Axonal Damage in Multiple Sclerosis

van der Star, B.J.

2014

document version
Publisher's PDF, also known as Version of record

CHAPTER 1

General Introduction

Partly adapted from Kipp *et al.*, 2012; Van der Star *et al.*, 2012 and Van der Star *et al.*, book chapter in: "Autoimmunity to Neuronal Proteins in Neurological Disorders", Nova Science Publishers 2011

Multiple sclerosis

Multiple Sclerosis (MS) is a chronic inflammatory, demyelinating and neurodegenerative disease of the central nervous system (CNS) frequently manifested by episodes of neurological disease (relapses) and recovery (remissions). Worldwide around 2.3 million people are estimated to be affected by MS and the disease is differentially distributed with a higher prevalence in North America and Europe. In the Netherlands 1 in 1000 of the population is affected by the disease (1). The first symptoms of the disease are generally observed in young adults between the age of 20 and 40 and more women are affected than men (2.3:1) (2). MS also occurs in younger adults and children as well as in older people.

Damage to the brain and the spinal cord of persons with MS results in a variety of signs and symptoms, depending on the area(s) affected. Symptoms include visual problems (3), loss of motor and sensory functions (4) and cognitive impairment (5). In many persons with MS the episodes of relapses and remissions lead to accumulation of neurological deficits over years with progression of disability and many patients eventually become wheelchair bound. Besides the variability of symptoms, the severity of the symptoms and duration of the disease differs between individuals with MS. Several forms for the clinical course of MS are defined (4, 6): Relapsing-Remitting MS (RRMS), Secondary Progressive MS (SPMS) and Primary Progressive MS (PPMS, Figure 1). The majority of the people (~80%) have RRMS which translates to the appearance of relapses and remissions (4). However, after years of relapses and remissions, the disease becomes more progressive due to the accumulation of symptoms, and is referred to as SPMS (Fig. 1A). It is estimated that 50-60% of the people with RRMS progress to develop SPMS (6). Approximately 10% of the people with MS have PPMS and experience less recovery from onset of first symptoms (Fig. 1B, gender ratio female to male 1.3:1) (7). Why the majority of the people with RRMS progress to SPMS and why others have PPMS is unknown.

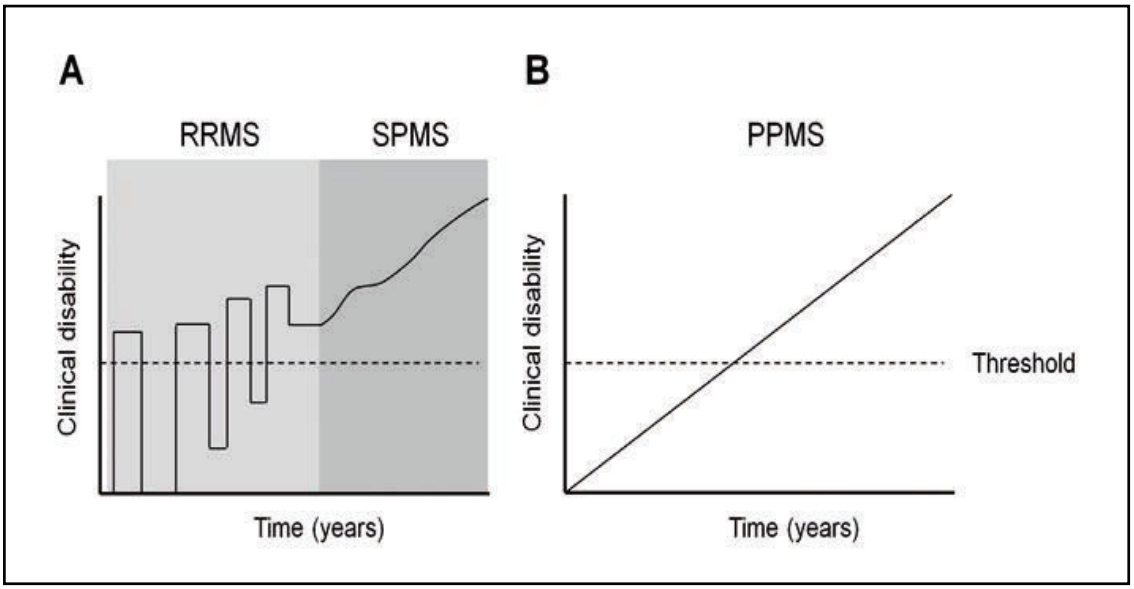

Figure 1. Different forms of MS. A) *The majority of the patients (~80%) develop relapsing-remitting MS (RRMS) with relapses as bars above the clinical threshold (dotted line) of clinical disability, and remission below the threshold. After decades, damage in the CNS accumulates with almost no recovery and is called secondary progressive MS (SPMS).* ***B)*** *In approximately 10% of the people with MS damage accumulates until it reaches the threshold of clinical disability and progresses in time without remissions (primary progressive MS, PPMS).*

Another form of MS which is less common than the forms described above is primary relapsing MS (PRMS) in which patients show progressive neurological disability with relapses (6). Individuals that experience a single relapsing-remitting episode are classified as clinically isolated syndrome (CIS). A recent study shows that 26% of the persons with CIS develop clinically definite MS after one year (8).
Several other uncommon subtypes of MS have also been described. These include acute and aggressive types such as Marburg's variant and Balò's concentric sclerosis (9) or a benign subtype MS, so-called clinically silent MS (10). It is estimated that between 1.7% and 5.6% of the people with MS are children under the age of 18, of which 95% of the cases follow a RRMS course (11, 12). Although several types of MS can be distinguished, the cause of MS has yet to be identified.

Genetics
Although the aetiology of MS is unclear, several lines of evidence indicate that genetic variations increase the risk of getting MS. Genome wide association studies have identified polymorphisms in genes coding for cytokines (e.g. Interleukin (IL)2-RA, IL-7R, IL22-RA2), co-stimulatory molecules (e.g. cluster of differentiation (CD)40, CD86) and signal transduction molecules (e.g. signal transducer and activator of transcription 3) (13, 14). The strongest association with the risk of MS is the human leukocyte antigen (HLA)-DRB1*1501 allele, located within the class II region of the major histocompatibility complex (MHC), which increases the risk of developing MS by approximately threefold (15, 16). Furthermore, the concordance rate of MS is 25%-30% in identical twins and the individual risk of getting MS increases 20- to 40- fold when a sibling has MS (17, 18). Mutations in the CYP27B1 gene are also associated with an increased risk of MS (19) and are thought to be due to the immunomodulatory effects of Vitamin D (20-23). Polymorphisms in killer immunoglobulin-like receptors (KIR), which are MHC class I-specific receptors, are associated with MS susceptibility as well. The KIR genes code for stimulating or inhibitory receptors and are expressed by natural killer (NK) cells and subsets of T lymphocytes. The absence of one of the inhibitory receptors, KIR2DL3, is associated with the development of CIS and MS (24, 25).
Nevertheless, the above mentioned genetic mutations do not fully explain the high incidence of MS, suggesting that other factors such as environmental factors might also contribute to the development of MS.

Environmental factors
Although MS occurs worldwide, geographical studies show that the prevalence of MS is not equally distributed (26). In general, the incidence of MS is low in tropical areas, higher further away from the equator but again low in the most northern regions. Migration early in life from a high-risk area to a low-risk area decreases the risk of getting MS (4, 20). Variations in Vitamin D levels are proposed as one of the contributors to the unequal distribution of MS prevalence. This is supported by differences in MS prevalence in Norway between fishing villages and farming villages inland, which is probably due to the high Vitamin D in the diet (16, 26). Contrary to the protective effect of Vitamin D is cigarette smoking, which is a risk factor for MS development (27-30), although a protective effect of nicotine is also proposed (31, 32). Other environmental factors, such as exposure to infections, might influence the risk of MS as well. Electron microscopical and virological studies support a role for infections in MS by revealing the presence of viruses such as measles (33), human Herpesvirus 6 (HHV-6)(34, 35) and Epstein-Barr Virus (EBV) (36) in the

CNS and cerebrospinal fluid (CSF) of people with MS. Furthermore, antibodies to EBV nuclear antigens are present in the serum of people with MS (37, 38), as well as B cell follicles containing EBV-infected B cells in post-mortem MS brain tissue. (36). However, these results are contradictory and the contribution of the EBV to the development of MS is still a matter of debate (39-43). A viral aetiology of MS is also supported by the discovery of structural similarities between viral antigens and myelin proteins in people with MS (44-46). In addition, several viruses e.g. measles and John Cunningham (JC) virus cause demyelination in humans (47, 48), but the exact impact of viruses in the development of MS is unclear.

Pathology

The major pathological characteristics of MS are inflammation, as reflected by the presence of innate immune activation and the presence of adaptive immune responses i.e. T and B cells, demyelination, axonal damage and sclerotic plaque formation. It is proposed that lesion development starts with the formation of pre-active lesions (49). These are small clusters of activated microglia which are HLA-DR$^+$ (Fig. 2A), resembling an activated cell type, and stressed oligodendrocytes, but without apparent myelin damage (Fig. 2B) (49, 50). At that stage, the blood-brain barrier (BBB) is still intact (49, 51). Pre-active lesions might develop into active lesions characterised by accumulation of activated and phagocytic HLA-DR$^+$ cells, oligodendrocyte loss, demyelination and axonal damage. At this stage, cells from the periphery including macrophages, dendritic cells and lymphocytes infiltrate the CNS, the BBB is damaged and lesions contain immunoglobulin (Ig) and complement deposits (49, 52-55). In active lesions, microglia and macrophages show a round morphology, contain intracellular (myelin and axonal) debris (Fig. 2C and D), and are also present in the perivascular space together with other leukocytes (52). HLA-DR$^+$ cells containing debris are also present in cervical lymph nodes, a site for antigen presentation, lymphocyte activation or lymphocyte tolerance (56, 57). Active lesions gradually progress into chronic active lesions which are characterised by a rim of phagocytic HLA-DR$^+$ cells (Fig. 2E, G) and a demyelinated hypocellular centre (Fig. 2F, H). Finally, due to an unknown trigger, phagocytosis of myelin and axonal proteins stops, leaving a rim of HLA-DR$^+$ cells (Fig. 2I and J) and a demyelinated centre (Fig. 2K) with reactive astrocytes (Fig. 2L) forming a sclerotic plaque. Contrary to plaque formation, regeneration of damaged axons and remyelination occurs as well (58, 59), although it is believed that these restored areas function less as before the damage.

