The role of myelin in neurodegeneration: implications for drug targets and neuroprotection strategies

Abstract: Myelination of axons in the central nervous system offers numerous advantages, including decreased energy expenditure for signal transmission and enhanced signal speed. The myelin sheaths surrounding an axon consist of a multi-layered membrane that is formed by oligodendrocytes, while specific glycoproteins and lipids play various roles in this formation process. As beneficial as myelin can be, its dysregulation and degeneration can prove detrimental. Inflammation, oxidative stress, and changes in cellular metabolism and the extracellular matrix can lead to demyelination of these axons. These factors are hallmark characteristics of certain demyelinating diseases including multiple sclerosis. The effects of demyelination are also implicated in primary degeneration in diseases such as glaucoma and Alzheimer’s disease, as well as in processes of secondary degeneration. This reveals a relationship between myelin and secondary processes of neurodegeneration, including resultant degeneration following traumatic injury and transynaptic degeneration. The role of myelin in primary and secondary degeneration is also of interest in the exploration of strategies and targets for remyelination, including the use of anti-inflammatory molecules or nanoparticles to deliver drugs. Although the use of these methods in animal models of diseases have shown to be effective in promoting remyelination, very few clinical trials in patients have met primary end points. This may be due to shortcomings or considerations that are not met while designing a clinical trial that targets remyelination. Potential solutions include diversifying disease targets and requiring concomitant interventions to promote rehabilitation.

Keywords: primary degeneration; remyelination; trans-synaptic degeneration; treatment targets

1 Myelin: its degeneration and repair

Axons are responsible for sending information in the form of electrical impulses across the brain. Some neurons within the peripheral and central nervous systems (PNS, CNS) are myelinated, where long segments of axons are wrapped with an extension of the plasma membrane into a multilamellar sheath (Nave 2010). The composition of the plasma membrane that forms the myelin sheath is a combination of mostly (∼70%) lipids, including cholesterol, and proteins. These proteins include myelin basic proteins (MBP) and proteolipid proteins (PLP) (Xue et al. 2021). The formation of the myelin sheath creates a seal on the surface of the axon where the sheath ends, defining the nodes of Ranvier between myelinated segments (Figure 1a) (Nave 2010).

In the CNS, myelin is formed by oligodendrocytes (OLG) who, when matured, can facilitate myelin wrapping (Quarles 2002). Certain glycoproteins associated with OLgs have roles regarding the formation, maintenance, and degeneration of myelin sheaths. These glycoproteins and their specific functions allow them to be utilised as therapeutic targets for interventions in myelin degeneration or neurodegeneration. For example, myelin-oligodendrocyte glycoproteins (MOG) are specific to myelin formation in the CNS. Its role in myelin regulation is implicated in the pathogenesis of certain demyelinating diseases, such as multiple sclerosis (MS) (Quarles 2002; Xue et al. 2021). MOGs are localised on the external surface of the myelin sheath and
oligodendrocytes, which allows it to communicate and interact with the extracellular environment and further maintain the myelin sheath structure. Because of its functions, MOGs have been explored as a potential primary antigen in autoimmune demyelinating diseases of the CNS (see Iglesias et al. (2001)).

The main function of myelin is long-term maintenance of axonal integrity and survival. The structure and metabolism of an axon is modulated by myelination. For example, axonal energy production is dependent on metabolic interactions between axons and myelin-forming cells (OLGs) (Ohno and Ikenaka 2019). Some of the benefits for a neuron to be myelinated, especially in those who have long axons, include energy reduction and increased conduction velocity (Nave 2010; Ohno and Ikenaka 2019). The formation of the myelin sheaths onto axons renders the action potentials and ion currents required for a neuron to send information restricted to a very small percentage of the axonal surface. This allows for the restoration of the ion gradient with a reduced level of adenosine triphosphate (ATP) consumption than in unmyelinated axons (Nave 2010). Alongside this, the speed at which the electrical current can be transmitted is significantly increased because of the addition of the myelin sheath. The size of an axon’s diameter dictates its conduction velocity, or how quickly the electrical stimulation can travel across an axon. This allows for rapid conduction and more complex connections to be formed in the higher nervous system. This rapid communication occurs as electrical impulses concentrated at the nodes of Ranvier, the unmyelinated segment between myelinated internodes, can easily travel from node to node (Dutta et al. 2006; Nave 2010).

1.1 Demyelination

Although there are many benefits for myelinated axons, its role in axonal survival and conservation is such that the loss of myelin can lead to pathologies associated with neurological disorders. Neurodegeneration in myelin diseases is influenced by interactions in neuronal and axonal microenvironments (Ohno and Ikenaka 2019). These interactions are critical for intrinsic factors that maintain the integrity and survival of axons and neurons. Inflammation and oxidative stress in the microenvironment invoke byproducts, including activated macrophages and antibodies, to be recruited to the area (Figure 1b) (Ohno and Ikenaka 2019; Stadelmann et al. 2011). The role of myelin in metabolic interactions and energy production of neurons are implicated in demyelination. Dysfunctions in these factors have been linked to mitochondrial abnormalities found in demyelinated lesions of multiple sclerosis (MS) patients (Dutta et al. 2006; Ohno and Ikenaka 2019).
MS is an inflammatory, demyelinating disease characterised by the presence of demyelinating lesions in the CNS, along with features of inflammation, axonal loss, and reactive gliosis (Dutta et al. 2006; Kuhlmann et al. 2008). This course of demyelination often occurs during the early stages of relapsing and remitting disease (RRMS), followed by the chronic, secondary progressive phase where irreversible damage often occurs (Bramow et al. 2010). The mechanisms of axonal loss due to demyelination in MS are multifaceted, occurring via direct and indirect pathways which are discussed here.

1.1.1 Iron and ferroptosis

Another important role that OPCs and OLGs have is their regulatory effects on iron metabolism. Iron is required for the oxygen utilization and ATP production and is essential for enzymes involved in the proliferation and differentiation of OPCs (Cheli et al. 2020; Schulz et al. 2012). Mature OLGs and OPCs account for the largest iron concentrations in the brain, with the primary intracellular storage for iron occurring in the protein ferritin. However, the exact mechanisms of iron transport and availability to OPCs are not well understood. One theory is that iron is transported from capillary endothelial cells to OPCs via the transporter ferroportin, where it is stored within the protein ferritin (Cheli et al. 2020; Jhelum et al. 2020; Schulz et al. 2012). When the iron metabolism in the brain is altered, it can impact these iron stores and result in deleterious effects on myelin upkeep. Increased levels of intracellular iron can become cytotoxic if it exceeds ferritin storage capacity, leading to cell death or ferroptosis (Jhelum et al. 2020). The iron released from these cells can create a noxious environment for other cells leading to further cytotoxicity. Alongside this, decreased iron levels can also be problematic as it is required for myelinating processes, as iron availability is required by OPCs to encourage proliferation and differentiation (Schulz et al. 2012). There is also evidence that iron deprivation can also alter the levels of cytokine and growth factor release, as treatment with an iron chelator reduced tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) expression in ferroportin knockout mice (Schulz et al. 2012). In summary, precise regulation of iron levels within OPCs is essential to maintaining myelin and facilitating myelin repair.

1.1.2 Inflammation and autoimmune response

There are some discrepancies from research in MS regarding the course of inflammation and demyelination, leading to a “chicken and egg” situation. There are theories that inflammation and demyelination are related such that inflammatory mechanisms and resulting products including antibodies and macrophages lead to demyelination. Contrariwise, there is also evidence that demyelination is caused by OLG and myelin damage which precedes inflammation (Stadelmann et al. 2011). In this case, inflammatory cells do not infiltrate until local microglial activation has occurred after the process of demyelination starts. This does not detract from the clear relationship between inflammation and demyelination but calls for further research into the exact mechanisms and cause and effect processes.

