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van der Star, B.J.

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CHAPTER 7

General Discussion and Future Perspectives

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) widely considered as an autoimmune disease in which autoaggressive T cells attack myelin. As well as demyelination, neurodegeneration is a major pathological feature that plays a crucial role in the clinical disability of people with MS (1). That neuronal and axonal damage contributes to the accumulation of neurological deficits, particularly in progressive forms of MS, has redirected attention to the cause of this damage as well as whether neurodegeneration is a cause or a consequence of demyelination. Recent studies have suggested that in addition to an autoimmune attack on myelin, autoimmunity to neuronal proteins present in people with MS, is a key mechanism leading to progressive neurodegeneration and axonal damage. One of the possible targets for the (auto)immune response is the neuron-specific cytoskeleton protein neurofilament light (NF-L) (2-6). However, whether these immune responses present in MS are merely biomarkers of axonal and neuronal damage or whether they play a pathogenic role and contribute to axonal damage in people with MS is the key question of this thesis.

Insights in the mechanisms contributing to neurodegeneration are crucial for the development of new drug targets to prevent neuronal damage and progressive neurological deficits. Therefore the goal of this thesis is to elucidate the role of immune responses to neuronal antigens, in particular NF-L. As depicted in 'Chapter 1', the thesis had several aims to test the hypothesis: **'Autoimmunity to NF-L contributes to axonal damage in MS'**. Each aim is discussed below in perspective of the results and recent knowledge. We conclude by discussing whether therapies targeting autoimmunity to neuronal antigens might be relevant for treating disease progression in MS.

Major questions:

1. *How can autoimmunity to neuronal antigens arise in MS?*
2. *What are the mechanism(s) by which NF-L-specific T cells cause axonal damage?*
3. *Can antibodies to NF-L serve as biomarkers of disease and/or do they contribute to axonal damage in MS?*
4. *Targeting autoimmunity to neuronal antigens; is this relevant for disease progression in MS?*

How can autoimmunity to neuronal antigens arise in MS?

Accumulating evidence indicates that, as well as myelin antigens, autoimmunity to neuronal antigens are present in MS and other neurodegenerative diseases. However, how these responses arise is unclear. One key feature for the generation of immune responses is antigen presentation. This could take place after neuronal or axonal damage, but also when micro-organism antigens show similarity with self-antigens (so-called 'molecular mimicry') (7). Molecular mimicry is demonstrated in experimental models for viral particles that share homology with myelin proteins, including proteolipid protein (PLP) (8), myelin basic protein (MBP) (9) and myelin oligodendrocyte glycoprotein (MOG) (10). Bacterial antigens are also shown to activate myelin-specific T cells, resulting in exacerbation of experimental autoimmune encephalomyelitis (EAE) (11). In humans, an example of axonal pathology following bacterial infections is Guillain-Barré Syndrome (GBS), although