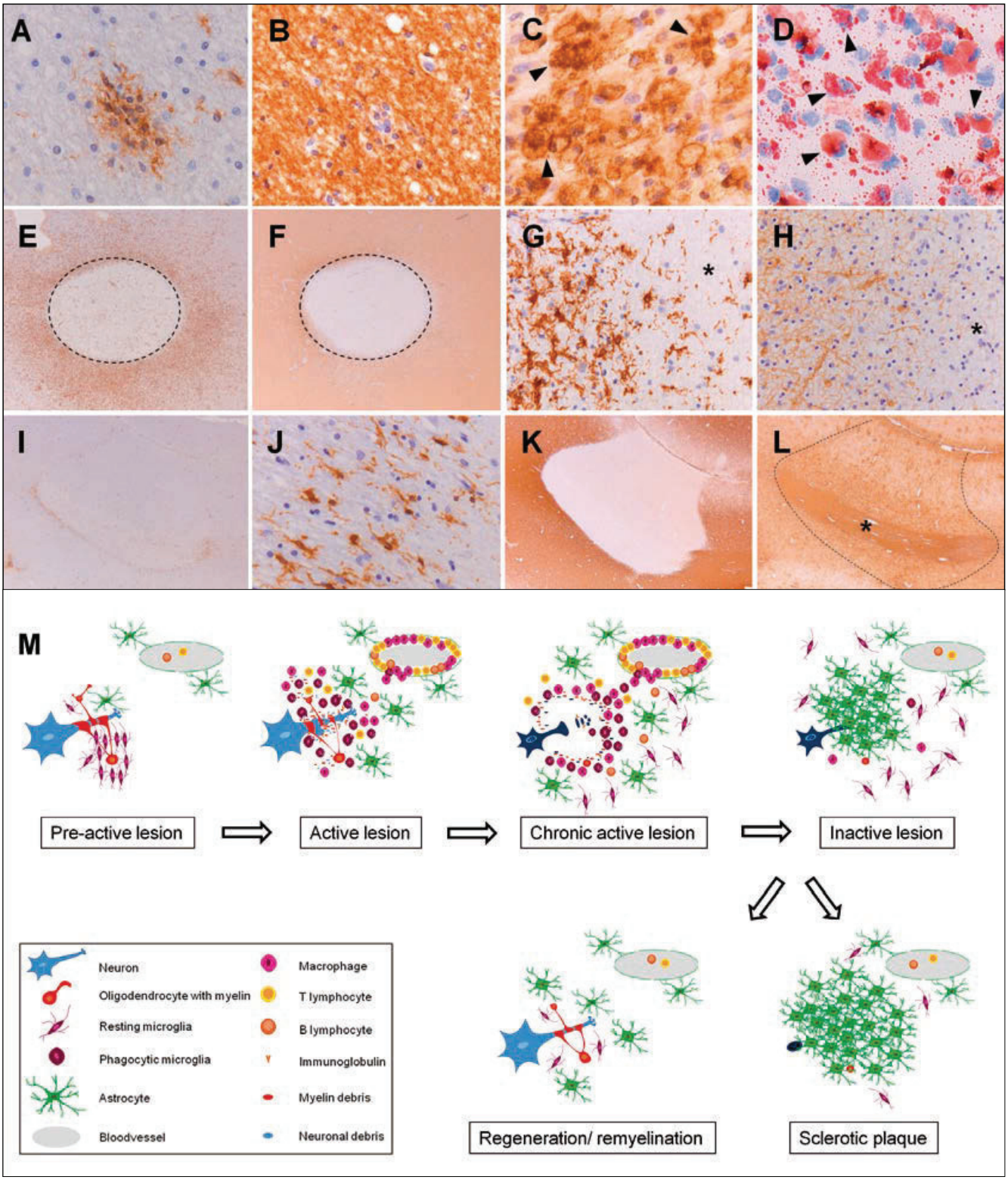

Figure 2. Pathology of MS and schematic representation of lesion development. A*) A small cluster of HLA-DR cells form pre-active lesions.* ***B****) No damage to myelin in pre-active lesions as shown by immunostaining of proteolipid protein (PLP). An active lesion with HLA-DR cells containing debris (****C****, arrowheads) or Oil-Red-O particles resembling uptake of neutral lipids (****D****, arrowheads). E-H) Chronic active lesion, circled by dotted line, is characterised by a rim of HLA-DR cells (****E, G****) and a demyelinated hypocellular centre as stained for PLP (****F, H****). Asterisks in G and H indicate the location of the lesion. I-L) Inactive lesion leaving a rim of ramified HLA-DR cells (****I, J****), a demyelinated centre as shown by PLP staining (****K****) and a centre of reactive astrocytes (****L****, asterisk, glial fibrillary acidic protein staining). The dotted line in L indicates the lesion. Cell nuclei are stained blue.* ***M****) Schematic and simplified representation of lesion development in MS.*

Axonal damage and neurodegeneration in MS

Although the extent of axonal injury is variable in lesions of people with MS, it is clearly present in all demyelinated lesions and normal appearing white matter (Fig. 3A-C) (53, 60-64). In chronic inactive lesions axonal density is reduced up to 70% (65, 66). Studies from post-mortem spinal cord tissue from people with MS reveal that axonal damage correlates with irreversible neurological disability (65) indicating that arresting progressive degeneration would be a crucial step in controlling disability. With the improvement of magnetic resonance imaging (MRI) and histochemical staining techniques, it has become clear that demyelination and axonal degeneration is not limited to the white matter (WM) but also occurs in the cortical regions (67). Since 1999 a classification system for grey matter (GM) lesions is used (67, 68). Leukocortical lesions (Type I lesions) are localised both in WM and GM (Fig. 3D). Intracortical lesions (Type II lesions) are localised in the cortex, thus subcortical WM and the surface of the cortex are not involved (Fig. 3E). Sub-pial cortical lesions (Type III lesions) only include superficial layers of the cortex (Fig. 3F) and finally transcortical lesions (Type IV lesions) affect the subcortical regions but not the WM (Fig. 3G) (68). In GM lesions, infiltration of macrophages and lymphocytes is lower compared to WM lesions, so low to be virtually absent (63, 69). This is also reflected at the border between the GM and WM part of leukocortical lesions, where sometimes a clear rim of inflammatory cells in the WM is seen, whereas the GM is devoid of these cells (63).

In summary, improvements of techniques to detect axonal damage have contributed to the knowledge about neurodegeneration in MS.

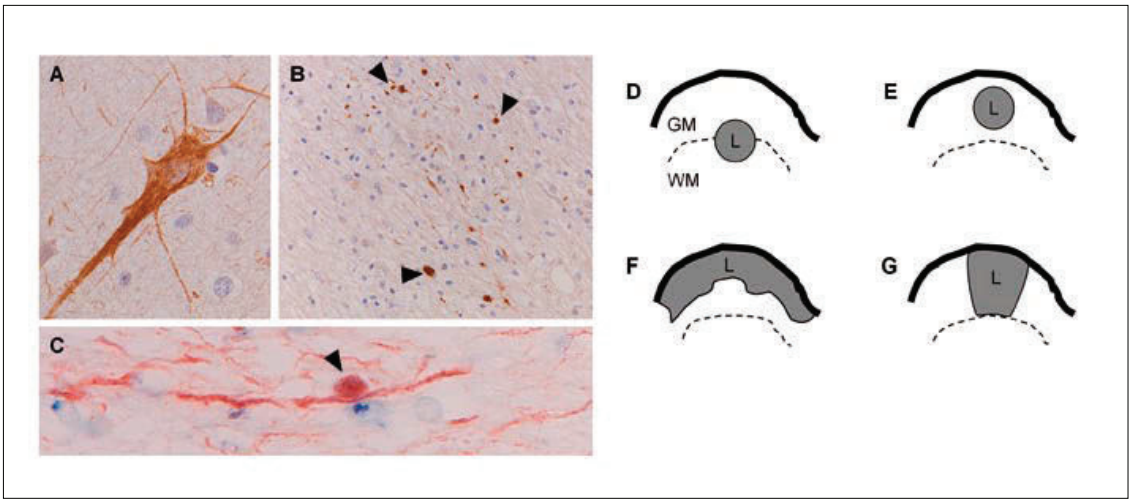

Figure 3. Axonal damage and grey matter lesions in MS. A*) A neuron with a thick axon extending from the cell body, stained with an antibody to neurofilament light.* ***B****) Axonal bulbs, identified with an antibody directed to amyloid precursor protein (APP), representing possible damage (arrowheads) in the rim of a chronic active MS lesion.* ***C****) Abnormal structure along the axon (arrowhead) indicating possible damage (antibody to APP). D-G) Schematic representation of grey matter MS lesion in the brain.* ***D****) Leukocortical lesions affect both the cortical GM and the WM.* ***E****) Intracortical lesions are localised in the cortex.* ***F****) Sub-pial lesions include only the superficial layers of the cortex.* ***G****) Transcortical lesions affect the subcortical regions but not the WM. L, lesion.*

Neurofilaments

The cytoskeleton of neurons and axons consists of actin microfilaments, microtubules and intermediate filaments (70). Neurofilaments, classified as intermediate filaments, are the most important proteins in the axonal cytoskeleton (71) that determine the axonal calibre and contribute to transport of proteins along the axons (72). Neurofilaments consist of the subunits neurofilament heavy (NF-H), medium (NF-M) and light (NF-L) (Fig. 4) and have their nomenclature related to their molecular weight. Regarding the structure of the subunits, all neurofilaments consist of a head (N-terminal), rod and tail (C-terminal) domain. While the head and rod domains are highly conserved between the subunits, the tail domain is variable (Fig. 4).

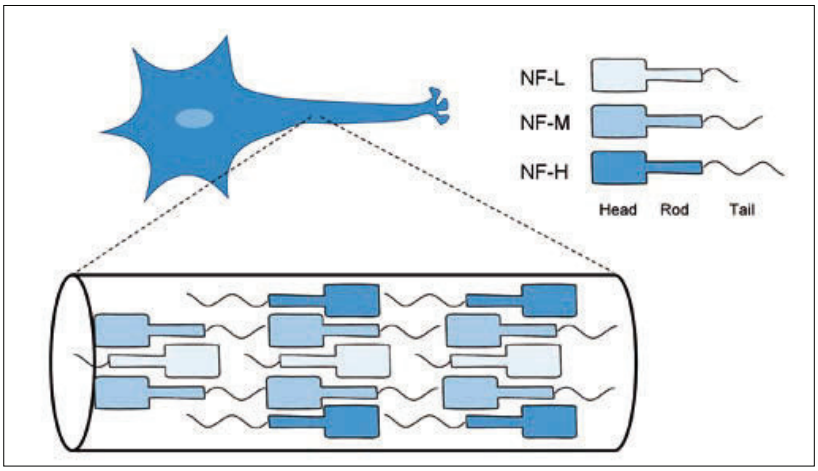

Figure 4. Simplified schematic representation of a neuron with assembly of neurofilament subunits. *The tail domain is highly variable, while the head and rod domain are conserved between the different subunits NF-L, NF-M and NF-H. Partly adapted from (71).*

Post-translational modifications e.g. phosphorylation and glycosylation of the neurofilament subunits, are involved in neurofilament assembly (71). Since NF-H is the most phosphorylated of the three subunits, monoclonal antibodies recognising non-phosphorylated or phosphorylated NF-H are used to detect changes in NF-H phosphorylation status in axons (71, 73). Especially in damaged brain areas dephosphorylated neurofilaments are detected (71, 73), whereas in a healthy environment the majority of neurofilament subunits are phosphorylated (71, 74). On the contrary, increased immunoreactivity to phosphorylated NF-H is also seen in brain tissues of people with Alzheimer's disease (75, 76) and in active MS brain lesions (74).

Mutations in the gene encoding NF-H are reported to cause aggregation of neurofilaments in a subset of people with amyotrophic lateral sclerosis (ALS) (77), the most common form of motor neuron disease in adults and a rapidly progressive disorder resulting in muscle weakness and muscle atrophy (78). In Charcot-Marie-tooth disease, an inheritable neuropathy, several mutations in the NF-L gene have been linked to aggregation (79-82).