Acute inflammation during the demyelinating stage of lesion formation in RRMS leads to direct neurodegeneration (Stadelmann et al. 2011). T-cells that are recruited as an inflammatory response can induce neuronal apoptosis by expressing cell death mediators. Specifically, CD8+ T-cells have been shown to target OLGs by expressing oligodendroglia and neuronal apoptosis factors (Skapenko et al. 2005; Stadelmann et al. 2011). CD8+ T-cells have a protective role against infections or other intrusions by inducing cytotoxic damage to its target cells. However, this can cause external or secondary damage to other cells by creating an environment for T-cell induced inflammation that is seen in autoimmune diseases (Skapenko et al. 2005).

An example of autoimmune neuropathy leading to demyelination is macrophage-mediated segmental demyelination. This process begins with the recognition of the target autoimmune epitope presented by an antigen-presenting cell; in this case targeting the myelin sheath (Kiefer et al. 2001; Stadelmann et al. 2011). Activated lymphocytes including T-cells and B-cells circulate and enter the nervous system. Once the autoantigens are recognised, clonal expansion, and cytokine release is induced. This activates macrophages and allows for monocytes to move to the area of inflammation. This creates the scene for the effector phase, where destruction of the autoimmune target leads to demyelination and/or axonal damage that is seen in segmental demyelination. When the inflammatory response has subsided, remyelination and axonal sprouting is induced in the recovery phase (Kiefer et al. 2001).

1.1.3 Mitochondrial dysfunction

Mitochondrial dysfunction is another component of neurodegeneration implied in diseases with and without features of demyelination or WM pathology. It contributes to axonal damage and neuronal loss in diseases such as MS, Leber’s hereditary optic neuropathy, and Alzheimer’s disease (Bargiela and Chinnery 2019; Madsen et al. 2017; Shiri et al. 2021). When reactive oxygen species associated with oxidative stress interact with DNA, proteins, or lipids, it can induce mitochondrial dysfunction and create abnormal
gene expressions or enzymatic activity (Bargiela and Chin
nery 2019; Shiril et al. 2021). This dysfunction can negatively impact neurons in many ways. For example, mitochondrial DNA (mtDNA) damage can affect transcription of respiratory chain subunits and decrease ATP production and has been seen in MS lesions. Oxidative stress which imbalances reactive oxygen species and antioxidant activity can also drive cell death, and is most threatening to OLG’s who are sensitive to reactive oxidative species (Bargiela and Chin
nery 2019).

Demyelination via mitochondrial dysfunction can also be caused by genetic alterations when mtDNA is impaired. For example, in a mouse model with genetically inserted double strand breaks in OLG mtDNA (PLP:mtPstI mouse model), increased damage to DNA also increased OLG degradation and in turn induced demyelination (Madsen et al. 2017). This was attributed to OLGs dependence on proteins related to oxidative phosphorylation which are coded by mtDNA. This further supports that uncontrolled oxidative stress with mitochondrial dysfunction creates an environment for cyclical demyelination.

1.2 Remyelination

Remyelination, or the restoration of damaged myelin, also restores its function of supporting axons and enhancing conduction velocity. Research in demyelinating diseases and animal models of demyelination have identified potential cells responsible for remyelination, including oligodendro
cyte precursor cells (OPCs) and matured OLGs (Lubetzki et al. 2020). In this theory, OPCs are highly proliferated and recruited to the damaged axon where OLG maturation occurs, as only matured OLGs can form myelin sheaths (Figure 1b). There are various pathways that occur to enable this process, including guidance cues that recruit OPCs and Notch signaling that promotes the OPC maturation processes (Lubetzki et al. 2020; Zhang et al. 2009). The Notch pathway is mediated by specific ligands, such as Jagged1 and Jagged2, which bind to Notch1 and Notch2 receptors (Figure 1b). These receptors influence OLG maturation and proliferation; however the balance between these two is disrupted in sites of inflammation such as in MS (Zhang et al. 2009). Another important component to remyelination is the clearing of myelin debris by macrophages and microgliia, as matured OLGs cannot efficiently or correctly wrap myelin when debris is still present (Figure 1b) (Lampron et al. 2015; Lubetzki et al. 2020).

The extracellular matrix surrounding myelin also plays an integral role in its preservation and capacity for repair, as the metabolic integrity of the axon and further neuron is maintained by the myelin sheath (Ghorbani and Yong 2021; Ohno and Ikenaka 2019; You and Gupta 2018). The composition of the extracellular matrix includes the interstitial matrix, a basement membrane surrounding blood vessels, and perineuronal nets (PNN) (Ghorbani and Yong 2021). This also includes components that play integral roles in myelin development, such that depletion or upregulation of these in diseases significantly decrease the ability of remyelination. One of these components is fibrinogen, a blood coagulation protein that can infiltrate the CNS if the blood brain barrier (BBB) is damaged (Ghorbani and Yong 2021; Petersen et al. 2017; You and Gupta 2018). This also includes components that play integral roles in myelin development, such that depletion or upregulation of these in diseases significantly decrease the ability of remyelination. One of these components is fibrinogen, a blood coagulation protein that can infiltrate the CNS if the BBB is damaged (Ghorbani and Yong 2021; Petersen et al. 2017; You and Gupta 2018). This can occur in neurodegenerative diseases such as in MS, or following traumatic or ischemic injury. Fibrinogen not only drives the inflammatory response in these diseases, but also impacts OPC maturation and further remyelination (Petersen et al. 2017). Petersen and colleagues (2017) found that fibrinogen induces downstream gene expressions that inhibits OPC differentiation and decreases MBP concentrations. However, other factors of the extracellular matrix can provide mediatory rather than deleterious roles in remyelination. This includes interactions between the extracellular matrix receptor dystroglycan, and laminin (Ghorbani and Yong 2021; Leiton et al. 2015). Laminin has been found to stimulate dystroglycan cleavage in OPCs which regulates proliferation and OLG development (Lei
ton et al. 2015).

Evidence of remyelination can be found in some MS lesions but is absent or limited to the lesion plaque border in most patients. In analysing tissue samples from 43 MS patients, Kuhlmann and colleagues (2008) found that during the early onset period of MS there is an increase in OPC and progenitor proliferation. However, there was a significantly decreased number of progenitor cells in lesions in patients with chronic MS. This was attributed to a “block” of OPC conversion into mature OLGs which restricts the extent of remyelination at later disease stages of MS (Kuhlmann et al. 2008). This reveals that remyelination does occur in MS, but the course of the disease obstructs or degrades that potential such that remyelinated areas may be incomplete (Bramow et al. 2010; Kuhlmann et al. 2008). Therefore there is a tight relationship between the severity and stage of disease, and the potential for remyelination in MS patients (Bramow et al. 2010). The role of OLGs in myelin formation and upkeep is an area of interest for research into neurodegenerative diseases.
diseases and provides a potential strategy for developing treatments for these diseases.

2 The role of myelin in primary and secondary neurodegeneration

The role of myelin in axons and axonal degradation has been connected to many degenerative diseases, even those not typically associated with the effects of demyelination. The emerging findings of demyelination in some of these diseases are included to highlight the influential role myelin has across neurodegenerative diseases and calls for further exploration into these theories. Although evidence of demyelination has been found in both primary and secondary forms of neurodegeneration, there is debate as to whether it plays a causal or consequential role, if any at all.

2.1 Primary, chronic neurodegeneration

Many neurodegenerative diseases have been associated with WM pathology, including changes to myelin content or structure. This includes diseases commonly associated with demyelination such as MS, along with other diseases that have more grey matter (GM) changes. Examples of these diseases include Huntington’s disease (HD), motor neuron disease and amyotrophic lateral sclerosis (ALS), and Parkinson’s disease (PD). The association between these disease pathologies and their impacts on myelin can occur through different mechanisms. For example, cellular pathologies including neuroinflammation and oxidative stress can account for classical PD characteristics and emerging WM changes (Cai et al. 2023). The pattern of myelin degeneration has to do with the path of neurodegeneration in PD being opposite from the pattern of brain myelination, such that the latest myelinated neurons are most vulnerable (Dean et al. 2016). Contrariwise, the demyelination hypothesis of HD suggests that the huntingtin mutation leads myelin breakdown in the earliest myelinated areas of the brain (Bartzokis et al. 2007). These neurons become overstimulated due to the failing afferent/efferent feedback loop and the myelin is eventually degraded. The huntingtin mutation is also associated with homeostatic changes in OLGs, whose heightened levels of ferritin iron content contribute to impairment and demyelination. Further OLG dysfunction also impairs any attempt of remyelination, characteristic of the chronic demyelination found in HD (Bartzokis et al. 2007; Casella et al. 2020).