mainly the peripheral nerves are affected (12). However, homologies between neurofilaments and micro-organisms have yet to be reported. Therefore, a more likely key event in the development of autoimmunity to neuronal antigens in MS is phagocytosis of neuronal debris. Several studies indicate a strong association between neuronal damage and immune cell activation in MS lesions. In **chapters 2 and 3** we demonstrated phagocytosis of neuronal antigens in the CNS of people with MS. Although engulfment of neuronal proteins was not as extensive as myelin proteins in active white matter lesions, phagocytosis of neuronal debris correlated with the extent of damaged axons. Thus, people with MS who have extensive neuronal damage could have a higher chance of autoimmunity to neuronal antigens including NF-L. On the contrary, phagocytosis of neuronal debris might lead to tolerance, since T cells and antibodies to myelin and neuronal antigens are also present in the serum of healthy controls (13). Tolerance following phagocytosis of debris is also supported by a Danish study that does not find a correlation between head injury and development of MS (14). More support for a tolerogenic effect after axonal damage is provided by Mutlu and colleagues who show that experimental brain injury significantly reduces the clinical signs of EAE, considered an animal model of MS (15). Nevertheless, uptake, degradation and presentation of neuronal antigens remain crucial steps for activation of autoreactive T cells and autoantibody production. We observed neuronal-antigen containing human leukocyte antigen (HLA)-DR⁺ cells in the cerebrospinal fluid (CSF) more frequently than in non-inflammatory disorders (**chapter 2**). A recent study by Schutzer and colleagues has identified neuronal proteins, rather than myelin proteins, in people with clinically isolated syndrome (CIS) suggesting involvement of grey matter early in MS development (16). More so, neuronal protein-containing cells are shown in cervical lymph nodes of people with MS expressing the co-stimulatory molecule cluster of differentiation-40 (CD40) (17). This is in line with our hypothesis that phagocytosis of NF-L might be the first step in the development of autoimmune responses to NF-L in MS. In addition, together with MBP and neurofilament medium, NF-L is eluted from major histocompatibility complex (MHC) class II molecules from people with MS, indicating presentation of these antigens (18). Whether NF-L antigen presentation induces an autoimmune response is still a subject of investigation. A preliminary experiment with monocyte-derived macrophages incubated with human white matter debris failed to demonstrate a proliferative effect on NF-L-specific T cells, although this experiment needs to be repeated (collaborative experiment with D.Y.S. Vogel, VUmc, and P. Nacken, Delta Crystallon BV).

In summary, neuronal antigens phagocytosed and presented by antigen presenting cells have the potential to attract and activate the immune system. In this way they might contribute to pathogenic immune responses and neuronal damage in people with MS.

What are the mechanism(s) by which NF-L-specific T cells cause axonal damage?

The presence of T cells in MS lesions and myelin-reactive T cells in the circulation of people with MS as well as T cell targeted therapies implicate T lymphocytes in the pathogenesis of MS. Furthermore, CD8⁺ T cells outnumber CD4⁺ T cells in MS lesions (19). Both myelin-reactive T cells and T cells reactive to neuronal antigens, e.g. NF-L, are present in people with MS and healthy controls (13). However, these results do not confirm a pathogenic role of T cells in MS. Most support for a pathogenic role of T cells in MS is generated by using animal models. For example,

adoptive transfer of myelin-specific T cells from myelin-immunised mice causes disease in naive recipients (20). However, little is known about neuronal-specific T cells. Previous studies showed that immunisation with recombinant NF-L induces spasticity and paralysis accompanied by grey matter pathology in the dorsal column of the spinal cord of Biozzi ABH (antibody high) mice (21, 22). In **chapter 4** we further characterised the T cell response in these mice and demonstrated an association of T cells with axonal damage in spinal cord lesions. In addition, transfer of NF-L-specific T cells into naive recipients induced clinical disease after 25 days. Although clinical disease was mild (partial loss of tone tail) and present in 29% of mice it reveals that NF-L-specific T cells have the potential to be pathogenic in this model.

The mechanisms by which T cells might contribute to axonal damage are discussed below.