This thesis focuses on one form of neurofilaments, namely NF-L, since antibodies to NF-L are present in the CSF and serum of people with MS (discussed below) and correlate with cerebral atrophy in people with MS (83). Antibodies to NF-L are suggested as biomarkers for disease progression in MS (84-88). In addition, NF-L is the most abundant of the neurofilament subunits (89) and immunisation of Biozzi antibody high (ABH) mice with the NF-L protein results in spasticity and paralysis, which are symptoms observed in people with MS (90, 91).

Therapies

First-line therapies of RRMS and SPMS with relapses include the disease-modifying treatments with interferon-β1a, interferon-β1b or glatiramer acetate (GA) (92). Patients treated with interferon-β1a show a ~30% decrease in relapses, fewer exacerbations and less gadolinium enhancing lesions on MRI than controls (93, 94). Also treatment with GA reduces the relapse rate with ~29% in patients with RRMS (95). Following the development of these disease-modifying therapies, drug development has progressed into target-specific therapies using monoclonal antibodies targeting specific structures on cells. The first officially approved humanised monoclonal antibody for RRMS is natalizumab, a monoclonal antibody that targets a receptor on leukocytes, Very Late Antigen 4 (VLA-4) (96). Blocking of VLA-4 obstructs leukocytes, including lymphocytes, to enter the CNS. Natalizumab decreases the annualised rate of relapses by 68%, reduces the mean number of hyperintense lesions by 83% and reduces progression of clinical disability compared to placebo (97). Unfortunately, patients positive for the JC virus treated with natalizumab have an increased risk of progressive multifocal leukoencephalopathy due to immunodeficiency (48, 98). Other therapies have been developed that inhibit lymphocyte trafficking, such as fingolimod, (99-101) or deplete lymphocytes, like alemtuzumab (102). Moreover, B lymphocyte depletion by a monoclonal antibody to CD20, rituximab, is currently used in trials for MS, and is already used for treatment of non-Hodgkin lymphoma and rheumatoid arthritis (103). Some people with MS benefit from plasma exchange, although temporarily (104-106). Keegan and colleagues propose the removal of potentially pathogenic antibodies as favourable in persons with complement and antibody deposits in the CNS (105).

Unfortunately, therapies such as GA, fail to have an effect on disease progression in people with PPMS (107). In another study, Coles and colleagues show a decrease in neuroinflammation in people with SPMS treated with alemtuzumab but ongoing accumulation of disability (102). Possible explanations might be that in people with progressive MS without relapses, inflammation is less involved or that in people with SPMS accumulation of axonal damage continues as a consequence of the active inflammatory episodes during the RRMS stage (4, 102).

In conclusion, the increase in therapies for RRMS so far has not been beneficial for people with progressive MS with accumulating neuronal damage. Therefore, unravelling the neuropathological mechanisms in MS, partly facilitated by experimental models, will contribute to development of new therapeutic agents.

Experimental models of MS

Although the primary cause of MS is unknown, the widely accepted view is that aberrant (auto)immune responses, possibly arising following infection(s), are responsible for the destructive inflammatory demyelination and neurodegeneration in the CNS. This notion, and the limited access of human brain tissue (early) in the course of MS, has led to the development of autoimmune, viral and toxin-induced demyelination animal models as well as the development of human CNS cell cultures and organotypic brain slice cultures in an attempt to understand events in MS. The autoimmune model, known as experimental autoimmune encephalomyelitis (EAE), and the viral models have shaped ideas of how environmental factors may trigger inflammation, demyelination and neurodegeneration in the CNS. Understandably, these models have also strongly influenced the development of therapies targeting the inflammatory aspect of MS.

Experimental autoimmune encephalomyelitis

The animal model EAE, characterised by inflammation, myelin damage and neurodegeneration induced following immunisation with brain antigens, strongly supports MS as an autoimmune disease (90, 91, 108, 109). Despite differences in the course and pathology between MS and EAE, EAE is still the most intensively used experimental model of MS. In addition, EAE studies have provided important contributions to our understanding of neuro-immune interactions within the CNS.

Immunisation of susceptible animals with CNS tissues and adjuvant elicits either a monophasic neurological episode of paralysis, from which the animals recover and are refractory to re-induction of disease, or chronic paralysis from which the animals do not recover (Table 1). While the use of adjuvants, such as complete Freund's adjuvant (CFA), boosts the immune response ($CD4^+$ T cell-mediated), injection of pertussis toxin (PT) from *Bordetella pertussis* is thought to make the BBB more permeable as well as to induce non-specific activation of T cells. EAE can also be induced following adoptive transfer of activated lymph node cells, or specific T cell lines and clones derived from myelin-immunised animals to naive recipients (110). While initial studies use $CD4^+$ T cells to induce EAE, the finding that $CD8^+$ T cells can also induce EAE (111) is important since $CD8^+$ T cells dominate inflammatory MS lesions (112-114). Depleting $CD4^+$ T cells or therapies inhibiting MHC class II interactions block the induction phase and severely reduce clinical relapses in EAE (115, 116).

Table 1. Spectrum of EAE

EAE	Animal	Clinical	Pathology	Reference
Hyperacute	Rats Adoptive transfer of lymphocytes after SCH immunisation	Hyperacute EAE one day after transfer and PT	Deposits of fibrin and neutrophils	(117)
Acute	Biozzi ABH mice Immunisation with MAG or MBP in CFA + PT	Acute monophasic disease	Minimal demyelination	(118, 119)
Acute	Lewis rats Immunisation with MBP in CFA	Acute monophasic disease	Minimal demyelination	(120)
Clinical optic neuritis	Rhesus monkeys Oligodendrocyte specific protein in CFA	Optic neuritis	Demyelination and inflammation in the optic nerve	(121)
Chronic	MOG 35-55 in C57BL/6 or Biozzi ABH mice in CFA + PT	Chronic disease no or very infrequent recovery	Extensive neuronal loss associated with inflammation in the spinal cord	(109)
Chronic	rMOG in CFA Native MOG in myelin in CFA	Chronic demyelinating	Demyelination in CNS	(122, 123)
Chronic relapsing	Biozzi mice Immunisation with rMOG, MOG 8-21 PLP, PLP 56-70 or SCH in CFA/CFA + PT	Chronic relapsing	Minimal demyelination in acute EAE and more extensive in relapses. Axonal damage and neurological deficit increases with time and number of relapse	(118, 124-126)
Chronic relapsing	Dark Agouti rats Immunisation with rMOG, SCH in IFA	Chronic relapsing	Mainly inflammatory with varying degrees of myelin damage	(120, 127)
Chronic relapsing	Guinea pig Hartley and Strain 13	Acute and chronic relapsing	Inflammation and extensive demyelination with remyelination in relapse EAE	(128, 129)
Secondary progressive	ABH mice Immunisation with SCH in CFA	Secondary progressive EAE	Marked gliosis, demyelination, remyelination and axonal and neuronal loss	(130, 131)
Spontaneous	Humanised double transgenic mice TCR for MBP and HLA-DR15	Monophasic with severe paralysis	Inflammation in CNS, limited demyelination	(132)
Spontaneous	Humanised double transgenic mice MOG TCR and HLA-DR15	Monophasic	Inflammation and demyelination in spinal cord and optic nerve	(133)
Spastic paresis	ABH mice Immunisation with NF-L in CFA + PT	Spastic paresis and paralysis	Axonal loss, grey matter damage and myelin damage	(90, 91)

SCH, spinal cord homogenate; MAG, myelin-associated glycoprotein; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; IFA, incomplete Freund's adjuvant; TCR, T cell receptor.

Whatever the induction regimen, the initial phase of clinical disease is usually termed the acute phase (Fig. 5A), and correlates with the presence of mononuclear cell infiltrates in the CNS. The scoring system ranges from 0 representing control or no clinical disease to 4 representing severe clinical paralysis commonly observed by flaccid paralysis of the hind limbs. For ethical reasons score 4 is generally the most severe score although in some reports score 5 is used to represent a moribund state or death from EAE. In species and strains where the animals recover, this recovery period is referred to as remission (Fig. 5A). If animals do not recover the disease is referred to as chronic EAE (Fig. 5B). Several animals, particularly strain 13 guinea pigs, Dark Agouti rats, SJL and ABH mice exhibit recurrent phases of neurological deficit (Fig. 5C). It is still unclear why some EAE animals develop relapses while others exhibit a chronic neurological disease. In the relapse phase myelin damage

and axonal loss are more prominent than in the acute phase (125, 129, 134). An advantage of chronic relapsing EAE is the development of a secondary progressive disease. The pathology of ABH mice immunised with spinal cord homogenate shows extensive demyelination, axonal and neuronal loss, and marked gliosis, all features of MS (130, 135). Omission of pertussis toxin decreases the clinical scores (Fig. 5D, black line) and less demyelination is observed (Fig. 5E). Injection of anti-myelin antibodies, such as anti-myelin oligodendrocyte glycoprotein (MOG) antibodies in MOG-immunised ABH mice (136), exacerbates EAE (Fig. 5D, dotted line) and results in extensive demyelination (Fig. 5F).
Besides the use of myelin antigens, immunisation with neuronal antigens can be used to induce autoimmune mediated neuronal damage.