Many neurodegenerative diseases are not considered to be demyelinating diseases, but there is overwhelming evidence of WM changes in certain diseases that calls for further investigation. Though demyelination may not be a primary cause or feature of neurodegeneration, there is evidence of such WM alterations that can contribute to the overall disease progression. Therefore, it remains a viable target for rehabilitative or neuroprotective treatments and should be investigated as such. The following sections detail the findings of demyelination and myelin changes in glaucoma and Alzheimer’s disease as well as describing points of interest for drug targets.

2.1.1 Glaucoma

Glaucoma is a neurodegenerative disease, characterised by the chronic loss of retinal ganglion cells (RGC) and axons (Xue et al. 2021; Zhang et al. 2015a). The pathogenesis of glaucoma is not fully understood, but intraocular pressure and autoimmune implications have been identified as potential risk factors (Reinehr et al. 2016; Xue et al. 2021). Research into the causes of glaucoma have led to the hypothesis that demyelination may play an important role in certain aspects of the disease pathology. However, there is no consensus on this idea as results are conflicting relating to the exact role of demyelination.

Animal models of glaucoma have corroborating evidence for demyelination. For example, RGC degeneration and failure in axonal regeneration as found in glaucoma pathology have been partially attributed to Nogo-A, a major myelin inhibitory protein (Liao et al. 2013; Mdzomba et al. 2018). This protein binds to the Nogo66 receptor, NGR1, and paired immunoglobulin-like receptor B (PirB), which has an inhibitory influence on remyelination. In an experimental ocular hypertension model of glaucoma, Liao and colleagues (2011) found a significant increased expression of Nogo-A in glaucomatous RGC’s, the inner plexiform layer (IPL), and outer plexiform layer (OPL) of the retina. These results indicate that Nogo-A expression may play a role in vision loss in glaucoma, such that increased expression may lead to synaptic function deficits and further RGC death in a downstream manner (Liao et al. 2011).

Using a form of high-field magnetic resonance, diffusion tensor imaging (DTI), the integrity and connectivity of the CNS can be assessed by measuring water molecule diffusion (Michelson et al. 2013; You et al. 2019; Zhang et al. 2015a). This tool uses a magnetic field gradient to measure the rate and direction of water molecule movement relative to the direction of the magnetic field (Aung et al. 2013). This can measure the fractional anisotropy (FA) which characterises the orientation of the axonal fiber that impedes or displaces...
water molecules. Other measurements can be extrapolated from FA using matrix diagonalisation operations to determine orthogonal (right angle) directionality, which translates to parallel and perpendicular movements (Aung et al. 2013; Zhang et al. 2015a). Therefore, measurements that combine the diffusivity (FA) and the direction of water molecule movement (RD or AD) can help detect disruptions to axonal architecture and potentially myelin degradation (Aung et al. 2013).

DTI measurements that correlate with axonal integrity and demyelination have been found in glaucoma patients. For example, in a study of 20 primary angle closure glaucoma (PACG) patients compared to 20 healthy control participants revealed that the optic nerves and optic radiations of the glaucoma patients had significantly higher mean diffusivity (MD), AD and RD measurements, and significantly reduced FA (Zhang et al. 2015a). Similar results were found in patients with normal-tension glaucoma and primary open-angle glaucoma (POAG), as both groups had significantly increased RD and decreased FA measurements (Michelson et al. 2013). These parameters were also correlated with Heidelberg retina tomography measurements, which showed that DTI measurements worsen with increased glaucoma severity and disease progression (Michelson et al. 2013).

Although these findings point to a direct influence of demyelination on glaucoma, there are discrepant findings that indicate whether it is a cause or effect of disease. Reinehr and colleagues (2016) assert that rather than demyelination being the cause of disease in glaucoma, it is a manifestation of axonal damage. In an experimental autoimmune glaucoma model (EAG), rats were immunised with a bovine optic nerve homogenate antigen, and the retina and optic nerves were analysed to determine the immune defence pathway of this model. Immunohistochemical staining against MBP within the optic nerve revealed a significant increase 3 days post immunisation, followed by a significant decrease at 14 days, when compared to control animals (Reinehr et al. 2016). Contrary to this, the effects of the autoimmune glaucoma model and subsequent activation of complement pathways was detected by day 7. This revealed that the optic nerve degeneration and RGC death found in the model occurred prior to demyelination detection, further confusing the role of myelin damage in glaucoma (Reinehr et al. 2016).

2.1.2 Alzheimer’s disease

Alzheimer’s disease (AD) is the most common form of dementia, whose symptoms are characterised by memory loss and behavioral changes due to deterioration of brain areas involved in memory, speech, and emotions (Desai et al. 2009). Although this disease is not traditionally associated with white matter (WM) degeneration, there is a significant amount of evidence that myelin may play a role in the typical amyloid-beta (Aβ) and Tau pathology. A popular theory of AD’s disease course, the amyloid hypothesis, defines the accumulation of Aβ plaques and neurofibrillary Tau tangles as causing the disruption of critical metabolic processes that ultimately cause neurodegeneration (Dean et al. 2017; Depp et al. 2023; Nasrabady et al. 2018). This deposition of Aβ and Tau tangles primarily occurs in the neocortex and hippocampus, which precedes the onset of symptoms and further development of dementia (Dean et al. 2017; Depp et al. 2023).

Despite evidence that myelin injury and AD pathology are related, there is debate as to the exact cause and effect relationship between the two, not dissimilar to that in glaucoma. In analysing post-mortem AD patients, their brains were chemically altered such that there was a decrease in myelin relevant proteins, including MBP, PLP, and Olig2+ cells (Nasrabady et al. 2018). This suggests that demyelination has occurred as part of AD pathology. One proposed theory for myelin loss in AD involves oxidative stress, which impairs the differentiation of OPCs. Oxidative stress has been shown to decrease gene expression of OLG differentiation promoters. This gene expression can also be modulated through Aβ pathology in AD, as toxicity towards OLGs can also occur via Aβ induced oxidative stress (Nasrabady et al. 2018).

Discrepancies in research regarding the time course of myelin impairments versus traditional AD pathology have leaned towards confirming the hypothesis that WM pathology precedes Aβ plaques and Tau tangle pathology in AD (Nasrabady et al. 2018). There is evidence that the regions that are most vulnerable to AD pathogenesis, including Aβ plaque formation and neurofibrillary Tau tangles, also have the most protracted or extended course of myelination (Dean et al. 2017; Desai et al. 2009; Nasrabady et al. 2018). The relationship between AD pathology and its impact on WM microstructure supports findings that show later myelinated brain regions are the first to degenerate in AD such that WM alterations are directly involved in AD pathogenesis (Figure 2) (Dean et al. 2017). This is reinforced by magnetic resonance imaging (MRI) analyses performed by Dean and colleagues (2017) on patients at risk for developing AD who have not developed cognitive symptoms. They found that these patients exhibited a decreased amount of myelin structures as evidenced by decreased myelin water fraction (MWF) and longitudinal/transverse relaxation rates (R1/R2), which implies decreased MBP concentrations. The MRI results were significantly negatively correlated with CSF measurements of Tau and Aβ related proteins (Dean et al. 2017). This relationship was most strongly seen in the
latest-myelinated areas, further supporting the relationship between myelin and AD pathology. For example, in the left hemispheric superior frontal gyreral white matter, higher levels of Tau and Aβ proteins were found alongside increased MWF decline. The combined findings further suggest that myelination defects precede amyloid and Tau pathologies in patients who are at risk of developing AD (Dean et al. 2017).