Mechanisms of axonal damage by T cells

Effector mechanisms of activated CD4⁺ and CD8⁺ T lymphocytes include secretion of perforin and granzyme B. Although CD4⁺ T cells are initially thought not to have cytotoxic abilities, studies indicate that a subset of CD4⁺ T cells is able to express cytotoxic molecules e.g. perforin and granzyme B (23, 24). In biological fluids of people with MS, perforin⁺ CD4⁺ and CD8⁺ T cells are identified (25, 26). Several studies show neurotoxicity following exposure of perforin (27-29), or destabilisation of microtubules (30). In perforin-deficient mice infected with Theiler's murine encephalomyelitis virus (TMEV), axons are preserved despite demyelination (31, 32) indicating that perforin contributes to axonal damage in this model. We showed that perforin expression was increased in NF-L immunised Biozzi ABH mice (**chapter 4**). After pore formation of the cell membrane caused by perforin, granzyme B enters the cell (33). Induction of neuronal death by granzyme B is reported *in vitro* (34-36) and *in vivo* (37). Granzyme B can also induce apoptosis in a perforin-independent manner through endocytosis (35). A role of granzyme B in CNS damage in MS is supported by an increased granzyme B mRNA expression in active and chronic active MS lesions compared to normal appearing white matter (35) as well as increased granzyme B protein expression in the CSF of people with relapsing-remitting MS (RRMS) who experience a relapse (38). In line with these studies are preliminary results that show the presence of granzyme B⁺ CD4⁺ T cells in an active MS brain lesion (Fig. 1). Whether these granzyme B⁺ granules are released is currently unknown. Furthermore, in **chapter 4** we show the presence of granzyme B⁺ cells in spinal cord lesions in NF-L immunised Biozzi ABH mice. These results indicate that besides perforin, secretion of granzyme B by NF-L-reactive T cells may contribute to axonal damage.

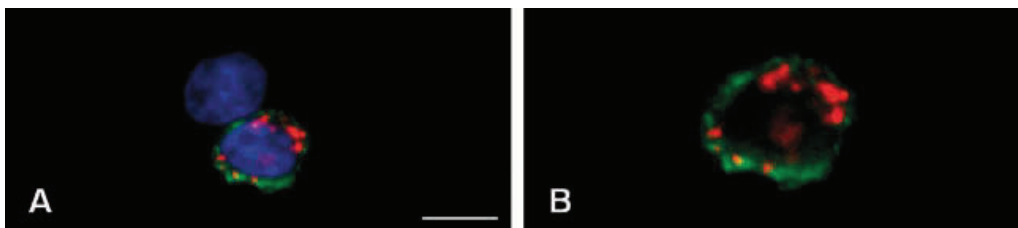


Figure 1. Granzyme B⁺ CD4⁺ T cell in the brain parenchyma in a MS lesion. A) Granzyme B expression (red) in CD4⁺ T cells (green) in an active MS lesion. **B)** Enlargement of (A). Nuclei are stained blue. Scale bar=5µm. In collaboration with prof. M. Kipp (RWTH, Aachen, Germany).

In highly inflammatory MS lesions, neurons are shown to express MHC class I (39), suggesting that neurons might also be targets for CD8⁺ T cells. Sauer and colleagues show direct axonal injury by CD8⁺ T cells through a MHC class I and granzyme B-dependent mechanism (36). Expression of MHC class I is up regulated in the presence of interferon-gamma (36, 40), a pro-inflammatory molecule which is produced by NF-L-reactive T cells (13). This indicates that neurons might also be targets for CD8⁺ T cells through the interaction with MHC class I molecules.

Other cell-cell contact dependent mechanisms by which T cells might contribute to axonal damage are through interaction of Fas- Fas ligand (FasL), tumor necrosis factor-related apoptosis inducing ligand (TRAIL), CD11a and CD40 (41-43). Giuliani and colleagues show decreased neuronal loss by CD4⁺ and CD8⁺ T cells following pre-treatment of T cells with antibodies to FasL, lymphocyte function-associated antigen-1 and CD40 (42). Furthermore, in MS lesions apoptotic spinal motor neurons are surrounded by TRAIL⁺ CD3⁺ T cells (43). To confirm a role for a TRAIL-dependent mechanism of neuronal loss, Vogt and colleagues compare neuronal loss in mice following adoptive transfer of MOG³⁵⁻⁵⁵-specific T cells from wild-type mice or TRAIL-deficient mice. Significantly more neuronal loss is observed in mice following adoptive transfer of MOG³⁵⁻⁵⁵-specific T cells, while the number of CD3⁺ T cells in the lesions is comparable (43).

To conclude, several mechanisms causing neuronal and axonal damage by T cells are described. Whether these mechanism are also part of NF-L-reactive T cells in the CNS of people with MS needs further study.