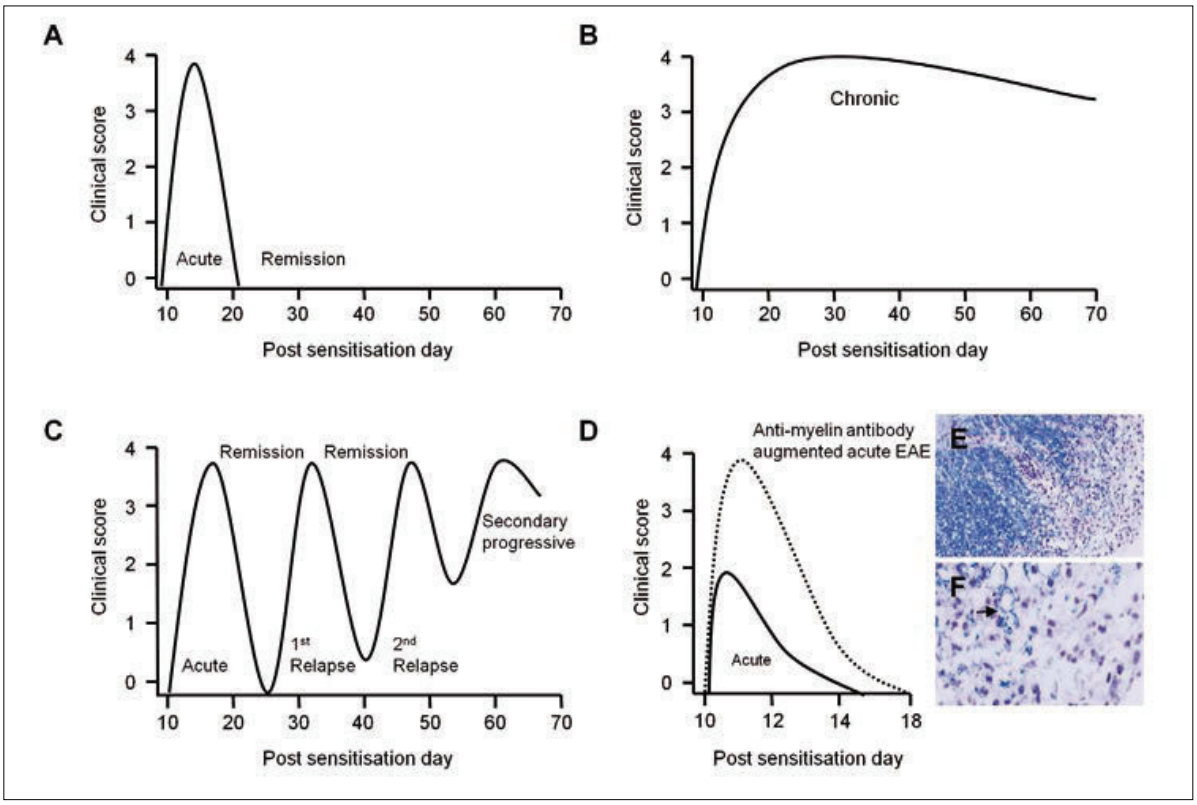

Figure 5. Disease courses in EAE. *Immunisation of animals with myelin antigens gives rise to a spectrum of EAE in which the clinical scores represent the neurological deficit observed. 0 Healthy, 1 limp tail, 2 impaired righting reflex, 3 partial hind-limp paralysis, 4 complete hind-limp paralysis.* ***A****) Acute phase in which animals recover (remission) yet do not develop further episodes of disease. In some cases animals do not recover from the acute phase and the disease is referred to as chronic EAE (****B****). In those models where animals recover, the remission phase is followed by relapse phases (****C****). In this case the animals develop subsequent relapses in which neurological deficit accumulate and eventually do not return to baseline (0) when the animals enter the secondary progressive EAE phase.* ***D****) Clinical course of acute EAE in ABH mice in which pertussis toxin has been omitted (black line, note the reduced clinical score compared to A) do not show extensive demyelination as shown by luxol fast blue (LFB) staining (****E****). To augment myelin damage injection of antibodies to myelin oligodendrocyte glycoprotein at the onset of EAE (day 10) exacerbates clinical disease (D, dotted line) concomitantly with myelin loss (****F****, arrow points to remnants of myelin, LFB staining).*

Autoimmune mediated neuronal damage
EAE models induced by immunisation with myelin proteins or peptides show preferential white matter pathology with some (reversible) neuronal damage (137,

138). Extensive axonal damage is also observed in the secondary-progressive EAE model in ABH mice immunised with spinal cord homogenate (130). Since in MS both GM and WM are affected (62, 67), animal models reflecting GM and WM pathology are more useful to investigate the mechanisms behind this aspect of the disease. Immunising animals with neuronal and axonal proteins or peptides induces more severe axonal pathology, depending on the animal, the strain and the antigen. Axonal pathology is often seen in the spinal cord and includes cellular infiltrates and Ig and complement deposits (90, 91). Immunisation of Biozzi mice with NF-L leads to paralysis and spasticity (91), which are clinical features of MS. In this model of axonal damage, Igs are observed in axons in the spinal cord of mice with disease but not in mice that do not exhibit overt clinical signs (91). This observation suggests that antibodies to the intracellular protein NF-L are able to reach their target and possibly contribute to the development of axonal damage.
In MOG-deficient mice, transfer of T cells with a MOG-specific T cell receptor (TCR) results in demyelination and GM pathology caused by an autoimmune response to one of the neurofilament subunits, NF-M (139). The authors identify shared epitope sequences between MOG^{35-55} and $NF\text{-}M^{18-80}$, responsible for recognition by the TCR. These findings indicate that myelin-specific T cells could also induce neuronal damage via cross-reactivity with neuronal antigens (139).
Furthermore, similar to antibodies to myelin, antibodies to neuronal antigens are demonstrated to cause damage in animals. For example, an antibody directed to neurofascin (NF) induces axonal pathology and exacerbation of EAE in Dark Agouti rats, but does not enhance inflammation or demyelination (140). In this study the authors use a monoclonal antibody directed to both NF-155 (myelin-related) and NF-186 (located at the nodes of Ranvier) isoforms *in vitro*, but could only find binding of the antibody to NF-186 *in vivo*, indicating NF-186 as the primary target (140). More recently, the pathogenicity of monoclonal antibodies to NF-155/186 and NF-186 alone as well as purified human Igs from MS sera was shown in myelinating spinal cord co-cultures, revealing that in some people with MS pathogenic antibodies contribute to pathology (141).
To conclude, depending on the question of interest, different antigens can be used for immunisation of animals to model certain aspects of MS pathology.

Proposed mechanisms of axonal damage in MS
As mentioned above, axonal pathology is present early in MS. Although the pathological mechanisms of axonal injury is unknown, two models have been proposed, based on a viral model in mice (142). These are the so-called 'inside-out' and 'outside-in' demyelination and neurodegeneration paradigms (Fig. 6) (142). In the inside-out model, pathological mechanisms lead to neurodegeneration, axonal damage, and dying back of axons, leaving so-called empty myelin sheaths that eventually degrade. In contrast, in the outside-in model damage to the myelin sheath leaves the naked axons vulnerable that, without the trophic support of myelin, degenerate as a secondary event.

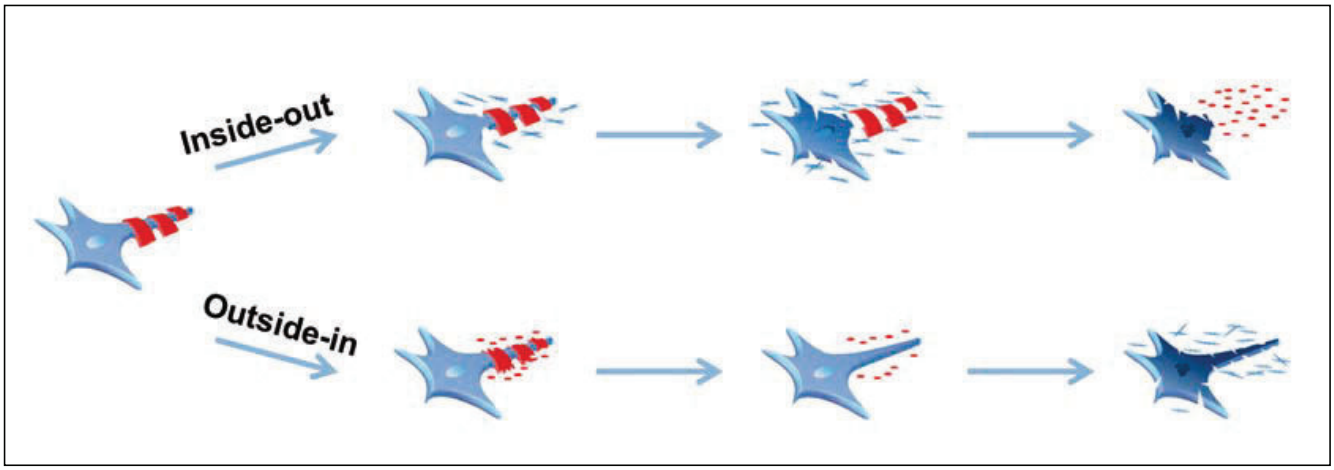

Figure 6. Proposed models of axonal damage in MS. *The neuron is depicted in blue, myelin in red. In the inside-out model, axonal damage is proposed as a primary event, followed by demyelination. In contrast, in the outside-in model it is proposed that primary demyelination causes axonal damage. Based on (142).*

Studies demonstrating an association between axonal damage and inflammation are in line with both models (64, 143, 144). Axonal damage (145, 146) and intracellular axonal changes, e.g. changes in post-translational modifications of neurofilaments (147) in the normal appearing white matter might be indications of an inside-out mechanism. Axonal injury might also be caused by autoimmunity to neuronal proteins (90, 148). In particular autoantibodies to neuronal antigens have been clearly demonstrated to be involved in axonal pathology in other diseases. For example, in paraneoplastic neurological syndromes (PNS), patients with tumours located outside the CNS experience neurological disability (149). In these disorders, tumour cells express neuronal antigens on their surface leading to an immune response causing encephalitis that is often associated with onconeural antibodies and T cells (149-151). Among the target antigens are surface proteins, such as the N-methyl-D-aspartate receptor (152) and gamma-aminobutyric acid-B receptor (153) or intracellular antigens including nuclear proteins such as Hu (154) or synaptic proteins like amphiphysin (155). In people with neuromyelitis optica, antibodies to aquaporin-4, which is expressed on astrocytes, lead to demyelination possibly through complement-dependent cytotoxicity (156-160). In Guillain-Barré syndrome, antibodies to gangliosides, including anti-GM1 and anti-GQ1, cause neurotoxicity through complement-mediated calcium influx resulting in paralysis (161). The realisation that MS may also be classified as a neurodegenerative disorder in which autoantibodies to neuronal antigens might play a role, may not only help to uncover the pathogenic role for such autoimmune responses but may also lead to novel therapeutic strategies. The question now is whether there is any evidence for the involvement of autoimmunity to neuronal antigens in MS.

Autoimmunity to neuronal/axonal antigens in MS

Evidence for a role of autoimmunity against neuronal antigens in MS is supported by the finding of axon-reactive T and B lymphocytes and antibodies in the serum and CSF of people with MS. Autoreactive T cells to the neuron specific proteins enolase, arrestin (162) and NF-L (163) are present in the serum of people with MS. However, T cell responses to NF-L are also present in healthy controls, indicating that these cells are part of the normal immune repertoire (163). This suggests that other factors may be involved in the development of autoimmunity to NF-L besides

only T cell (re)activation (163). The presence of antibodies in the serum and CSF of people with MS might be one of the possible contributors of autoimmunity to NF-L in MS. Igs are detected in the CSF, referred to as oligoclonal bands (OCBs) and are one of the diagnostic criteria for MS (164, 165). Furthermore, B cells and Igs have also been detected in MS brain lesions (36, 52, 166). Antibodies to neuronal antigens including NF-L have been demonstrated in the serum and CSF of people with MS by several groups (167-169). Levels of antibodies to NF-L are increased in the serum and CSF of people with progressive disease (167, 168) and correlate with clinical disability (168) and cerebral atrophy (83). Nevertheless, it is unknown how Igs in the CSF arise and whether they are part of the disease mechanism in MS.

Mechanisms of axonal damage in MS

Several studies indicate a strong correlation between axonal damage and the degree of inflammation in the CNS of people with MS (59-61, 170). The release of toxic factors is one of the mechanisms by which activated inflammatory cells may contribute to axonal injury. For example, the expression of inducible nitric oxide (NO) synthase is increased in acute MS lesions (171, 172), which could lead to neurotoxic levels of NO. Subsequently, NO may inhibit mitochondrial function causing reduced adenosine triphosphate production (173). This is supported by mitochondrial dysfunction in MS lesions (174) which contributes to axonal damage and neuronal loss (175). Also an imbalanced glutamate homeostasis, reflected by an increased glutamate production, might contribute to axonal injury (176, 177).

Furthermore, recognition of neuronal antigens and thus T cell activation leading to neurological damage requires the expression of key molecules for antigen presentation, such as MHC and co-stimulatory molecules. Although it has become clear that $CD8^+$ T cells outnumber $CD4^+$ T cells in MS lesions (178), little is known whether, or how, T cells kill neurons in the CNS of people with MS. In the healthy brain, neurons express negligible levels of MHC class I (179). However during inflammation neurons and axons increase the expression of MHC class I molecules (179, 180). In MS lesions axons positive for MHC class I are detected (181). Fissolo *et al.* purified MHC class I and MHC class II molecules from brain autopsy samples from people with MS and analysed the eluted peptides from these molecules using mass spectrometry. Interestingly, the NF-L, NF-M and α-synuclein peptides are found in this analysis (182). This suggests that T cells may become (re)activated through HLA class I and class II molecules and attack neuronal components resulting in neurodegeneration. *In vitro*, axons up regulate MHC class I after interferon-gamma and tetrodotoxin treatment, resulting in transection of neurites by cytotoxic T cells (183).