2.2 Secondary degeneration

2.2.1 Trauma

Following an initial injury to the CNS, the surrounding neurons are subsequently damaged and degenerate resulting in loss of white matter and neuronal function of the surrounding area (Ek et al. 2012; Payne et al. 2011, 2012). Secondary demyelination has been described in traumatic injuries including spinal cord injury (SCI), ischemic stroke, and following optic nerve damage. In SCI, OLG injury and death leads to the demyelination and sometimes dysmyelination that can be found in traumatic or ischemic CNS injuries (Ek et al. 2012; Hassannejad et al. 2019; Huang et al. 2014; McDonald and Belegu 2006). Dysmyelination has been shown to cause certain motor symptoms and pain, as the dysregulation of myelin leads to dysregulation in signal relay (McDonald and Belegu 2006). For example, in a rat model of SCI complete demyelination as well as dysmyelination characterised by empty or collapsed myelin sheaths were found in the dorsal column of the spinal cord along with the ventrolateral tracts (Ek et al. 2012). Demyelination following SCI has been attributed to OLG damage and apoptosis, as OLG apoptosis has been identified in widely distributed patterns of white matter lesion sites following experimental CSCI in rats (Figure 3a) (Huang et al. 2014). The implication that OLG apoptosis is directly involved in secondary degeneration further highlights the integral role myelin plays in maintenance. These findings not only determine one pathology of secondary demyelination following CNS injury, but it also presents a potential target for restorative strategies.

Secondary demyelination has also been shown in CNS damage within the optic nerve. In an animal model of secondary degeneration, optic nerve transection was performed to leave the central portion of the optic nerve undamaged but vulnerable secondary degeneration (Payne et al. 2011). Following one month post injury, immunohistochemical staining of MBP within the vulnerable portion of the optic nerve had significantly decreased, and further decreased at 3 months. Alongside this, the percentage of normally myelinated axons also significantly decreased by 3 months, while the percentage of abnormally myelinated axons increased (Payne et al. 2011). These abnormalities were characterised by fully de-compacted, partially de-compacted, or excessively myelinated axons. Furthermore, in a similar model of chronic secondary degeneration, the number of compacted myelin doubled by 3 months following injury and persisted up to 6 months, indicating persistent swelling of myelinated axons (Payne et al. 2012). Functional deficits that manifested 6 months post-injury were attributed to chronic secondary degeneration, including visual function loss and fast reset responses (Payne et al. 2012). These results further support the role of secondary degeneration and myelin loss, but also highlight the compounding effects of chronic secondary degeneration as evidenced by animal models of traumatic injury to the CNS.
2.2.2 Ischemia

White matter stroke (WMS) results in the ischemia or loss of blood flow to parts of the neurovascular system, where oxygen deprivation creates cell death lesions that can expand with time (Marin and Carmichael 2019; Ren et al. 2012). This injury breaks down the BBB, allowing for infiltration of peripheral or alien products, including myeloid cells and auto-antigens (Ren et al. 2012). This creates an environment for exacerbated inflammation, which can lead to the characteristics of demyelination found around the paranode of the stroke location (Figure 3a) (Marin and Carmichael 2019). For example, the innate immune response against the invasion of leukocytes that target MOG or MBP in the form of autoantibodies, are responsible for inducing EAE in animal models. This is due to the proinflammatory immune response which recruits TNFs and IFN-γ, which are known to play a role in demyelination (Ren et al. 2012). Interestingly, there is a close relationship regarding ischemic induced demyelination and the development of AD. In an animal model with induced ischemic injury, the areas with myelin and axonal injury were also susceptible to developing Aβ pathology (Zhan et al. 2015). This was evidenced by co-localisation of myelin and Aβ aggregates within the area of stroke. These results highlight the demyelinating potential following ischemic stroke, as well as further the hypothesis that myelin plays a role in multiple neurodegenerative diseases.

2.2.3 Infection

Acute disseminated encephalomyelitis (ADEM) refers to a group of non-infectious, inflammatory demyelinating diseases of the CNS, mainly found in children aged three to seven (Cole et al. 2019; Pohl et al. 2016). ADEM diagnoses requires specific criteria of detected symptoms and clinical findings, including polyfocal CNS events of inflammatory demyelination, encephalopathy, acute brain MRI abnormalities with demyelination, and an acute range of MRI findings (within 3 months from onset) (Krupp et al. 2013; Pohl et al. 2016). Further criteria determine more specific diagnoses, such as the occurrence of single or multiple polyfocal CNS events, and the presence of other features such as optic neuritis (ADEM-ON) (Pohl et al. 2016). The characteristics of demyelination in ADEM in comparison to MS can be unclear for initial diagnoses, but the larger criteria for diagnosis and presence of sleeves of perivenous demyelination have become the “gold standard” for differentiation (Cole et al. 2019; Pohl et al. 2016; Young et al. 2010).

The mechanisms of demyelination in this disease are not fully understood, but there is significant evidence that it is caused by the inflammatory response found in ADEM after the incurring infection (Cole et al. 2019; Esposito et al. 2015). This response includes T cell cross-activation against myelin related proteins, including MBP, PLP, and MOG, via molecular mimicry. Another possibility is a non-specific
autoimmune sensitisation of T-cells against these myelin related proteins (Esposito et al. 2015).

2.2.4 Transsynaptic degeneration

Transsynaptic degeneration (TSD) or trans-neuronal degeneration refers to the process of neuronal atrophy after the cells they project to have degenerated or died (Cowan 1970). The course of this atrophy or degeneration dictates the afferent and efferent directionality: retrograde refers to degeneration from a posterior to anterior direction, while anterograde refers to the reverse (Figure 3b) (Balk et al. 2015; Cowan 1970; Gabilondo et al. 2014; Jindahra et al. 2012). The processes and mechanisms of TSD have been studied extensively in the previous decades since its first identification in 1850, but not without debate regarding its existence outside of primates or other-nonhuman animals. This has culminated to multiple animal models of TSD, as well as a better understanding of how TSD can occur in human diseases.

The visual pathway has been previously used as a model of neuronal degeneration because of its well-established and simple organisation within the brain. In this model, the process of TSD is defined as damage spreading to or from the distal or proximal visual pathway via the synapses by connecting neurons. Following this, retrograde TSD (rTSD) defines damage to the visual cortex spreading to the retina or optic nerves, and anterograde TSD (aTSD) is defined as damage to the retina spreading to the visual cortex (Gabilondo et al. 2014; Jindahra et al. 2012).

Evidence of both rTSD and aTSD has been shown in MS patients, as an example of bi-directional TSD (Balk et al. 2015). In MS patients with optic neuritis (MSNON), they exhibited decreased peripapillary retinal nerve fibers layer (pRNFL) and the ganglion cell complex (GCC) (Balk et al. 2015). This indicates that damage to the anterior or distal pathway, such as lesions to the OR, spreads to the retina or to the proximal pathway. This confirms that not only can the visual pathway be successfully used as a model of TSD, but both forms can be found in a single disease model of MS.

2.2.4.1 The role of myelin in TSD

The process of TSD, as being well established in the visual system and diseases that affect it, has become a target for potential therapies in demyelinating diseases. One reason for this is that distal regions of axons can act as a metabolic compartment, such that homeostatic impairment of axonal metabolism in their associated myelinated glia is related to subsequent axonal degeneration (Nave 2010; Ohno and Ikenaka 2019). These factors can be utilised and manipulated to prevent demyelinating pathways. For example, by better understanding the mechanisms of Wallerian degeneration, a form of aTSD, and its associations with axonal metabolism dysregulation, potential targets for axonal survival could be adapted into therapeutics for demyelinating diseases (Ohno and Ikenaka 2019).