Can antibodies to NF-L serve as biomarkers of disease and/or do they contribute to axonal damage in MS?

Around 90% of people with MS have oligoclonal bands (OCB) in the CSF, which are a result of local immunoglobulin (Ig) production. It is unknown whether these Igs contribute to disease, although a pathogenic role of intrathecal Igs is supported by an increased risk of conversion to MS in people with CIS positive for OCB (44, 45). Oligoclonal antibody production in the CSF is not specific for MS (46, 47) and there is discussion whether OCB determination should still be part of the diagnostic tools for MS (47, 48). On the contrary, Igs in the CSF might serve as useful biomarkers to predict disease progression.

In the CSF and serum of people with MS, several studies report the presence of antibodies to neuronal antigens including NF-L (2, 4, 6). A recent study by Sádaba and colleagues has shown the binding of IgM and IgG on axons in active, chronic active and inactive MS lesions (49). Furthermore, levels of antibodies to NF-L are increased in the CSF and serum of people with progressive disease (2, 6) and correlate with clinical disability (6) and cerebral atrophy (3), suggesting the potential of antibodies to NF-L as biomarkers. Not all studies show a correlation between antibodies to NF-L and disability (**chapter 5** and (46)). These inconsistencies might be a result of differences in cohorts or assays used (50) as well as the medical history of the subjects (46). Also important to mention is that antibodies to NF-L are likely to bind NF-L proteins, which are also present in biological fluids (51, 52), and form complexes, which might interfere with the assay used for detection (50). Thus, it should be noted that measurements of NF-L antibodies might underestimate the actual numbers. In **chapter 5** we investigated the influence of treatment on serum antibody levels. We reported reduced levels of serum antibodies to NF-L in people with RRMS treated with natalizumab. These results are in line with a study

by Bartos and colleagues, who report that levels of intrathecal NF-L antibodies are specifically reduced following immunosuppressive and/or immunomodulatory treatment (46). Decreased NF-L protein levels in the CSF of people with RRMS following natalizumab treatment (53) as well mitoxantrone and rituximab treatment in people with progressive MS (54) are also reported. This might suggest that reduction of anti-NF-L antibodies is secondary to a decrease in axonal damage and therefore a decline in the release of neurofilament protein or that the treatment decreases the levels of NF-L antibodies which results in a positive effect on axonal damage. The latter argument is supported by a negative correlation with NF-L antibody levels and time since last relapse as shown in **chapter 5**.

Thus, antibodies to NF-L may serve as useful biomarkers for treatment responses and reflect axonal damage, but there are also indications that argue for a potential pathogenic role of NF-L-specific antibodies. The question whether antibodies to NF-L might contribute to axonal damage was the focus in **chapter 6**. In this chapter we demonstrated that a monoclonal antibody (mAb) to NF-L caused axonopathy in spinal cord co-cultures and a mouse model of optical neuritis. Recently, Elliott and colleagues have shown demyelination and axonal reduction using the same spinal cord co-culture system following MOG and neurofascin-specific antibodies, respectively (55). Whether mAb to NF-L also have an effect on myelination would be of interest for future studies, although in NF-L-immunised ABH mice mainly axons are affected (22). To localise NF-L antibodies, stereotactic intracerebral injection with labelled mAb to NF-L was performed in a mouse. This revealed internalisation of mAb to NF-L in neurons, although the isotype control was also detected inside neurons following stereotactic injection. Uptake of control Ig is shown before (56, 57). In the study by Greenlee and colleagues, anti-Yo antibodies targeting cytoplasmic proteins e.g. ribosomes in Purkinje cells, are taken up and accumulate intracellularly. This accumulation results in neuronal death whereas the control Ig does not accumulate in the Purkinje cell and therefore does not result in neuronal death (56). Fc receptor-mediated and clathrin-mediated endocytosis are possible non-epitope specific mechanisms by which antibodies (to NF-L) may be internalised by neurons (58-60). A recent study has shown that antibodies to an intracellular antigen enter neurons by endocytosis and cause depletion of adenosine triphosphate (ATP) and an increase in caspase levels leading to apoptosis and cell death (61). Activation of Fc-gamma receptor 1 by IgG-immune complex is shown to cause an increase in intracellular calcium and enhances excitability in sensory neurons (62). On the contrary, uptake of Ig by neurons might also serve as a protective mechanism because their internalisation inhibits complement activation (59) and assists in protein clearance (58).