More evidence for the impact of T cells on neurons comes from research on the interaction of proteolipid protein (PLP)-specific T cells in brain slice cultures. Activated PLP-specific T cells induce calcium changes in neurons leading to neuronal damage (184). Likewise myelin basic protein (MBP)-specific T cells are more effective in activating microglia that have axon-damaging ability as observed in organotypic CNS cultures (185), suggesting that CNS-specific T cells augment CNS damage.

As mentioned above, autoantibodies are present in the CSF and serum of people with MS. Mathey *et al.* report the presence of antibodies to NF-155/186, a neuronal protein expressed at the nodes of Ranvier, in the serum of people with MS (140). Antibodies to contactin-2, also located at the nodes of Ranvier, are also identified in the CSF and serum of people with MS (186). In this latter study, Derfuss and

colleagues use a proteomic approach to reveal that this axonal protein is recognised by both autoantibodies and T helper 1 cells (Th1) and Th17 cells. Antibodies directed to NF-L (187-189), NF-M (190), NF-H (191), tau (192) and tubulin (193) are also detected in the serum and/or CSF of people with MS. Among all subgroups in MS, people with progressive disease have elevated levels of antibodies to NF-L in serum or CSF (167, 168). In one study, people with PPMS have higher levels of antibodies to NF-L in the serum, but not in the CSF, than people with SPMS (167). In another study, people with PPMS or SPMS have higher levels of antibodies to NF-L in CSF than people with RRMS (168). These data indicate that anti-neuronal immune responses may be important in a subset of people with MS. The level of antibodies to NF-L in people with MS, especially in the relapsing-remitting subgroup, correlates with MRI measures for axonal loss (83). However, in that study no adjustments are made for possible confounding factors, such as age which is known to influence both NF-L antibody levels and cerebral atrophy (167, 194).
Concluding, the contribution of antibodies (to neuronal antigens) to the disease mechanism in MS is still unclear. Understanding the mechanisms by which autoimmunity to neuronal antigens contribute to MS pathology is key to the development of effective therapies to prevent progression of disease and irreversible damage.

Outline of the thesis

Hypothesis

We hypothesise that autoimmunity to NF-L contributes to axonal damage in MS.

Aim

The research has several aims:

1. Investigate how autoimmunity to neuronal antigens in people with MS might arise.
2. Use EAE as a model to investigate the mechanism(s) by which NF-L-specific T cells cause axonal damage.
3. Determine whether antibodies to NF-L are biomarkers of disease or whether they contribute to axonal damage in people with MS.

Neurodegeneration is a pathological hallmark of MS contributing to irreversible neurological disability. In actively demyelinating lesions, myelin is phagocytosed by microglia and macrophages, while the fate of degenerating or damaged axons is unclear. In **chapter 2** we investigated phagocytosis and degradation of neuronal debris by microglia/macrophages. In MS, grey matter pathology is characterised by less pronounced microglia activation and lymphocyte infiltration compared to white matter lesions. Such differences are highlighted by leukocortical lesions that extend across white and grey matter offering an opportunity to examine differences in grey and white matter microglia activation in one lesion. In **chapter 3** we examined grey and white matter parts of leukocortical lesions with respect to microglia activation, axonal damage and phagocytosis of axonal debris. *In vitro* studies were performed with white and grey matter-derived microglia from mice to compare these cells from both regions in functional assays. To further characterise the immune response to NF-L we used a proposed animal model of MS, EAE (**chapter 4**). In this chapter we focussed on T- and B-cell responses to NF-L peptides and the pathogenicity of immunodominant eptiopes in the NF-L amino acid sequence. As mentioned above,

antibodies to NF-L are suggested as biomarkers. Therefore we evaluated the NF-L antibody levels in sera of people with MS with clinical variants and investigated whether these antibody levels change during treatment with natalizumab (**chapter 5**). Besides that antibodies to NF-L are suggested as surrogate markers, it is at present unknown whether antibodies to NF-L are pathogenic. In **chapter 6** we investigated whether monoclonal antibodies to NF-L and purified human Igs of people with MS with high levels of NF-L antibodies were pathogenic *in vitro* and *in vivo*.

Finally, we conclude with **chapter 7** where we discuss our aims and the results of this thesis and put the results in perspective of the recent literature and suggest further research goals.

References

1. MSIF. 2013. Atlas of MS: Mapping Multiple Sclerosis Around the World.
2. Alonso, A., and Hernan, M.A. 2008. Temporal trends in the incidence of multiple sclerosis: a systematic review. *Neurology* 71:129-135.
3. Petzold, A., de Boer, J.F., Schippling, S., Vermersch, P., et al. 2010. Optical coherence tomography in multiple sclerosis: a systematic review and meta-analysis. *Lancet Neurol* 9:921-932.
4. Compston, A., and Coles, A. 2008. Multiple sclerosis. *Lancet* 372:1502-1517.
5. Hulst, H.E., Steenwijk, M.D., Versteeg, A., Pouwels, P.J., et al. 2013. Cognitive impairment in MS: impact of white matter integrity, gray matter volume, and lesions. *Neurology* 80:1025-1032.
6. Antel, J., Antel, S., Caramanos, Z., Arnold, D.L., and Kuhlmann, T. 2012. Primary progressive multiple sclerosis: part of the MS disease spectrum or separate disease entity? *Acta Neuropathol* 123:627-638.
7. Kremenchutzky, M., Rice, G.P., Baskerville, J., Wingerchuk, D.M., and Ebers, G.C. 2006. The natural history of multiple sclerosis: a geographically based study 9: observations on the progressive phase of the disease. *Brain* 129:584-594.
8. Giorgio, A., Battaglini, M., Rocca, M.A., De Leucio, A., et al. 2013. Location of brain lesions predicts conversion of clinically isolated syndromes to multiple sclerosis. *Neurology* 80:234-241.
9. Hu, W., and Lucchinetti, C.F. 2009. The pathological spectrum of CNS inflammatory demyelinating diseases. *Semin Immunopathol* 31:439-453.
10. Ramsaransing, G.S., and De Keyser, J. 2006. Benign course in multiple sclerosis: a review. *Acta Neurol Scand* 113:359-369.
11. Pena, J.A., and Lotze, T.E. 2013. Pediatric multiple sclerosis: current concepts and consensus definitions. *Autoimmune Dis* 2013:673947.
12. Yeh, E.A., Chitnis, T., Krupp, L., Ness, J., et al. 2009. Pediatric multiple sclerosis. *Nat Rev Neurol* 5:621-631.
13. Kreft, K.L., Verbraak, E., Wierenga-Wolf, A.F., van Meurs, M., et al. 2012. The IL-7Ralpha pathway is quantitatively and functionally altered in CD8 T cells in multiple sclerosis. *J Immunol* 188:1874-1883.
14. Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C.C., et al. 2011. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 476:214-219.
15. Hauser, S.L., Fleischnick, E., Weiner, H.L., Marcus, D., et al. 1989. Extended major histocompatibility complex haplotypes in patients with multiple sclerosis. *Neurology* 39:275-277.
16. O'Gorman, C., Lucas, R., and Taylor, B. 2012. Environmental risk factors for multiple

sclerosis: a review with a focus on molecular mechanisms. *Int J Mol Sci* 13:11718-11752.
17. Ebers, G.C. 2005. A twin consensus in MS. *Mult Scler* 11:497-499.
18. Ebers, G.C., Sadovnick, A.D., and Risch, N.J. 1995. A genetic basis for familial aggregation in multiple sclerosis. Canadian Collaborative Study Group. *Nature* 377:150-151.
19. Ramagopalan, S.V., Dyment, D.A., Cader, M.Z., Morrison, K.M., et al. 2011. Rare variants in the CYP27B1 gene are associated with multiple sclerosis. *Ann Neurol* 70:881-886.
20. Ascherio, A., Munger, K.L., and Lunemann, J.D. 2012. The initiation and prevention of multiple sclerosis. *Nat Rev Neurol* 8:602-612.
21. Burton, J.M., Kimball, S., Vieth, R., Bar-Or, A., et al. 2010. A phase I/II dose-escalation trial of vitamin D3 and calcium in multiple sclerosis. *Neurology* 74:1852-1859.
22. Mahon, B.D., Gordon, S.A., Cruz, J., Cosman, F., and Cantorna, M.T. 2003. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. *J Neuroimmunol* 134:128-132.
23. Smolders, J., Peelen, E., Thewissen, M., Cohen Tervaert, J.W., et al. 2010. Safety and T cell modulating effects of high dose vitamin D3 supplementation in multiple sclerosis. *PLoS One* 5:e15235.
24. Jelcic, I., Hsu, K.C., Kakalacheva, K., Breiden, P., et al. 2012. Killer immunoglobulin-like receptor locus polymorphisms in multiple sclerosis. *Mult Scler* 18:951-958.
25. Trachtenberg, E.A. 2009. Understanding the role of natural killer cell receptors and their human leukocyte antigen ligands in multiple sclerosis. *Ann Neurol* 65:626-628.
26. Ebers, G.C. 2008. Environmental factors and multiple sclerosis. *Lancet Neurol* 7:268-277.
27. Hedstrom, A.K., Baarnhielm, M., Olsson, T., and Alfredsson, L. 2009. Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis. *Neurology* 73:696-701.
28. Hedstrom, A.K., Ryner, M., Fink, K., Fogdell-Hahn, A., et al. 2014. Smoking and risk of treatment-induced neutralizing antibodies to interferon beta-1a. *Mult Scler* 20:445-450.
29. Hernan, M.A., Olek, M.J., and Ascherio, A. 2001. Cigarette smoking and incidence of multiple sclerosis. *Am J Epidemiol* 154:69-74.
30. Riise, T., Nortvedt, M.W., and Ascherio, A. 2003. Smoking is a risk factor for multiple sclerosis. *Neurology* 61:1122-1124.
31. Hedstrom, A.K., Hillert, J., Olsson, T., and Alfredsson, L. 2013. Nicotine might have a protective effect in the etiology of multiple sclerosis. *Mult Scler* 19:1009-1013.
32. Naddafi, F., Reza Haidari, M., Azizi, G., Sedaghat, R., and Mirshafiey, A. 2013. Novel therapeutic approach by nicotine in experimental model of multiple sclerosis. *Innov Clin Neurosci* 10:20-25.
33. Salmi, A.A., Panelius, M., and Norrby, E. 1972. Multiple sclerosis and measles virus. *Lancet* 2:1088-1089.
34. Moore, F.G., and Wolfson, C. 2002. Human herpes virus 6 and multiple sclerosis. *Acta Neurol Scand* 106:63-83.
35. Virtanen, J., Wohler, J., Fenton, K., Reich, D., and Jacobson, S. 2014. Oligoclonal bands in multiple sclerosis reactive against two herpesviruses and association with magnetic resonance imaging findings. *Mult Scler* 20:27-34.
36. Serafini, B., Rosicarelli, B., Franciotta, D., Magliozzi, R., et al. 2007. Dysregulated