The interactions between TSD and demyelination have been previously defined in other systems and models of degeneration, such as following spinal cord injuries (SCI). In this example, following SCI there is a significant decrease in axons and neurodegeneration is spread via TSD (Hassannejad et al. 2019). In the myelinated axons following injury, the myelin sheath thickness is significantly decreased, followed by a period of rapid remyelination. However, the thickness and overall length of the repaired myelin is smaller. This leads to functional abnormalities or overall dysfunction of the axon, such as slowed conduction times (Figure 3b). One reason for this inadequate remyelination is the rapid death of OLGs via apoptosis, which leads to further demyelination and diminishes the ability of remyelination (Hassannejad et al. 2019).

Previous studies in MS and glaucoma patients have investigated the role of demyelination and TSD in the disease pathology of the visual system. By using imaging and electrophysiological tests, such as MRI, occipital coherence tomography (OCT), and multi-focal visual evoked potential (mVEP), You and colleagues found that myelin pathology is closely involved in and potentially precedes neurodegeneration (2019). This combination of methodologies allowed them to measure the RNFL (Siger et al. 2008) and presence of demyelination (You et al. 2011) through OCT and mVPE analyses, respectively. Specifically, it was reported that there was an increased mVPE latency in glaucoma patients, which correlates with increased RD within the OR and is a sign of aTSD (You et al. 2019). The decreased conduction that produced the shifted VEP latency is attributed to myelin loss and damage to myelin membranes. This highlights the facilitating role that demyelination and other myelin pathology has on TSD. One method of TSD proposed in this work concerns the interactions between OPCs, and their role in axonal synapses (Lubetzki et al. 2020; You et al. 2019). The lack of OPCs and inhibition of OPC differentiation into mature OLGs may be impacted by synaptic dysfunctions, which can further spread trans-synaptically producing the degeneration identified in distant areas of demyelination.
2.2.4.2 TSD, myelin, and neuroplasticity

Recent research into TSD has revealed an interesting relationship between TSD and neuroplasticity, and there are further implications to the role this plays in demyelinating diseases. Firstly, neuroplasticity is the ability or capacity for the nervous system to respond to certain stimuli by reorganising or redistributing its functions, connections, etc. (Cramer et al. 2011). The ability for neurons and their accompanying processes to remodel itself has been studied extensively in the visual pathway, and has more recently become a target for therapeutics (Balk et al. 2014; Cramer et al. 2011; Yang et al. 2020).

Neuroplasticity has also been implicated in demyelinating diseases, specifically MS, in findings that show the plastic abilities of certain portions of the visual system can prevent the spread of neurodegeneration through TSD. Balk and colleagues (2014) found that the diffusion of degeneration through rTSD does not surpass the inner nuclear layer (INL) of the retina in MS patients following optic neuritis (ON) episodes. This is attributed to the capability of plasticity that can be found in the retina. Contrariwise to this, aTSD can fully extend through V1 and V2 of the visual cortex because the capacity for neuroplasticity in this area is cut off early in life (Balk et al. 2014; Daw 1998). The opposite is also true in MS patients who have not had ON, where rTSD mainly affects physiologically wired connections that are no longer plastic, such as connections from RCGs to V1 (Balk et al. 2015; Daw 1998; Yang et al. 2020).

The fact that there is a relationship between neuroplasticity in the visual system, and the effects of myelin or subsequent demyelination in TSD, sheds light on a potential system for recovery in demyelinating diseases. As previously mentioned, certain inhibitory proteins including Nogo-A are implicated in axonal regeneration failure in diseases such as glaucoma (Liao et al. 2011; Mdzomba et al. 2018). Studies in glaucomatous RGC's following retinal injury show that they exhibit a significant increase in Nogo-A expression (Liao et al. 2011). Extending this idea, it was found that Nogo-A inactivation increased the capacity for visual plasticity and potentiated vision recovery after N-methyl-D-aspartate (NMDA) induced retinal damage in mice, similar to damage seen in glaucoma patients (Mdzomba et al. 2018). With high doses of NMDA and more severe induced injury, Nogo-A inhibited axonal regeneration by activating Ngr1 and transforming protein RhOa within RGCs (Mdzomba et al. 2018). This significantly limited the capacity for neuronal plasticity in RGCs and further impaired cortical neuronal activation in V1. This is an example of one potential way that Nogo-A can inhibit the plasticity of RGCs in the brain following retinal injury. However, it does not necessarily mean that targeting Nogo-A will be a viable neuroprotective strategy. The authors state that Anti-Nogo-A aimed treatments may only be relevant to treating injuries that are strictly limited to RGCs, such as in glaucoma (Mdzomba et al. 2018). This calls into question the feasibility of targeting myelin components in neuroplasticity to repair neurodegeneration, and further exploration into these targets will need to be considered.

3 Strategies and targets for remyelination

3.1 Anti-inflammatory targets

3.1.1 Activin-A and macrophage phenotypes

Inflammation induced demyelination occurs when inflammatory cells, such as activated macrophages, are attracted to the area of injury (Ohno and Ikenaka 2019; Stadelmann et al. 2011). In macrophage-mediated demyelination, macrophages remove the myelin sheath from the axon rendering the axon unable to safely conduct nerve impulses (Kiefer et al. 2001; Stadelmann et al. 2011). However, the role of macrophages can be both deleterious and reconstructive, as macrophages are involved in demyelination and remyelination processes. Macrophages and microglia are components to the regeneration of myelin by phagocytosing myelin debris and secreting growth and neurotrophic factors (Miron et al. 2013). Macrophages can be polarised into one of two phenotypes, M1 or M2, depending on factors such as the activation pathway and external biological processes (Atri et al. 2018; Miron et al. 2013). For example, M1 phenotype macrophages that have been “classically” activated are often found in inflammatory environments, where they exhibit phagocytic activity and guide inflammatory responses by producing proinflammatory cytokines (Atri et al. 2018). Alternatively activated M2 macrophages are also involved in inflammation but have a more regulatory response as they can recruit neutrophils, monocytes, and lymphocytes. M2 macrophages are also able to downregulate proinflammatory cytokines in an anti-inflammatory manner (Atri et al. 2018).

The effects of macrophage phenotypes have been detected in remyelination processes, evidenced by analysing the microglia and macrophages in demyelination induced mice. It was found that resident microglia and macrophages in the area had switched from M1 to M2 dominant phenotypes when remyelination was triggered (Miron et al. 2013). Therefore, it has been theorised that by modulating the amount of M2 phenotype macrophages and microglia, the...
process of remyelination could be more efficient. Activin-A is a member of the transforming growth factor-beta (TGFβ) family which is produced by inflammatory macrophages (Table 1) (Miron et al. 2013; Zheng et al. 2021). It has been shown to promote neuronal survival by inhibiting apoptosis, as well as promote differentiation of OPCs (Zheng et al. 2021). Its role in the restorative process following inflammation allows it to be used as a marker for M2 polarisation. OPCs in remyelinating lesions have been found to directly bind and allows it to be used as a marker for M2 polarisation. OPCs in high protein expression, including MOG and MBP, which

encourages remyelination. This provides support for cell or drug-based treatments that may further manipulate M2 polarisation or increased activin-A expression and consequently increase remyelination.

