesions on MRI\textsuperscript{128} Persisting antibodies against natalizumab are associated with a loss of clinical benefit and infusion-related hypersensitivity reactions\textsuperscript{129}. 
1.8 Endogenous IFN-β pathways and disease activity in MS

Apart from the role of IFN products in modulating disease activity in RR-MS patients, a role for endogenous type I IFN pathways in MS disease course have been suggested. Endogenous IFN-β, which is in ‘normal’ circumstances produced in a stable, low level, is rapidly produced in response to a viral infection in almost all nucleated cells. IFNs play an essential role in the innate immunity by inhibiting the replication and spread of viral, bacterial and parasitic pathogens. They modulate immune responses and exert anti-proliferative effects in some cell-lineages. IFNs are used in the clinic to treat chronic hepatitis B and C infections and some types of cancer (hairy cell leukaemia for example). There are different IFN subclasses, of which IFN-alpha (IFN-α) and IFN-β belong to the type I IFNs, and for example IFN-gamma to type II IFNs. There are several genes encoding for IFN-α, in contrast there is a single gene encoding for human IFN-β which contains no introns, consequently, no splice variants exist for IFN-β\textsuperscript{130}. Both IFN-α and IFN-β use a heterodimeric receptor to exhibit their effects, composed of the IFNAR1 and IFNAR 2 chains. The key difference in terms of recognition by their receptor is a difference in affinity: IFN-β binds both subunits with high affinity, whereas IFN-α has a low affinity for IFNAR 1. Therefore, the tertiary complex (IFN-IFNAR1/IFNAR2 complex) of IFN-β has a longer lifetime (approximately 100 sec) compared to IFN-α (1-5 sec)\textsuperscript{131} (Figure 2. IFNAR receptor and IFN pathway). The intracellular domains of both receptor subunits (Tyk2 for IFNAR1 and Jak 1 for IFNAR2) are brought in close proximity and an intracellular cascade of events follows. Several genes and gene products are up- and down-regulated. For example, a robust production of Myxovirus resistance substance A (MxA) messenger RNA (mRNA) is measured in response to IFN-β stimulation, together with many other gene products. Most of these are acute phase proteins or inflammatory biomarkers such as neopterin, IL-10, β-2 microglobulin, soluble VCAM\textsuperscript{132}. If the level of activity of endogenous type I IFN pathways is related to differences in disease severity in MS, is unknown. Recently, a role for Interleukin receptor 7 (IL7R) in MS susceptibility was confirmed, besides the strongest and most consistent found genetic factor for MS susceptibility, the major histocompatibility complex (MHC) HLA region\textsuperscript{134}. IL7R has been shown to play a critical role in the development and differentiation of T cell lymphocytes\textsuperscript{134}. The promoter region of IL7R contains a functional interferon regulatory element\textsuperscript{134}. Defects in the IL7R are a cause of severe combined immunodeficiency (SCID), characterized with an absence of T-cell-mediated cellular immunity\textsuperscript{135}. A polymorphism at position 244 (SNP rs6897932) containing either a C allele or a T allele strongly influences susceptibility to MS\textsuperscript{133,136}. The C allele results in an approximately two-fold increase in the skipping of exon 6, leading to an increased production of a soluble form of IL7R. Unknown are the consequences of IL7R splice variants, leading to a different ratio of soluble and membrane-bound IL7R receptor, in the disease course of MS patients.
Chapter 1

1.9 Thesis outline

Chapter two focuses on the impact of a reduced biological availability of recombinant IFN-β on treatment response in relapsing-remitting MS. First, we confirmed the association between MxA mRNA expression, as a measure for the biological activity of recombinant IFN-beta, and the occurrence of NAbs, using a method developed in the VU medical center. Does the development of NAbs cause a titer dependent reduction of IFN-β biological activity? The relationship between NAbs and a poor response to IFN-β was previously established, for NAbs positive patients were more likely to have clinical relapses and showed more disease activity on MRI. Biological availability of recombinant IFN-β, as measured with MxA mRNA expression was less clearly associated with IFN-β treatment response. Therefore, we investigated if the absence of a MxA mRNA response to IFN-β treatment was associated with the occurrence of relapses in RR-MS patients. Thirdly, we studied two other possible factors, leading to variations in IFN-β treatment response, besides antibody-mediated reduced biological activity, namely IRF5 and IL7R polymorphisms. Previous study showed that the individual response to IFN-β treatment could be determined by assessing the magnitude or pattern of IFN-regulated genes before and during treatment. If a patient demonstrates an active INF type I 'signature' before treatment, induction of these IFN-regulated genes are expected to be low or absent after treatment initiation and related to a poor clinical treatment response. We investigate the role of IRF5 polymorphisms in IFN-β treatment response, a major type I IFN transcriptional regulator which was recently suggested to be associated with IFN-β treatment response. We questioned if IRF5 gene variants in IFN-β treated RR-MS patients were related to their pharmacological response to IFN-β, which was defined as the response (up and down regulation) of several IFN-regulated genes after initiation of IFN-β treatment. We postulated that IRF5 gene variants were related to clinical and radiological measures of disease activity during IFN-β treatment. Furthermore, we wanted to confirm the added role of IL7R as a predisposing factor for MS to HLA DRB1*1501. As IL7R expression is influenced by type I IFN stimulation, we postulated that IL7R splice variants, leading to a change in ratio of soluble and non-soluble IL7R, could be related to disease activity and response to IFN-β treatment in RR-MS. IL7R mRNA expression was measured and soluble/non-soluble IL7R ratio was related to relapse rate, progression on EDSS and MRI, and response to IFN-β treatment in a Dutch cohort of MS and CIS patients. In chapter three, the influence of endogenous IFN-β as a disease modulator in RR-MS is evaluated. First we looked if spontaneous expressed MxA mRNA, as a measure for endogenous IFN-β biological activity, was related to clinical relapses and disability progression in untreated RR-MS patients. Finally, we wanted to confirm that anti-IFN-β NAbs are able to persist- in the absence of antigen exposure-, i.e. long after IFN-β treatment cessation. We studied if persisting Nabs, with possible long term blocking of endogenous IFN-β, were associated with a more severe disease course in this subgroup of MS patients. In chapter four we discuss overall conclusions of this thesis and we make suggestions for future perspectives.
References


Chapter 1