Epstein-Barr virus infection in the multiple sclerosis brain. *J Exp Med* 204:2899-2912.
37. Larsen, P.D., Bloomer, L.C., and Bray, P.F. 1985. Epstein-Barr nuclear antigen and viral capsid antigen antibody titers in multiple sclerosis. *Neurology* 35:435-438.
38. Lunemann, J.D., Huppke, P., Roberts, S., Bruck, W., et al. 2008. Broadened and elevated humoral immune response to EBNA1 in pediatric multiple sclerosis. *Neurology* 71:1033-1035.
39. Willis, S.N., Stadelmann, C., Rodig, S.J., Caron, T., et al. 2009. Epstein-Barr virus infection is not a characteristic feature of multiple sclerosis brain. *Brain* 132:3318-3328.
40. Jafari, N., van Nierop, G.P., Verjans, G.M., Osterhaus, A.D., et al. 2010. No evidence for intrathecal IgG synthesis to Epstein Barr virus nuclear antigen-1 in multiple sclerosis. *J Clin Virol* 49:26-31.
41. Peferoen, L.A., Lamers, F., Lodder, L.N., Gerritsen, W.H., et al. 2010. Epstein Barr virus is not a characteristic feature in the central nervous system in established multiple sclerosis. *Brain* 133:e137.
42. Sargsyan, S.A., Shearer, A.J., Ritchie, A.M., Burgoon, M.P., et al. 2010. Absence of Epstein-Barr virus in the brain and CSF of patients with multiple sclerosis. *Neurology* 74:1127-1135.
43. Lassmann, H., Niedobitek, G., Aloisi, F., and Middeldorp, J.M. 2011. Epstein-Barr virus in the multiple sclerosis brain: a controversial issue--report on a focused workshop held in the Centre for Brain Research of the Medical University of Vienna, Austria. *Brain* 134:2772-2786.
44. Cheng, W., Ma, Y., Gong, F., Hu, C., et al. 2012. Cross-reactivity of autoreactive T cells with MBP and viral antigens in patients with MS. *Front Biosci (Landmark Ed)* 17:1648-1658.
45. Fujinami, R.S., and Oldstone, M.B. 1985. Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science* 230:1043-1045.
46. Lunemann, J.D., Jelcic, I., Roberts, S., Lutterotti, A., et al. 2008. EBNA1-specific T cells from patients with multiple sclerosis cross react with myelin antigens and co-produce IFN-gamma and IL-2. *J Exp Med* 205:1763-1773.
47. Fox, A., Hung, T.M., Wertheim, H., Hoa le, N.M., et al. 2013. Acute measles encephalitis in partially vaccinated adults. *PLoS One* 8:e71671.
48. Langer-Gould, A., Atlas, S.W., Green, A.J., Bollen, A.W., and Pelletier, D. 2005. Progressive multifocal leukoencephalopathy in a patient treated with natalizumab. *N Engl J Med* 353:375-381.
49. De Groot, C.J., Bergers, E., Kamphorst, W., Ravid, R., et al. 2001. Post-mortem MRI-guided sampling of multiple sclerosis brain lesions: increased yield of active demyelinating and (p)reactive lesions. *Brain* 124:1635-1645.
50. Van Noort, J.M., Bsibsi, M., Gerritsen, W.H., van der Valk, P., et al. 2010. Alphab-crystallin is a target for adaptive immune responses and a trigger of innate responses in preactive multiple sclerosis lesions. *J Neuropathol Exp Neurol* 69:694-703.
51. Van Horssen, J., Singh, S., van der Pol, S., Kipp, M., et al. 2012. Clusters of activated microglia in normal-appearing white matter show signs of innate immune activation. *J Neuroinflammation* 9:156.
52. Breij, E.C., Brink, B.P., Veerhuis, R., van den Berg, C., et al. 2008. Homogeneity of active demyelinating lesions in established multiple sclerosis. *Ann Neurol* 63:16-25.
53. Ferguson, B., Matyszak, M.K., Esiri, M.M., and Perry, V.H. 1997. Axonal damage in

acute multiple sclerosis lesions. *Brain* 120 (Pt 3):393-399.
54. Henderson, A.P., Barnett, M.H., Parratt, J.D., and Prineas, J.W. 2009. Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. *Ann Neurol* 66:739-753.
55. Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E., et al. 2006. Dendritic cells in multiple sclerosis lesions: maturation stage, myelin uptake, and interaction with proliferating T cells. *J Neuropathol Exp Neurol* 65:124-141.
56. Mutlu, L., Brandt, C., Kwidzinski, E., Sawitzki, B., et al. 2007. Tolerogenic effect of fiber tract injury: reduced EAE severity following entorhinal cortex lesion. *Exp Brain Res* 178:542-553.
57. Van Zwam, M., Huizinga, R., Melief, M.J., Wierenga-Wolf, A.F., et al. 2008. Brain antigens in functionally distinct antigen-presenting cell populations in cervical lymph nodes in MS and distinct EAE models. *Submitted for publication.*
58. Barkhof, F., Bruck, W., De Groot, C.J., Bergers, E., et al. 2003. Remyelinated lesions in multiple sclerosis: magnetic resonance image appearance. *Arch Neurol* 60:1073-1081.
59. Bruck, W. 2005. The pathology of multiple sclerosis is the result of focal inflammatory demyelination with axonal damage. *J Neurol* 252 Suppl 5:v3-9.
60. Bitsch, A., Schuchardt, J., Bunkowski, S., Kuhlmann, T., and Bruck, W. 2000. Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. *Brain* 123 (Pt 6):1174-1183.
61. Frischer, J.M., Bramow, S., Dal-Bianco, A., Lucchinetti, C.F., et al. 2009. The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain* 132:1175-1189.
62. Kutzelnigg, A., Lucchinetti, C.F., Stadelmann, C., Bruck, W., et al. 2005. Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 128:2705-2712.
63. Peterson, J.W., Bo, L., Mork, S., Chang, A., and Trapp, B.D. 2001. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Ann Neurol* 50:389-400.
64. Trapp, B.D., Peterson, J., Ransohoff, R.M., Rudick, R., et al. 1998. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 338:278-285.
65. Bjartmar, C., Kidd, G., Mork, S., Rudick, R., and Trapp, B.D. 2000. Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. *Ann Neurol* 48:893-901.
66. Mews, I., Bergmann, M., Bunkowski, S., Gullotta, F., and Bruck, W. 1998. Oligodendrocyte and axon pathology in clinically silent multiple sclerosis lesions. *Mult Scler* 4:55-62.
67. Kidd, D., Barkhof, F., McConnell, R., Algra, P.R., et al. 1999. Cortical lesions in multiple sclerosis. *Brain* 122 (Pt 1):17-26.
68. Bo, L., Geurts, J.J., Mork, S.J., and van der Valk, P. 2006. Grey matter pathology in multiple sclerosis. *Acta Neurol Scand Suppl* 183:48-50.
69. Bo, L., Vedeler, C.A., Nyland, H., Trapp, B.D., and Mork, S.J. 2003. Intracortical multiple sclerosis lesions are not associated with increased lymphocyte infiltration. *Mult Scler* 9:323-331.
70. Fuchs, E., and Cleveland, D.W. 1998. A structural scaffolding of intermediate filaments in health and disease. *Science* 279:514-519.
71. Petzold, A. 2005. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. *J Neurol Sci* 233:183-198.
72. Singh, T.J., Grundke-Iqbal, I., McDonald, B., and Iqbal, K. 1994. Comparison of the

phosphorylation of microtubule-associated protein tau by non-proline dependent protein kinases. *Mol Cell Biochem* 131:181-189.

73. Sternberger, L A., and Sternberger, N.H. 1983. Monoclonal antibodies distinguish phosphorylated and nonphosphorylated forms of neurofilaments in situ. *Proc Natl Acad Sci U S A* 80:6126-6130.
74. Petzold, A., Gveric, D., Groves, M., Schmierer, K., et al. 2008. Phosphorylation and compactness of neurofilaments in multiple sclerosis: indicators of axonal pathology. *Exp Neurol* 213:326-335.
75. Rudrabhatla, P., Grant, P., Jaffe, H., Strong, M.J., and Pant, H.C. 2010. Quantitative phosphoproteomic analysis of neuronal intermediate filament proteins (NF-M/H) in Alzheimer's disease by iTRAQ. *Faseb J* 24:4396-4407.
76. Sternberger, N.H., Sternberger, L.A., and Ulrich, J. 1985. Aberrant neurofilament phosphorylation in Alzheimer disease. *Proc Natl Acad Sci U S A* 82:4274-4276.
77. Al-Chalabi, A., Andersen, P.M., Nilsson, P., Chioza, B., et al. 1999. Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis. *Hum Mol Genet* 8:157-164.
78. Van den Berg-Vos, R.M., Franssen, H., Visser, J., de Visser, M., et al. 2002. Disease severity in multifocal motor neuropathy and its association with the response to immunoglobulin treatment. *J Neurol* 249:330-336.
79. Brownlees, J., Ackerley, S., Grierson, A.J., Jacobsen, N.J., et al. 2002. Charcot-Marie-Tooth disease neurofilament mutations disrupt neurofilament assembly and axonal transport. *Hum Mol Genet* 11:2837-2844.
80. De Jonghe, P., Mersivanova, I., Nelis, E., Del Favero, J., et al. 2001. Further evidence that neurofilament light chain gene mutations can cause Charcot-Marie-Tooth disease type 2E. *Ann Neurol* 49:245-249.
81. Mersiyanova, I.V., Perepelov, A.V., Polyakov, A.V., Sitnikov, V.F., et al. 2000. A new variant of Charcot-Marie-Tooth disease type 2 is probably the result of a mutation in the neurofilament-light gene. *Am J Hum Genet* 67:37-46.
82. Perez-Olle, R., Lopez-Toledano, M.A., Goryunov, D., Cabrera-Poch, N., et al. 2005. Mutations in the neurofilament light gene linked to Charcot-Marie-Tooth disease cause defects in transport. *J Neurochem* 93:861-874.
83. Eikelenboom, M.J., Petzold, A., Lazeron, R.H., Silber, E., et al. 2003. Multiple sclerosis: Neurofilament light chain antibodies are correlated to cerebral atrophy. *Neurology* 60:219-223.
84. Kuhle, J., Malmestrom, C., Axelsson, M., Plattner, K., et al. 2013. Neurofilament light and heavy subunits compared as therapeutic biomarkers in multiple sclerosis. *Acta Neurol Scand* 128:e33-36.
85. Kuhle, J., Plattner, K., Bestwick, J.P., Lindberg, R.L., et al. 2013. A comparative study of CSF neurofilament light and heavy chain protein in MS. *Mult Scler* 19:1597-1603.
86. Norgren, N., Rosengren, L., and Stigbrand, T. 2003. Elevated neurofilament levels in neurological diseases. *Brain Res* 987:25-31.
87. Teunissen, C.E., and Khalil, M. 2012. Neurofilaments as biomarkers in multiple sclerosis. *Mult Scler* 18:552-556.
88. Lycke, J.N., Karlsson, J.E., Andersen, O., and Rosengren, L.E. 1998. Neurofilament protein in cerebrospinal fluid: a potential marker of activity in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 64:402-404.
89. Heins, S., Wong, P.C., Muller, S., Goldie, K., et al. 1993. The rod domain of NF-L determines neurofilament architecture, whereas the end domains specify filament assembly and network formation. *J Cell Biol* 123:1517-1533.