In summary, antibodies to NF-L might serve as biomarkers but that does not exclude their possible pathogenic effects in inflammatory neurodegenerative diseases.

Mechanisms of axonal damage by antibodies

Antibodies directed to targets in the CNS contribute to pathology in several autoimmune disorders. In people with neuromyelitis optica, antibodies to aquaporin-4, a protein expressed on astrocytes, lead to demyelination possibly through complement-dependent cytotoxicity (63-68). In GBS antibodies to specific lipids, gangliosides, present on the surface of neurons, including anti-GM1 and anti-GQ1b cause neurotoxicity through complement-mediated calcium influx resulting in paralysis (12). Furthermore, the neuronal protein neurofascin is also demonstrated as a target for autoimmunity in MS (69, 70). Neurofascin has two

isoforms (155 kDa or 186 kDa) and is located at the nodes of Ranvier and functions in cell adhesion (69). Co-transfer of anti-neurofascin specific monoclonal antibodies and MOG-specific T cells results in complement-dependent, although reversible, axonal damage in rats. The authors show that anti-neurofascin antibodies interfere with electrophysiological properties of hippocampal neurons *in vitro* (70).

In the above mentioned examples the target antigen is located extracellular, but antibody-mediated axonal damage to intracellular located antigens is also reported. For example, in stiff person syndrome, passive transfer of antibodies to amphiphysin, an intracellular protein involved in vesicle release, is pathogenic in rats (71, 72). Geis and colleagues also demonstrate epitope-specific uptake of anti-amphiphysin antibodies by neurons and disturb synaptic activity (71). Whether antibodies to NF-L have an impact on neuronal communication and electrophysiology could be relevant for future studies by using e.g. patch clamping (Box 2). Other mechanisms of antibody-mediated axonal damage include disruption of axonal transport (73, 74) and alterations in the function of the target antigen (75). Antibodies to enolase, an enzyme involved in glycolysis, inhibit enolase function resulting in ATP depletion and apoptosis of retinal cells (75, 76). Whether antibodies to NF-L are able to disrupt axonal transport would be interesting for future research (Box 2). A previous study showed accumulation of mitochondria in axons located in the spinal cord following immunisation with NF-L in ABH mice (22). Accumulation of mitochondria is demonstrated early in the course of Wallerian degeneration (77) suggesting that internalised antibodies to NF-L might cause an increase in mitochondria interfering with neuronal function. It could be of interest to investigate mitochondrial function in spinal cord co-cultures following incubation with NF-L antibodies or investigate co-localisation of intracellular antibodies to NF-L and mitochondria accumulation in NF-L-immunised mice. The presence of antibodies to NF-L in the CNS might interfere with axon regeneration similar as has been reported for GM1-specific antibodies, one of the targets of autoimmunity in GBS patients (78, 79). On the contrary, antibody internalisation might also lead to degradation in lysosomes, which decreases the chance of a pathogenic effect of antibodies (59).

To conclude, besides the use of antibodies to NF-L as a biomarker, accumulating data supports a role for these antibodies in axonal damage.

Targeting autoimmunity to neuronal antigens; is this relevant for disease progression in MS?

The association of active white matter MS lesions with inflammatory cells suggests that in people with MS, the balance between protection and autoimmunity is lost. Nevertheless, grey matter pathology in the CNS of people with MS is accompanied by less inflammation. This suggests that pathological mechanisms independent of inflammation might be possible as well or that the grey matter is more vulnerable to pro-inflammatory cytokines (**chapter 3**).