### 3.1.2 IL4-I1 facilitates T-cell activity

As inflammation is one of many causes of demyelination, unresolved inflammation can also be detrimental to any attempts at recovery leading to remyelination failure and further axonal injury. Alternative activated macrophages (M2) express IL-4I1 (Table 1). IL4-I1 is one of many factors required to facilitate anti-inflammation and promote myelin repair by modulating T-cell activity during remyelination (Atri et al. 2018; Psachoulia et al. 2016). Injections of IL-4I1 into lysolecithin-induced demyelinated mouse spinal cord

<table>
<thead>
<tr>
<th>Target</th>
<th>Examples of intervention</th>
<th>Mechanism(s)</th>
<th>Remyelinating outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophage mediated inflammation</td>
<td>Activin-A (Miron et al. 2013; Zheng et al. 2021)</td>
<td>–Promote neuronal survival –Promotes myelin-related protein expression (e.g. MOG, MBP) –Anti-inflammatory properties –Modulate T-Cell activity –Interactions with M2 macrophages</td>
<td>–Increases OPC differentiation and protein expression to encourage remyelination and recovery</td>
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<tr>
<td>IL-4I1 (Psachoulia et al. 2016)</td>
<td></td>
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<tr>
<td>Antioxidants</td>
<td>Arbutin (Ebrahim-Tabar et al. 2020)</td>
<td>–Inhibit COX and LOX mediated inflammation –Decrease pro-inflammatory mediators</td>
<td>—Increases potential for remyelination</td>
</tr>
<tr>
<td>A-Lipoic acid (Fiedler et al. 2021; Loy et al. 2018)</td>
<td></td>
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<tr>
<td>RXR pathway</td>
<td>9cRA (Huang et al. 2011; Yang et al. 2017)</td>
<td>–Roles in cellular development and metabolism –Stimulates MBP expression in CG-4 cells –Increase OPC differentiation –Olig2 activating RXR pathway –Unspecific RXR antagonism</td>
<td>–RXR pathway regulates cellular metabolism, and can upregulate myelin related protein expression (e.g. MBP, OPCs) –Acceleration of cell proliferation and differentiation into mature OLGs to promote remyelination</td>
</tr>
<tr>
<td>Bexarotene (Santos-Gil et al. 2021)</td>
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<tr>
<td>Nanoparticles</td>
<td>9CDHRA (Ruhl et al. 2015, 2018)</td>
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<tr>
<td>Antihistamine</td>
<td>Clemastine (Li et al. 2015; Mei et al. 2014; Xie et al. 2021)</td>
<td>–Antimuscarinic properties –Modulates OPC metabolism</td>
<td>–Increase OPC survival, proliferation, and differentiation –Increased OLG maturation –Increased MBP expression</td>
</tr>
</tbody>
</table>

Table 1: Remyelination targets and their general method of action.

Summarised are the general mechanisms of the discussed remyelination targets and their related outcomes.
led to significant decreases in inflammation and malformed myelin (Psachoulia et al. 2016). This positive effect was attributed to M2 macrophage mediating IL-41’s ability to accelerate inflammation resolution. Therapeutic treatment of IL-41 was also used in experimental autoimmune encephalomyelitis (EAE), an animal model of autoimmune inflammation (Constantinescu et al. 2011; Psachoulia et al. 2016). This treatment significantly decreased the density of CD4+ T-cells in the spinal cord and spleen, a marker of the innate immune response, which in return reduced disease severity (Psachoulia et al. 2016). This provides evidence that inflammation control via IL-41 may decrease disease severity or modify the course of disease in MS or other demyelinating diseases.

### 3.1.3 Antioxidants against inflammation and oxidative stress

The use of antioxidants to control inflammation and oxidative stress in MS has also been explored as therapeutic or palliative treatments. This is in part due to their abilities as anti-inflammatories and its pleiotropic capacity (Theodosis-Nobelos and Rekka 2022). Natural phenolic antioxidants have anti-inflammatory potential because they inhibit cyclooxygenase (COX) and lipoxygenase (LOX), which are key players in enzymatic pathways that control inflammation. Antioxidants can also decrease the expression of pro-inflammatory mediators such as IL-1b and TNF-α (Theodosis-Nobelos and Rekka 2022). Examples of these include arbutin, an antioxidant and anti-inflammatory molecule which after treatment in demyelination induced rats was found to decrease demyelination and increase myelin repair (Table 1) (Ebrahim-Tabar et al. 2020). This effect was confirmed by quantifying myelin related products, which revealed increased MBP and Olig2 expression in the optic chiasm. Arbutin treatment also decreased expression of inflammatory cytokines while increasing antioxidant and anti-inflammatory mediators. These reparative effects were attributed to its ability to modulate the transcription of these components (Ebrahim-Tabar et al. 2020; Theodosis-Nobelos and Rekka 2022).

Sulfur containing antioxidants, such as α-lipoic acid, also has anti-inflammatory capabilities (Table 1). One reason for this is that lipoic acid can inhibit pro-inflammatory cytokine secretion, such as IL-1b and (TNF-α) (Fiedler et al. 2021). In a study of MS patients and healthy controls conducted by Fiedler and colleagues (2021), serum and tissue were collected from both groups and treated with lipoic acid. MS cells had a significant decrease in inflammatory phenotypes following treatment, as monocyte derived macrophages and secretion of cytokines were inhibited by lipoic acid (Fiedler et al. 2021). In a 2 year in-patient study of MS, physical functions were also repaired by lipoic acid treatment but only significantly in those with lower levels of disability (Loy et al. 2018). Similarly, 2 year treatment with lipoic acid had an insignificant improvement in gait features, but had significant improvements on brain atrophy as annual percent change of brain volume (PCBV) was reduced by 68 % (Spain et al. 2017). The evidence from these studies shows that treatment with lipoic acid antioxidants is beneficial in reducing inflammation in MS patients, but only improves the attributed symptoms of MS at low levels of disability or in early disease onset. Therefore, similar treatments could be considered as a palliative addition to first line therapies.

### 3.2 Retinoid-X receptor pathway targets

#### 3.2.1 9-cis-retinoic acid

The retinoid-X receptor (RXR) is one of many nuclear hormone receptors which influence cellular development, metabolism, and death (Dawson and Xia 2012). One of its functions is as a transcription factor that binds to specific hormone gene promoter regions, including thyroid receptors, and vitamin D (Krezel et al. 2019). This also includes retinoic acid receptors (RAR), which regulate genes that control cell proliferation and differentiation (Dawson and Xia 2012; Huang et al. 2011; Yang et al. 2017). 9-cis-retinoic acid (9cRA) activates RXR-γ signaling, which is also expressed in OLG cells (Table 1) (Huang et al. 2011; Yang et al. 2017). In focal demyelination induced rats, treatment with 9cRA improved CNS remyelination and increased the percentage of MBP positive membrane sheets. This allowed for further promotion of OPC differentiation (Huang et al. 2011). 9cRA has previously been shown to increase MBP expression in a dose-dependent manner, as it stimulates MBP expression in central glia-4 cells (CG-4) after differentiation to OLGs within the rat optic nerve (Pombo et al. 1999). Further supporting 9cRA mediated RXR activation as a promoter of OLG differentiation and MBP expression, treatment in demyelination induced rats also increased OPC differentiation by promoting maturation (Yang et al. 2017).

#### 3.2.2 Bexarotene and MS

Other RXR agonists, such as bexarotene, have also been shown to increase remyelination in a triple-transgenic mouse model of AD (Table 1) (Santos-Gil et al. 2021). These transgenic mice were shown to have significantly decreased levels of Olig2 in areas of the hippocampus. After treatment
with Bexarotene, OPC levels were restored to normal control animal levels, including the levels of intermediary OLG progenitors Olig2 and Olig4. This was accomplished by Olig2 expression activating the RXR pathway, which accelerated cell proliferation and differentiation. Alongside this, bexarotene also increased the expression of MBP positive mature OLGs, indicating that remyelination has been promoted (Santos-Gil et al. 2021). As bexarotene treatment has been shown to be effective in remyelination in animals, a recent MS patient trial was undertaken to determine its safety and efficacy. In this trial, bexarotene was administered at 300 mg/m² body surface per day, compared with matching placebo (Brown et al. 2021). This revealed poor tolerability as all patients who took bexarotene experienced negative side effects resulting in five out of the 26 dosed patients to withdraw from the study (Brown et al. 2021). Although there is some evidence that it did promote remyelination, as mVEP latency significantly decreased towards normal levels, this treatment did not prove to be a viable option for MS patients (Brown et al. 2021).

