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Axonal Damage in Multiple Sclerosis

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Summary

Multiple Sclerosis (MS) is a chronic inflammatory, demyelinating and neurodegenerative disease of the central nervous system (CNS). Neurodegeneration and accumulation of axonal damage contribute to the irreversible neurological disability. Although the cause of MS is unknown, possible mechanisms leading to axonal damage include autoimmunity to neuronal antigens. Antibodies to the neuronal cytoskeleton protein neurofilament light (NF-L) are present in the cerebrospinal fluid (CSF) and serum of people with MS. Increased knowledge about the contribution and mechanism(s) of these responses to disease progression in MS might improve therapeutic strategies inhibiting neurological decline in people with MS. We hypothesised that autoimmunity to NF-L contributes to axonal damage in MS. In actively demyelinating lesions, myelin is phagocytosed by microglia and macrophages, while the fate of degenerating or damaged axons is unclear. Phagocytosis is essential for clearing neuronal debris to allow repair and regeneration. However, phagocytosis may lead to antigen presentation and autoimmunity. In **chapter 2** we show that neuronal debris was engulfed, phagocytosed and degraded by microglia/macrophages. We observed that phagocytosis of neuronal debris by HLA-DR⁺ cells was associated with axonal damage. Nevertheless, phagocytosis of neuronal debris was less frequent than phagocytosis of myelin. This observation was supported by phagocytosis of NF-L and myelin basic protein in the mouse macrophage cell line J774.2. Degradation of NF-L was confirmed by showing co-localisation of NF-L with the lysosomal membrane protein LAMP1. More phagocytosis of myelin compared with neuronal debris was also observed in cerebrospinal fluid cells from people with MS, suggesting neuronal debris is drained by this route following axonal damage. Although uptake is essential for clearing neuronal debris, phagocytic cells could also play a role in augmenting autoimmunity to neuronal antigens.

Furthermore, 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 the grey and white matter of leukocortical MS lesions with respect to microglia activation, axonal damage and phagocytosis of amyloid precursor protein positive structures. As expected, we observed less inflammation in the grey matter. However, there was no difference in the extent of axonal damage between the white and grey matter in these lesions. Microglia isolated from white and grey matter brain areas from mice were very distinct regarding morphology, but no differences were observed in relation to phagocytosis. On the contrary, white matter microglia isolated from mice systemically challenged with the endotoxin *Lipopolysaccharide*, showed increased expression of genes involved in the innate and adaptive immune system compared to grey matter microglia. Differences observed in the activation status of microglia derived from the white matter compared to the grey matter might explain the axonal damage that occurs without apparent inflammation in the grey matter in people with MS.

Autoimmunity to neuronal proteins occurs in several neurological syndromes where cellular and humoral responses are directed to surface, as well as intracellular antigens. In **chapter 4** we studied the immune response to the axonal protein NF-L in the animal model experimental autoimmune encephalomyelitis, considered an animal model of MS. The interaction of T cells and axons was analysed by

confocal microscopy of central nervous system tissues. We show that CD4⁺ T cells were associated with spasticity, axonal damage and neurodegeneration in NF-L immunised mice. Furthermore, both CD4⁺ and CD8⁺ T cells were observed alongside damaged axons in the lesions of NF-L immunised mice. CD4⁺ T cells dominated the areas of axonal injury in the dorsal column of spastic mice in which the expression of the cytotoxic molecules granzyme B and perforin was detected. T-cell and B-cell responses to the NF-L protein were investigated and the immunodominant NF-L epitopes induced mild neurological signs similar as observed with the NF-L protein, yet distinct from those characteristic of neurological disease induced with myelin oligodendrocyte glycoprotein. Immune therapies targeting neuronal-specific T cells could thus be beneficial in reducing neurodegeneration in inflammatory disorders such as MS.

While neuronal-specific T cells are observed in people with MS, antibodies to NF-L are associated with neurodegeneration in MS. In **chapter 5** we evaluated the NF-L antibody levels in serum of people with MS with clinical variants and treatment responses. We observed a significant increase of NF-L antibody levels in the sera of people with MS compared to healthy controls. NF-L antibody levels were significantly decreased after two years of natalizumab treatment compared with baseline measurements. Whether this is caused by a decrease in axonal damage or as a consequence of decreased B-cell function remains to be elucidated.

Although antibodies directed to NF-L are widely considered to be surrogate biomarkers of axonal injury in MS, it is unknown whether these antibodies are pathogenic and contribute to neurodegeneration. In **chapter 6**, we show axonopathy in rat spinal cord co-cultures by a monoclonal antibody to NF-L. We also show that this antibody induced and promoted neurodegeneration in a mouse model of optic neuritis related to MS. To examine if such pathogenic antibodies are present in MS, we purified the IgG fraction from sera of people with MS. The pathogenicity of the antibodies was revealed by their ability to augment clinical neurological experimental autoimmune encephalomyelitis in ABH mice. Antibodies to NF-L may be involved in disease progression in MS and other immune-mediated neurodegenerative diseases where NF-L antibodies are present.

In conclusion, the studies described in this thesis show that:

- 1) Phagocytosis of neuronal antigens by HLA-DR⁺ cells might be a first step in the development of an immune response to NF-L in people with MS.
- 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.
- 3) Immunodominant epitopes in the NF-L sequence can induce experimental disease as well.
- 4) Antibodies to NF-L in serum reflect treatment responses in MS.
- 5) Monoclonal antibodies to NF-L are pathogenic *in vitro* and *in vivo* while hIgG from MS serum exacerbates experimental neurological disease in mice.

Therefore, antibodies to NF-L should not be ruled out as part of the disease mechanism in (a subgroup of) people with MS.