Treatment strategies prohibiting entry of T cells in the CNS show that MS is more complex than originally thought, namely reactivation of CNS-specific T cells and in particular myelin-reactive T cells. The complex interplay between both the innate and adaptive immune system suggests that targeting of whole cell populations has major implications for the immune response. This is in line with an increased risk of neurotropic viruses attacking the CNS, such as increased risk of progressive multifocal leukoencephalopathy following treatment with natalizumab (80).

Increased knowledge regarding B cells and antibodies has led to targeting B cells in people with MS. Rituximab, a monoclonal antibody to CD20, reduces relapse rate

and newly forming lesions in people with RRMS (81, 82). Rituximab depletes CD20⁺ B cells which also leads to reduced levels of T lymphocytes in the CNS, although the treatment does not have an effect on OCB or CSF-IgG (83). Nevertheless, removal of humoral factors including antibodies using plasma exchange is beneficial for some people with MS, suggesting that perhaps in a (small) group of people with MS, pathogenic antibodies contribute to the disease. Unfortunately, plasma exchange provides only a temporary relief and can also worsen disability in some cases (84, 85). Depletion of B cells and antibodies might impede repair as well, since B cells and antibodies also aid in the removal of debris (86, 87) and promote repair (87, 88). A positive effect of antibodies is supported by favourable results in people with MS following administration of naturally occurring antibodies (intravenous immunoglobulin, IVIg) (89), although controversial (90).

In people with progressive MS with little inflammatory activity, anti-inflammatory agents do not seem to be effective (91) or even make the disease worse (92). Although progressive MS gains more and more attention, it is unclear why disability continues while inflammation in the CNS is less than in the CNS of people with RRMS (93). One hypothesis suggests a secondary response to inflammation present earlier in disease, for example in people with SPMS following RRMS (94).

A study by Ehling and colleagues and Silber and colleagues shows increased levels of antibodies to NF-L in progressive MS compared to people with RRMS (2, 6). This observation might be explained by the possibility that these antibodies are a remainder of an autoimmune response to NF-L in the active phase of the disease and B cell clones in the CNS produce these antibodies locally. This is in line with studies identifying B cell maturation and expansion in the CSF of people with MS (95, 96). Repair of the blood-brain barrier might contribute to this so-called compartmentalisation of antibodies (94). Another possibility includes the selection of subjects. Perhaps the selected people with progressive MS in these two studies show more inflammation than other people with progressive MS, although antibodies to other antigens, e.g. the above mentioned gangliosides, have also been shown to be increased in progressive MS (97).

To conclude, currently we know little about ongoing progression of neurological deficits in people with MS. Therapies targeting the inflammatory response are effective in diminishing neuroinflammation, but have no effect on continuation of axonal damage. Possibly a more specific targeting of the pathological mechanisms, such as antibodies to NF-L, in combination with neuroprotective agents and promoting repair mechanisms (94, 98) may be more effective. In addition, early treatment is a must to decrease clinical disability in later stages (98), which also urges the need for reliable biomarkers. Also the identification and targeting of pathogenic components in people with MS will be a challenge for future research.

Conclusions and future perspectives

We hypothesise that autoimmunity to NF-L contributes to axonal damage in MS (Fig. 2). Our research has implicated that in MS, autoimmunity to NF-L indeed might contribute to axonal damage. This conclusion is supported by our main findings as summarised in Box 1. We show that in the CNS of people with MS HLA-DR⁺ cells phagocytose neuronal antigens. This could lead to antigen presentation by professional antigen presenting cells that could serve as a key event in development of autoimmunity to NF-L. Furthermore, using an experimental mouse model for inflammatory-mediated neurodegeneration, we show that T cells secreted cytotoxic molecules that harm neurons and that immunodominant epitopes in the NF-L

sequence also induced experimental disease in mice. Antibodies to NF-L may be added to the growing panel of biomarkers to reflect treatment responses in MS, but are also shown to be pathogenic *in vitro* and *in vivo*. Therefore, antibodies to NF-L should not be ruled out as part of the disease mechanism in (a subgroup of) people with MS.