One potential reason that this treatment successfully encouraged remyelination but also induced adverse events was the general agonistic behaviour of bexarotene. Bexarotene is a non-selective agonist and therefore recognises the alpha (α), beta (β), and gamma (γ) RXR pathways (Brown et al. 2021; Dawson and Xia 2012). Such widespread agonism of all RXR isoforms would subsequently lead to widespread effects, as specific isoforms are found in different areas of the body. For example, RXR-γ is predominantly expressed in the brain and muscles, whereas RXR-α predominates the epidermis, intestines, kidneys, and liver, and RXR-β is largely non-specifically expressed (Dawson and Xia 2012). Therefore, bexarotene will non-selectively agonise RXR-γ in the brain as well as RXR-α and RXR-β throughout the body.

3.3 Other targets and techniques

3.3.1 Nanoparticles

Nanoparticles are a form of nanotechnology that produces materials at a nanoscale, less than 100 nm, which contain a core and shell layer, and a functional surface layer (Khan et al. 2019). This surface layer can be customised depending on the desired function by utilising other small molecules, as well as metal ions, surfactants, or polymers. They can be used in the creation and/or application of pharmaceuticals and other treatments, as their nanostructure allows for very accurate and efficient delivery at optimum doses as “personalised medicine” (Table 1) (Khan et al. 2019; Zhang et al. 2015b). Depending on the size and shape of the nanoparticle it can enter the cells by different pathways, including phagocytosis or endocytosis, which further dictates its speed and amount of uptake (Zhang et al. 2015b).

Certain types of nanoparticle-based delivery have been used to deliver factors directly into target cells. For example, leukemia inhibitory factor (LIF) has been previously shown to directly prevent OLG death (Butzkueven et al. 2002; Rittchen et al. 2015). This is achieved by LIF activating LIF receptor-β (LIFR-β) which inhibits IFN-γ induced OLG death as part of the endogenous anti-inflammatory defence mechanisms (Butzkueven et al. 2002). Rittchen and colleagues (2015) were able to engineer nanoparticles carrying LIF with accompanying antibodies for proteoglycans that are expressed on OPCs. This enabled the nanoparticles to bind directly to the OPCs and induce differentiation into matured OLGs (Rittchen et al. 2015). This further supports the abilities of nanoparticles to not only effectively administer drugs, but also the potential application of engineered nanoparticles in restricting or preventing the course of demyelinating diseases.

Commonly used silver nanoparticles (AgNP) are shown to have antimicrobial properties, but prolonged use can induce oxidative stress in myelin (Dabrowska-Bouta et al.
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2019). An alternative to AgNP could be the use of gold nanoparticles (AuNP), which has better biocompatibility and biodistribution in comparison, allowing for a safer and more targeted administration (Table 1) (Lira-Diaz et al. 2022; Papastefanaki et al. 2015). This technique is also a viable option for demyelination via secondary degeneration pathways, such as following SCI, by administering anti-inflammatory or neuroprotective factors. Polyethylene glycol (PEG) has these capabilities but can be toxic and biologically unavailable for uptake (Papastefanaki et al. 2015). However, when coupled with AuNP it can overcome these limitations and is able to increase remyelination and promote motor recovery by reducing the oxidative stress that causes demyelination following SCI (Papastefanaki et al. 2015). Continuing the evidence of AgNP as a remyelinating treatment, a clinical trial utilising nanocrystalline gold to remedy remyelination failure in MSON patients was registered in 2018 to determine its safety and efficacy (Clene Nanomedicine 2018, NCT03536559).

3.3.2 Antidiabetics (Metformin)

Metformin is a first-line drug commonly used to treat type-2 diabetes, and has recently been explored for its potential in other diseases such as cancer, AD, and poly-cystic ovarian syndrome (PCOS) (Abdi et al. 2021; Dziedzic et al. 2020). Metformin plays a mediating role in multiple pathways, including those with anti-inflammatory and antioxidant capacities (Table 1). One of these pathways includes inhibition of oxidative phosphorylation and CD4+ T-cell activation in an anti-inflammatory manner by indirectly activating adenosine monophosphate-activated protein kinase (AMPK) pathways (Abdi et al. 2021; Dziedzic et al. 2020). Metformin has been proposed as a potential effective treatment for MS because of its ability to mediate inflammation and oxidative stress. For example, metformin was able to inhibit oxidative phosphorylation and CD4+ T cell activation in a mouse model of lupus, which could be further applied to MS (Dziedzic et al. 2020).

In a CZ-induced model of MS, it was found that metformin treatment significantly increased the localisation and maturation of OLGs. It was also able to control the pathways for inflammation and oxidative stress by down-regulating pro-inflammatory macrophage and microglia markers (Mac-3 mRNA) (Sanadgol et al. 2020). This was attributed to direct activation of AMPK which reduced inflammation in a neuroprotective manner. Other effects of metformin mediated AMPK activation are the inhibition of NF-κB, whose translocation promotes the transcription of inflammatory cytokines, including IL-1 and IL-6 (Abdi et al. 2021). In this case, metformin was able to protect from demyelination following CZ treatment, as it downregulated pro-inflammatory genes and NF-κB activation levels were significantly decreased.

Another attribute of metformin integral in its treatment of type-2 diabetes is its fasting metabolism-mimicking abilities. The AMPK pathway also plays a role in central nutrient signaling, which when modulated via metformin treatment mimics the act of fasting on a cellular level (Neumann et al. 2019). In this sense, fasting encourages a more regenerative permissive environment by reversing certain ageing factors of OPCs. It was found that in aged rats, metformin treatment was able to activate the AMPK pathway and restore OPC mitochondrial functions and further proliferation in a manner similar to rats that underwent alternate day fasting (Neumann et al. 2019). This is another aspect of how AMPK activation can encourage OPC differentiation, as it increases mitochondrial function in a rejuvenating manner in older OPCs and encourages remyelination. These pre-clinical animal studies have shown the potential for metformin as a potential remyelinating target, and there is currently a study recruiting patients with progressive MS to determine the safety of metformin use (Patel 2022, NCT05349474).

3.3.3 Antibody therapies

Antibodies to target proteins related to myelin have also been used as a potential treatment in MS to encourage remyelination. For example, leucine-rich repeat neuronal protein 1 (LINGO) is a protein expressed exclusively on oligodendrocytes and inhibits its differentiation (Ruggieri et al. 2017; Vasileiou and Fitzgerald 2023). Blocking or decreasing endogenous levels of LINGO would therefore promote OLG differentiation and maturation, along with increased axonal branching and myelin sheath development (Ruggieri et al. 2017). The anti-LINGO antibody, opicinumab (BIIB033) was confirmed in animal studies, as treatment with the antibody effectively blocked LINGO functions and enhanced remyelination and functional recovery.

Although promising outcomes were seen in animal studies, translation to in human studies has not been wholly successful. In a 2013–2014 study of relapsing MS patients, were treated with opicinumab or matching placebo. There were no significant changes to disability scores or MRI and DTI measurements in opicinumab treated patients compared to placebo treatments, except for in a small subgroup with shorter disease duration and lower brain lesion baselines (Cadavid et al. 2019). In a similar study of MSON patients, those given opicinumab treatment within the per-protocol population did exhibit some level of remyelination as VEP latency was significantly improved at 32 weeks (Cadavid et al. 2017). However, the researchers involved in this clinical trial did note that...
the findings may be skewed by patient population size and influences of statistical power (Cadavid et al. 2017). Therefore, the potential for opicinumab or other antibodies targeting LINGO as a remyelination strategy will require further research and more clinical studies.

3.3.4 Antihistamine (Clemastine)

Clemastine fumarate is an H1-antihistamine approved for use in allergic reactions (Li et al. 2015; Mei et al. 2014; Xie et al. 2021). Its remyelination properties were identified in (2014) by Mei and colleagues, who found that it enhanced OLG differentiation and wrapping of micropillars, without altering the number of OLG cells at low doses (Table 1). They attributed this to the antimuscarinic properties of clemastine, as blocking muscarinic receptors can promote remyelination. M1 Muscarinic receptors are expressed by oligodendroglia cells and have modulating effects on OPC survival, proliferation, and further differentiation into mature OLGs (Mei et al. 2014). Therefore, by antagonising these muscarinic receptors with clemastine treatment, it will in turn enforce OPC differentiation and encourage remyelination. This was validated in mice with induced toxic injury and demyelination of the spinal cord, which revealed enhanced OLG differentiation, and accelerated remyelination following clemastine treatment (Mei et al. 2014). In CZ treated mice modelling MS type demyelination, clemastine significantly increased MBP expression throughout the brain, suggesting that remyelination was enhanced (Li et al. 2015). This was further corroborated when the number of mature OLGs was quantified and revealed a significant increase. The authors attributed this effect to clemastine’s ability to encourage OLG differentiation (Li et al. 2015).