90. Huizinga, R., Gerritsen, W., Heijmans, N., and Amor, S. 2008. Axonal loss and gray matter pathology as a direct result of autoimmunity to neurofilaments. *Neurobiol Dis* 32:461-470.
91. Huizinga, R., Heijmans, N., Schubert, P., Gschmeissner, S., et al. 2007. Immunization with neurofilament light protein induces spastic paresis and axonal degeneration in Biozzi ABH mice. *J Neuropathol Exp Neurol* 66:295-304.
92. Ali, R., Nicholas, R.S., and Muraro, P.A. 2013. Drugs in development for relapsing multiple sclerosis. *Drugs* 73:625-650.
93. Jacobs, L.D., Cookfair, D.L., Rudick, R.A., Herndon, R.M., et al. 1996. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Ann Neurol* 39:285-294.
94. Ebers, G.C., Sibley, W A., and Paty, D.W. 1995. Immunotherapy for MS: clinical aspects and trials. *Acta Neurol Scand Suppl* 161:54.
95. Johnson, K.P., Brooks, B.R., Cohen, J.A., Ford, C.C., et al. 1995. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. *Neurology* 45:1268-1276.
96. Yednock, T A., Cannon, C., Fritz, L.C., Sanchez-Madrid, F., et al. 1992. Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. *Nature* 356:63-66.
97. Polman, C.H., O'Connor, P.W., Havrdova, E., Hutchinson, M., et al. 2006. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 354:899-910.
98. Rudick, R., Polman, C., Clifford, D., Miller, D., and Steinman, L. 2006. Natalizumab: bench to bedside and beyond. *JAMA Neurol* 70:172-182.
99. Brinkmann, V., Davis, M.D., Heise, C.E., Albert, R., et al. 2002. The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *J Biol Chem* 277:21453-21457.
100. Cohen, J.A., Barkhof, F., Comi, G., Hartung, H.P., et al. 2010. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med* 362:402-415.
101. Radue, E.W., O'Connor, P., Polman, C.H., Hohlfeld, R., et al. 2012. Impact of fingolimod therapy on magnetic resonance imaging outcomes in patients with multiple sclerosis. *Arch Neurol* 69:1259-1269.
102. Coles, A.J., Cox, A., Le Page, E., Jones, J., et al. 2006. The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. *J Neurol* 253:98-108.
103. Castillo-Trivino, T., Braithwaite, D., Bacchetti, P., and Waubant, E. 2013. Rituximab in relapsing and progressive forms of multiple sclerosis: a systematic review. *PLoS One* 8:e66308.
104. Weinshenker, B.G., O'Brien, P.C., Petterson, T.M., Noseworthy, J.H., et al. 1999. A randomized trial of plasma exchange in acute central nervous system inflammatory demyelinating disease. *Ann Neurol* 46:878-886.
105. Keegan, M., Konig, F., McClelland, R., Bruck, W., et al. 2005. Relation between humoral pathological changes in multiple sclerosis and response to therapeutic plasma exchange. *Lancet* 366:579-582.
106. Kaynar, L., Altuntas, F., Aydogdu, I., Turgut, B., et al. 2008. Therapeutic plasma exchange in patients with neurologic diseases: retrospective multicenter study. *Transfus Apher Sci* 38:109-115.
107. Wolinsky, J.S., Narayana, P.A., O'Connor, P., Coyle, P.K., et al. 2007. Glatiramer acetate in primary progressive multiple sclerosis: results of a multinational,

multicenter, double-blind, placebo-controlled trial. *Ann Neurol* 61:14-24.
108. Allen, S.J., Baker, D., O'Neill, J.K., Davison, A.N., and Turk, J.L. 1993. Isolation and characterization of cells infiltrating the spinal cord during the course of chronic relapsing experimental allergic encephalomyelitis in the Biozzi AB/H mouse. *Cell Immunol* 146:335-350.
109. Smith, P.A., Heijmans, N., Ouwerling, B., Breij, E.C., et al. 2005. Native myelin oligodendrocyte glycoprotein promotes severe chronic neurological disease and demyelination in Biozzi ABH mice. *Eur J Immunol* 35:1311-1319.
110. Miller, S.D., Karpus, W.J., and Davidson, T.S. 2010. Experimental autoimmune encephalomyelitis in the mouse. *Curr Protoc Immunol* Chapter 15:Unit 15 11.
111. Sun, D., Whitaker, J.N., Huang, Z., Liu, D., et al. 2001. Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J Immunol* 166:7579-7587.
112. Johnson, T.A., Jirik, F.R., and Fournier, S. 2010. Exploring the roles of CD8(+) T lymphocytes in the pathogenesis of autoimmune demyelination. *Semin Immunopathol* 32:197-209.
113. Traugott, U., Reinherz, E.L., and Raine, C.S. 1983. Multiple sclerosis. Distribution of T cells, T cell subsets and Ia-positive macrophages in lesions of different ages. *J Neuroimmunol* 4:201-221.
114. Hauser, S.L., Bhan, A.K., Gilles, F., Kemp, M., et al. 1986. Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann Neurol* 19:578-587.
115. O'Neill, J.K., Baker, D., Davison, A.N., Allen, S.J., et al. 1993. Control of immune-mediated disease of the central nervous system with monoclonal (CD4-specific) antibodies. *J Neuroimmunol* 45:1-14.
116. Sakai, K., Zamvil, S.S., Mitchell, D.J., Hodgkinson, S., et al. 1989. Prevention of experimental encephalomyelitis with peptides that block interaction of T cells with major histocompatibility complex proteins. *Proc Natl Acad Sci U S A* 86:9470-9474.
117. Levine, S., and Sowinski, R. 1973. Hyperacute allergic encephalomyelitis. A localized form produced by passive transfer and pertussis vaccine. *Am J Pathol* 73:247-260.
118. Amor, S., O'Neill, J.K., Morris, M.M., Smith, R.M., et al. 1996. Encephalitogenic epitopes of myelin basic protein, proteolipid protein, myelin oligodendrocyte glycoprotein for experimental allergic encephalomyelitis induction in Biozzi ABH (H-2Ag7) mice share an amino acid motif. *J Immunol* 156:3000-3008.
119. Heijmans, N., Smith, P.A., Morris-Downes, M.M., Pryce, G., et al. 2005. Encephalitogenic and tolerogenic potential of altered peptide ligands of MOG and PLP in Biozzi ABH mice. *J Neuroimmunol* 167:23-33.
120. Swanborg, R.H. 2001. Experimental autoimmune encephalomyelitis in the rat: lessons in T-cell immunology and autoreactivity. *Immunol Rev* 184:129-135.
121. Bajramovic, J.J., Brok, H.P., Ouwerling, B., Jagessar, S.A., et al. 2008. Oligodendrocyte-specific protein is encephalitogenic in rhesus macaques and induces specific demyelination of the optic nerve. *Eur J Immunol* 38:1452-1464.
122. Genain, C.P., and Hauser, S.L. 2001. Experimental allergic encephalomyelitis in the New World monkey Callithrix jacchus. *Immunol Rev* 183:159-172.
123. Jagessar, S.A., Smith, P.A., Blezer, E., Delarasse, C., et al. 2008. Autoimmunity against myelin oligodendrocyte glycoprotein is dispensable for the initiation although essential for the progression of chronic encephalomyelitis in common marmosets. *J Neuropathol Exp Neurol* 67:326-340.
124. Amor, S., Baker, D., Groome, N., and Turk, J.L. 1993. Identification of a major encephalitogenic epitope of proteolipid protein (residues 56-70) for the induction of

experimental allergic encephalomyelitis in Biozzi AB/H and nonobese diabetic mice. *J Immunol* 150:5666-5672.

125. Amor, S., Groome, N., Linington, C., Morris, M.M., et al. 1994. Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. *J Immunol* 153:4349-4356.
126. Baker, D., O'Neill, J.K., Davison, A.N., and Turk, J.L. 1992. Control of immune-mediated disease of the central nervous system requires the use of a neuroactive agent: elucidation by the action of mitoxantrone. *Clin Exp Immunol* 90:124-128.
127. Lorentzen, J.C., Issazadeh, S., Storch, M., Mustafa, M.I., et al. 1995. Protracted, relapsing and demyelinating experimental autoimmune encephalomyelitis in DA rats immunized with syngeneic spinal cord and incomplete Freund's adjuvant. *J Neuroimmunol* 63:193-205.
128. Gambi, D., Di Cesare, N., Di Trapani, G., Macchi, G., and Sbriccoli, A. 1989. Experimental allergic encephalomyelitis in guinea pig: variability of response to intradermal emulsion injection. *Ital J Neurol Sci* 10:33-41.
129. Raine, C.S., Traugott, U., and Stone, S.H. 1978. Chronic relapsing experimental allergic encephalomyelitis: CNS plaque development in unsuppressed and suppressed animals. *Acta Neuropathol* 43:43-53.
130. Hampton, D.W., Anderson, J., Pryce, G., Irvine, K.A., et al. 2008. An experimental model of secondary progressive multiple sclerosis that shows regional variation in gliosis, remyelination, axonal and neuronal loss. *J Neuroimmunol* 201-202:200-211.
131. Park, I.K., Hiraki, K., Kohyama, K., and Matsumoto, Y. 2008. Differential effects of decoy chemokine (7ND) gene therapy on acute, biphasic and chronic autoimmune encephalomyelitis: implication for pathomechanisms of lesion formation. *J Neuroimmunol* 194:34-43.
132. Ellmerich, S., Mycko, M., Takacs, K., Waldner, H., et al. 2005. High incidence of spontaneous disease in an HLA-DR15 and TCR transgenic multiple sclerosis model. *J Immunol* 174:1938-1946.
133. Bettelli, E., Pagany, M., Weiner, H.L., Linington, C., et al. 2003. Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. *J Exp Med* 197:1073-1081.
134. Baker, D., O'Neill, J.K., Gschmeissner, S.E., Wilcox, C.E., et al. 1990. Induction of chronic relapsing experimental allergic encephalomyelitis in Biozzi mice. *J Neuroimmunol* 28:261-270.
135. Al-Izki, S., Pryce, G., O'Neill, J.K., Butter, C., et al. 2012. Practical guide to the induction of relapsing progressive experimental autoimmune encephalomyelitis in the Biozzi ABH mouse. *Multiple Sclerosis and Related Disorders* 1:29-38.
136. Morris-Downes, M.M., Smith, P.A., Rundle, J.L., Piddlesden, S.J., et al. 2002. Pathological and regulatory effects of anti-myelin antibodies in experimental allergic encephalomyelitis in mice. *J Neuroimmunol* 125:114-124.
137. Nikic, I., Merkler, D., Sorbara, C., Brinkoetter, M., et al. 2011. A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. *Nat Med* 17:495-499.
138. Vogt, J., Paul, F., Aktas, O., Muller-Wielsch, K., et al. 2009. Lower motor neuron loss in multiple sclerosis and experimental autoimmune encephalomyelitis. *Ann Neurol* 66:310-322.
139. Krishnamoorthy, G., Saxena, A., Mars, L.T., Domingues, H.S., et al. 2009. Myelin-specific T cells also recognize neuronal autoantigen in a transgenic mouse model of