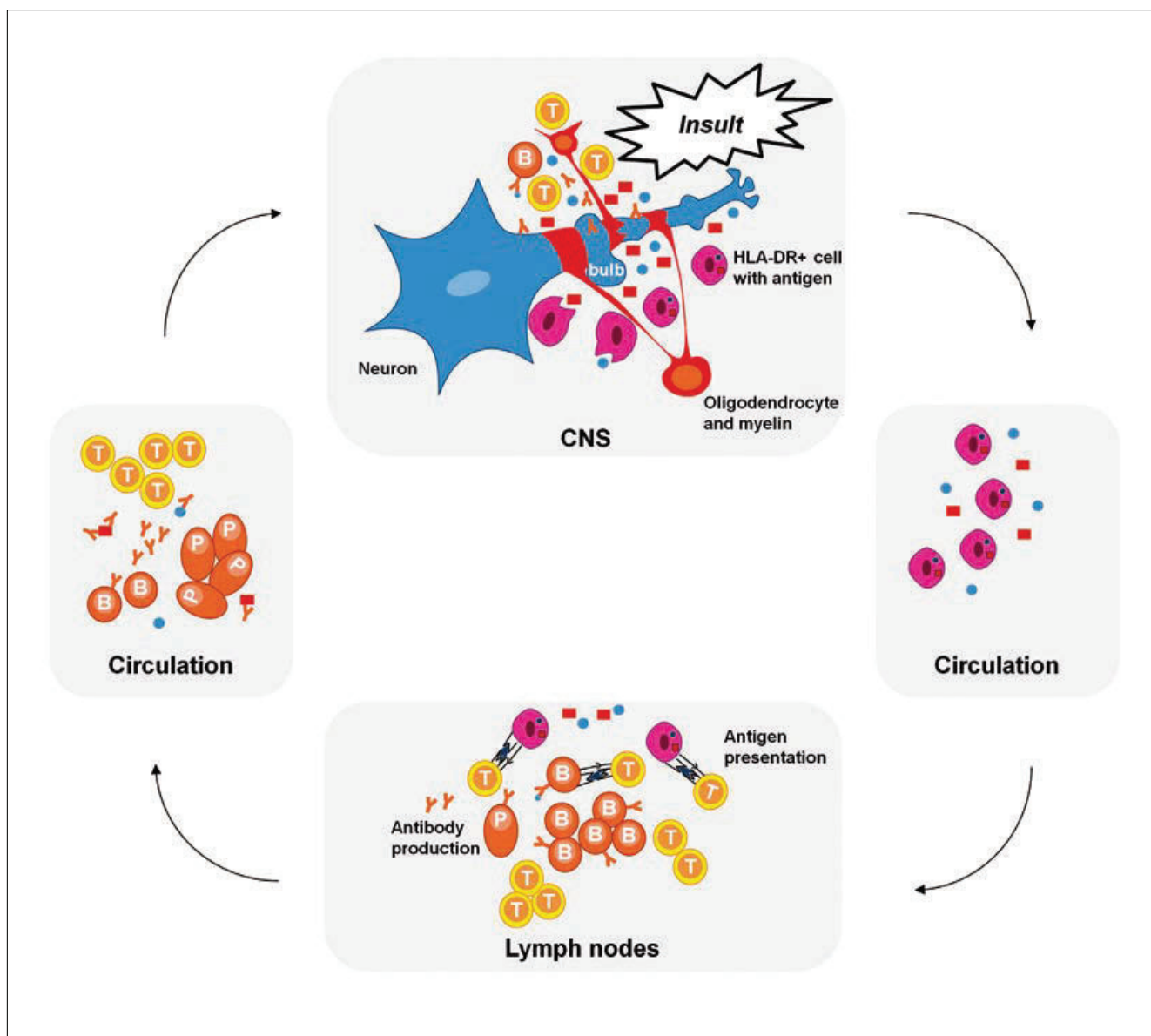


Figure 2. Hypothesis of autoimmunity to NF-L in inflammatory neurodegenerative diseases. Axonal and myelin damage in the CNS caused by an unknown insult (or multiple insults). Neuronal debris (blue, rounds) and myelin debris (red, squares) are cleared by HLA-DR cells which enter the circulation and reach the lymph nodes. Antigen presentation and (re)activation of T cells takes place followed by B cell activation and maturation in plasma cells and antibody production. Activated cells and antibodies enter the circulation and reach the CNS where T cells might contribute to neuronal damage through secretion of inflammatory molecules and antibodies that interfere with (intra)cellular processes leading e.g. to the formation of axonal bulbs. B, B lymphocyte; T, T lymphocyte; P, plasma cell.

Box 1 Conclusions of this thesis

- HLA-DR⁺ cells phagocytose neuronal antigens including NF-L in MS lesions and there is a higher frequency of HLA-DR⁺ cells containing NF-L in the CSF of people with MS (**Chapter 2**).
- Functional differences between HLA-DR⁺ cells in the grey and white matter might be responsible for the extent of axonal damage observed in the grey matter part of leukocortical MS lesions, despite the lower degree of inflammation compared with the white matter (**Chapter 3**).
- Immunodominant epitopes in the NF-L sequence can induce experimental disease as well (**Chapter 4**).
- Antibodies to NF-L are increased in the serum of people with MS compared to healthy controls and are decreased following natalizumab treatment (**Chapter 5**).
- Monoclonal antibodies to NF-L cause axonopathy *in vitro* and *in vivo* (**Chapter 6**).

Further research regarding autoimmunity to NF-L in MS should focus on the pathogenicity *in vitro* and *in vivo* of NF-L-specific antibodies purified from the serum and CSF of people with MS and other inflammatory neurodegenerative disorders. For example, the myelinating spinal cord co-cultures could be used as an *in vitro* model as we show in chapter 6 and by Elliott and colleagues (55). This model could be used to examine the impact of NF-L-specific antibodies on axonal development and regeneration as well as to investigate whether antibodies to NF-L interfere with NF-L assembly and function in axons.

A limitation of our studies is the unknown binding affinity of NF-L-specific antibodies to the three neurofilament subunits as well as whether steric hindrance of the other neurofilament subunits influence the binding capacity of neurofilament antibodies. These questions should be addressed in the future.

To conclude, the results in this thesis add to the growing knowledge about the pathological impact of neuronal-reactive antibodies and the mechanisms that may operate in progressive neurological deficits in MS.

Box 2 Outstanding questions

- Do NF-L-containing HLA-DR⁺ cells in MS tissue express co-stimulatory molecules? And can NF-L containing HLA-DR⁺ cells activate T cells or do they induce T cell tolerance?
- Which NF-L peptide epitopes are recognised by NF-L-reactive T cells from people with MS?
- Is exacerbation of EAE by purified hIgG with high antibody levels to NF-L from the sera of people with MS due to pathogenic NF-L antibodies? Is there still exacerbation of EAE when antibodies to NF-L are absorbed out from the hIgG?
- Do people with MS with high antibodies to neuronal proteins including NF-L also have high levels of antibodies to other proteins such as myelin proteins?
- Can antibodies to NF-L interfere with remyelination and/or neuronal communication?
- What is the mechanism by which antibodies enter neurons? Are antibodies still taken up by Fc receptor-deficient neurons or if F(ab')₂ fragments are used?
- Once internalised, do antibodies to NF-L interfere with neurofilament assembly and axonal transport?

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