multiple sclerosis. *Nat Med* 15:626-632.
140. Mathey, E.K., Derfuss, T., Storch, M.K., Williams, K.R., et al. 2007. Neurofascin as a novel target for autoantibody-mediated axonal injury. *J Exp Med* 204:2363-2372.
141. Elliott, C., Lindner, M., Arthur, A., Brennan, K., et al. 2012. Functional identification of pathogenic autoantibody responses in patients with multiple sclerosis. *Brain* 135:1819-1833.
142. Tsunoda, I., and Fujinami, R.S. 2002. Inside-Out versus Outside-In models for virus induced demyelination: axonal damage triggering demyelination. *Springer Semin Immunopathol* 24:105-125.
143. Howell, O.W., Rundle, J.L., Garg, A., Komada, M., et al. 2010. Activated microglia mediate axoglial disruption that contributes to axonal injury in multiple sclerosis. *J Neuropathol Exp Neurol* 69:1017-1033.
144. Singh, S., Metz, I., Amor, S., van der Valk, P., et al. 2013. Microglial nodules in early multiple sclerosis white matter are associated with degenerating axons. *Acta Neuropathol* 125:595-608.
145. Aboul-Enein, F., Krssak, M., Hoftberger, R., Prayer, D., and Kristoferitsch, W. 2010. Reduced NAA-levels in the NAWM of patients with MS is a feature of progression. A study with quantitative magnetic resonance spectroscopy at 3 Tesla. *PLoS One* 5:e11625.
146. Bjartmar, C., Kinkel, R.P., Kidd, G., Rudick, R.A., and Trapp, B.D. 2001. Axonal loss in normal-appearing white matter in a patient with acute MS. *Neurology* 57:1248-1252.
147. Petzold, A., Tozer, D.J., and Schmierer, K. 2011. Axonal damage in the making: neurofilament phosphorylation, proton mobility and magnetisation transfer in multiple sclerosis normal appearing white matter. *Exp Neurol* 232:234-239.
148. Huizinga, R., Linington, C., and Amor, S. 2008. Resistance is futile: antineuronal autoimmunity in multiple sclerosis. *Trends Immunol* 29:54-60.
149. Braik, T., Evans, A.T., Telfer, M., and McDunn, S. 2010. Paraneoplastic neurological syndromes: unusual presentations of cancer. A practical review. *Am J Med Sci* 340:301-308.
150. Poh, M.Q., Simon, N.G., Buckland, M.E., Salisbury, E., and Watson, S. 2013. Evidence of T-cell mediated neuronal injury in stiff-person syndrome with anti-amphiphysin antibodies. *J Neurol Sci* 337:235-237.
151. Voltz, R., Dalmau, J., Posner, J.B., and Rosenfeld, M.R. 1998. T-cell receptor analysis in anti-Hu associated paraneoplastic encephalomyelitis. *Neurology* 51:1146-1150.
152. Dalmau, J., Tuzun, E., Wu, H.Y., Masjuan, J., et al. 2007. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol* 61:25-36.
153. Lancaster, E., Lai, M., Peng, X., Hughes, E., et al. 2010. Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *Lancet Neurol* 9:67-76.
154. Lucchinetti, C.F., Kimmel, D.W., and Lennon, V.A. 1998. Paraneoplastic and oncologic profiles of patients seropositive for type 1 antineuronal nuclear autoantibodies. *Neurology* 50:652-657.
155. Geis, C., Weishaupt, A., Hallermann, S., Grunewald, B., et al. 2010. Stiff person syndrome-associated autoantibodies to amphiphysin mediate reduced GABAergic inhibition. *Brain* 133:3166-3180.
156. Roemer, S.F., Parisi, J.E., Lennon, V.A., Benarroch, E.E., et al. 2007. Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. *Brain* 130:1194-1205.

157. Saadoun, S., and Papadopoulos, M.C. 2010. Aquaporin-4 in brain and spinal cord oedema. *Neuroscience* 168:1036-1046.
158. Lennon, V.A., Wingerchuk, D.M., Kryzer, T.J., Pittock, S.J., et al. 2004. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* 364:2106-2112.
159. Lennon, V.A., Kryzer, T.J., Pittock, S.J., Verkman, A.S., and Hinson, S.R. 2005. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* 202:473-477.
160. Lucchinetti, C.F., Mandler, R.N., McGavern, D., Bruck, W., et al. 2002. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. *Brain* 125:1450-1461.
161. Willison, H.J. 2005. The immunobiology of Guillain-Barre syndromes. *J Peripher Nerv Syst* 10:94-112.
162. Forooghian, F., Cheung, R.K., Smith, W.C., O'Connor, P., and Dosch, H.M. 2007. Enolase and arrestin are novel nonmyelin autoantigens in multiple sclerosis. *J Clin Immunol* 27:388-396.
163. Huizinga, R., Hintzen, R.Q., Assink, K., van Meurs, M., and Amor, S. 2009. T-cell responses to neurofilament light protein are part of the normal immune repertoire. *Int Immunol* 21:433-441.
164. Andersson, M., Alvarez-Cermeno, J., Bernardi, G., Cogato, I., et al. 1994. Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. *J Neurol Neurosurg Psychiatry* 57:897-902.
165. Polman, C.H., Reingold, S.C., Edan, G., Filippi, M., et al. 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol* 58:840-846.
166. Sadaba, M.C., Tzartos, J., Paino, C., Garcia-Villanueva, M., et al. 2012. Axonal and oligodendrocyte-localized IgM and IgG deposits in MS lesions. *J Neuroimmunol* 247:86-94.
167. Ehling, R., Lutterotti, A., Wanschitz, J., Khalil, M., et al. 2004. Increased frequencies of serum antibodies to neurofilament light in patients with primary chronic progressive multiple sclerosis. *Mult Scler* 10:601-606.
168. Silber, E., Semra, Y.K., Gregson, N A., and Sharief, M.K. 2002. Patients with progressive multiple sclerosis have elevated antibodies to neurofilament subunit. *Neurology* 58:1372-1381.
169. Fialova, L., Bartos, A., Svarcova, J., Zimova, D., et al. 2013. Serum and cerebrospinal fluid light neurofilaments and antibodies against them in clinically isolated syndrome and multiple sclerosis. *J Neuroimmunol* 262:113-120.
170. Kuhlmann, T., Lingfeld, G., Bitsch, A., Schuchardt, J., and Bruck, W. 2002. Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. *Brain* 125:2202-2212.
171. Bo, L., Dawson, T.M., Wesselingh, S., Mork, S., et al. 1994. Induction of nitric oxide synthase in demyelinating regions of multiple sclerosis brains. *Ann Neurol* 36:778-786.
172. Liu, J.S., Zhao, M.L., Brosnan, C.F., and Lee, S.C. 2001. Expression of inducible nitric oxide synthase and nitrotyrosine in multiple sclerosis lesions. *Am J Pathol* 158:2057-2066.
173. Brorson, J.R., Schumacker, P.T., and Zhang, H. 1999. Nitric oxide acutely inhibits neuronal energy production. The Committees on Neurobiology and Cell Physiology. *J Neurosci* 19:147-158.
174. Witte, M.E., Bo, L., Rodenburg, R.J., Belien, J.A., et al. 2009. Enhanced number

and activity of mitochondria in multiple sclerosis lesions. *J Pathol* 219:193-204.
175. Witte, M.E., Nijland, P.G., Drexhage, J.A., Gerritsen, W., et al. 2013. Reduced expression of PGC-1alpha partly underlies mitochondrial changes and correlates with neuronal loss in multiple sclerosis cortex. *Acta Neuropathol* 125:231-243.
176. Werner, P., Pitt, D., and Raine, C.S. 2000. Glutamate excitotoxicity--a mechanism for axonal damage and oligodendrocyte death in Multiple Sclerosis? *J Neural Transm Suppl*:375-385.
177. Werner, P., Pitt, D., and Raine, C.S. 2001. Multiple sclerosis: altered glutamate homeostasis in lesions correlates with oligodendrocyte and axonal damage. *Ann Neurol* 50:169-180.
178. Babbe, H., Roers, A., Waisman, A., Lassmann, H., et al. 2000. Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J Exp Med* 192:393-404.
179. Neumann, H., Cavalie, A., Jenne, D.E., and Wekerle, H. 1995. Induction of MHC class I genes in neurons. *Science* 269:549-552.
180. Redwine, J.M., Buchmeier, M.J., and Evans, C.F. 2001. In vivo expression of major histocompatibility complex molecules on oligodendrocytes and neurons during viral infection. *Am J Pathol* 159:1219-1224.
181. Hoftberger, R., Aboul-Enein, F., Brueck, W., Lucchinetti, C., et al. 2004. Expression of major histocompatibility complex class I molecules on the different cell types in multiple sclerosis lesions. *Brain Pathol* 14:43-50.
182. Fissolo, N., Haag, S., de Graaf, K.L., Drews, O., et al. 2009. Naturally presented peptides on major histocompatibility complex I and II molecules eluted from central nervous system of multiple sclerosis patients. *Mol Cell Proteomics* 8:2090-2101.
183. Medana, I., Martinic, M A., Wekerle, H., and Neumann, H. 2001. Transection of major histocompatibility complex class I-induced neurites by cytotoxic T lymphocytes. *Am J Pathol* 159:809-815.
184. Nitsch, R., Pohl, E.E., Smorodchenko, A., Infante-Duarte, C., et al. 2004. Direct impact of T cells on neurons revealed by two-photon microscopy in living brain tissue. *J Neurosci* 24:2458-2464.
185. Gimsa, U., Peter, S.V., Lehmann, K., Bechmann, I., and Nitsch, R. 2000. Axonal damage induced by invading T cells in organotypic central nervous system tissue in vitro: involvement of microglial cells. *Brain Pathol* 10:365-377.
186. Derfuss, T., Parikh, K., Velhin, S., Braun, M., et al. 2009. Contactin-2/TAG-1-directed autoimmunity is identified in multiple sclerosis patients and mediates gray matter pathology in animals. *Proc Natl Acad Sci U S A* 106:8302-8307.
187. Newcombe, J., Gahan, S., and Cuzner, M.L. 1985. Serum antibodies against central nervous system proteins in human demyelinating disease. *Clin Exp Immunol* 59:383-390.
188. Terryberry, J.W., Thor, G., and Peter, J.B. 1998. Autoantibodies in neurodegenerative diseases: antigen-specific frequencies and intrathecal analysis. *Neurobiol Aging* 19:205-216.
189. Almeras, L., Lefranc, D., Drobecq, H., de Seze, J., et al. 2004. New antigenic candidates in multiple sclerosis: identification by serological proteome analysis. *Proteomics* 4:2184-2194.
190. Bartos, A., Fialova, L., Soukupova, J., Kukal, J., et al. 2007. Antibodies against light neurofilaments in multiple sclerosis patients. *Acta Neurol Scand* 116:100-107.
191. Fialova, L., Bartos, A., Svarcova, J., Zimova, D., and Kotoucova, J. 2013. Serum and cerebrospinal fluid heavy neurofilaments and antibodies against them in early

multiple sclerosis. *J Neuroimmunol* 259:81-87.
192. Fialova, L., Bartos, A., Svarcova, J., and Malbohan, I. 2011. Increased intrathecal high-avidity anti-tau antibodies in patients with multiple sclerosis. *PLoS One* 6:e27476.
193. Svarcova, J., Fialova, L., Bartos, A., Steinbachova, M., and Malbohan, I. 2008. Cerebrospinal fluid antibodies to tubulin are elevated in the patients with multiple sclerosis. *Eur J Neurol* 15:1173-1179.
194. Resnick, S.M., Pham, D.L., Kraut, M.A., Zonderman, A.B., and Davatzikos, C. 2003. Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. *J Neurosci* 23:3295-3301.