Not only could this treatment be beneficial for demyelinating diseases, but it can also be an additional therapeutic in diseases with implied myelin dysfunction. For example, in a mouse model of AD, clemastine treatment significantly reduced Aβ deposition along with increasing OPC and OLG maturation, which decreased the amount of damaged myelin (Li et al. 2021; Xie et al. 2021). This was also achieved in older mice, as clemastine treatment was able to enhance autophagy or cellular degradation of Aβ which rescued neuroinflammation and accompanying cognitive deficits (Xie et al. 2021). Clemastine treatment was also responsible for upregulating autophagy of Aβ and preventing OPC senescence, leading to decreased plaques and enhanced remyelination (Xie et al. 2021).

In a double-blind, randomised trial, MS patients were treated with 36 mg clemastine fumarate five times daily, to determine not only the safety and efficacy of this treatment but also determine its remyelinating potential (Green et al. 2017). Aligning with evidence from pre-clinical animal studies as previously mentioned (You et al. 2019), all patients had a decreased P100 VEP latency at the trial endpoint, indicating remyelination had been instigated (Green et al. 2017). However, MRI measurements showed no change (positive or negative) when comparing baseline analyses to end of treatment. The improved VEP latency evidenced remyelination, and lack of serious adverse events reported by participants of the clinical trial shows the potential for clemastine as an effective remyelinating therapy for MS or other demyelinating disease patients (Green et al. 2017).

3.4 Remyelination strategies outside of MS

Although MS is the disease most associated with demyelination, it has previously been shown that myelin plays an integral role in the disease pathology of multiple different diseases, including AD and glaucoma. Clinical trials and other therapeutic measures considering other diseases therefore use a different variety of targets. However, this has proven difficult, as finding medications or cellular factors to target a specific pathway is akin to throwing drugs at the wall to see what sticks.

3.4.1 Glaucoma

Treatments for glaucoma that may not have initially targeted myelin have more recently been proven to hold remyelinating properties. For example, cytidine 5'-diphosphocholine (citicoline) is an endogenous mononucleotide which modulates biosynthesis of phospholipids in the CNS and increases certain neurotransmitters (Roberti et al. 2015; Sahin et al. 2022; Skripuletz et al. 2015). Using oral citicoline treatment in primary open angle glaucoma patients, it was shown that the RNFL was significantly thicker in the average and inferior quadrants. This is in comparison to patients who were given a placebo, and experienced significant thinning of the retinal section of the RNFL (Sahin et al. 2022). Alongside these neuroprotective features, citicoline has been shown to induce remyelination by increasing OPCs in EAE and CZ animal models (Skripuletz et al. 2015). This is achieved by citicoline inducing protein kinase C (PKC) phosphorylation, which in turn upregulates cell proliferation (Skripuletz et al. 2015).

Although the extent of citicoline induced remyelination in glaucoma patients has not yet been explored, there is evidence that its neuroprotective capacities could be beneficial for these patients.
3.4.2 Schizophrenia

Other studies outside of MS include psychiatric conditions, including borderline-personality disorder (BPD) and schizophrenia. Schizophrenia patients have been shown to exhibit disturbed myelin and overall WM structure, however this was originally believed to be a consequence of common antipsychotic drugs (Livni et al. 1979; Takahashi et al. 2011). Although it has subsequently been proven that WM pathology is not a consequence of treatment, there is debate as to the exact demyelination and whether it is a consequence or cause of schizophrenia (Takahashi et al. 2011; Yu et al. 2022). Antipsychotics have been investigated to determine their potential myelin-protection and OLG stimulating properties (Kroken et al. 2014). Risperidone is one approved antipsychotic that following long-acting injection resulted in increased intracortical myelin volumes within the frontal lobe in first-episode schizophrenia patients (Bartzokis et al. 2012; IRCCS 2022, NCT05322031). One proposed method of action of remyelination via risperidone treatment is by inhibiting glycogen synthase kinase-3 β (GSK3β) when dopamine receptors are blocked by antipsychotic treatment (Bartzokis et al. 2012). GSK3β is an integrating molecule that negatively regulates Akt mediated proliferation and differentiation. Inhibiting its signals has been shown to promote OPC differentiation and OLG maturation to promote remyelination (Azim and Butt 2011). Interestingly, GSK3β activity has also been implicated as risk factors for developing AD, Parkinson’s disease, and MS, as many GSK3β variants have been found in these patients (Galimberti et al. 2011).

4 Neuroprotective strategies targeting remyelination

4.1 Limitations to neuroprotection

An overwhelming number of studies in human and animal models of human diseases have been conducted in order to establish a drug or treatment capable of reversing neurodegeneration. This includes various attempts to target remyelination as a source of neuroprotection. The failure to replicate the restorative properties found in these studies highlights the need for further, more effective, and well-designed projects. An estimated 90% of clinical drug trials fail, and when paired with the high cost and high-risk associated with clinical trials reveals the dire need for these studies to be re-optimised and re-evaluated (Ploughman et al. 2022; Sun et al. 2022). The predominant reasons for these failures are a lack of clinical efficacy, or the inability to meet clinical end-points, and unmanageable toxicity in patients (Sun et al. 2022). In order to rescue these efforts and save future trials, new strategies have been suggested including co-delivery of therapies and non-invasive strategies.

4.1.1 Only utilising monotherapies

Although there is pre-clinical, animal based data that shows multiple drugs as being capable of promoting remyelination through a variety of pathways, no drugs have been approved as a remyelinating therapy (Gingele and Stangel 2020). One reason for this is that these clinical trials only utilise one potential drug. The process of remyelination is incredibly complex, and targeting only one of the pathways may not be sufficient to promote remyelination. Therefore, co-delivery or combination therapies should be further investigated to cover all avenues of remyelination (Fessel 2022; Gingele and Stangel 2020). Although there is pre-clinical, animal based data that shows multiple drugs as being capable of promoting remyelination through a variety of pathways, no drugs have been approved as a remyelinating therapy (Gingele and Stangel 2020). One reason for this is that these clinical trials only utilise one potential drug. The process of remyelination is incredibly complex, and targeting only one of the pathways may not be sufficient to promote remyelination. Therefore, co-delivery or combination therapies should be further investigated to cover all avenues of remyelination (Fessel 2022; Gingele and Stangel 2020). Evidence of this idea has also been shown in the use of remyelinating factors as an AD treatment. Although multiple drugs have been trialled in controlling Aβ pathogenesis in AD, it is proposed that remyelinating compounds should be used in combination with these drugs (Fessel 2022). This is attributed to the correlation of myelin disruption and the complicated factors surrounding Aβ pathology, such that a combination of treatments designed to target both paths may be required to effectively treat the pathology process in its entirety.

Evidence of success in combination therapies has previously been shown in clinical trials for MS patients (Minagar et al. 2008). This study utilised interferon β-1a (IFNβ) and doxycycline, as IFNβ can control the inflammatory process and doxycycline has immunomodulatory and neuroprotective abilities (Minagar et al. 2008). IFNβ has been prescribed to MS patients for over 25 years, while doxycycline is an antibiotic with immunomodulating and neuroprotective features and works through multiple possible pathways, including down-regulating major histocompatibility complexes and inducing T-cell production of IL-10 (Jakimovski et al. 2018; Minagar et al. 2008). It was
found that the combination of treatments was effective and safe, as there were only mild adverse events reported by participants and most patients had reductions in lesion numbers and decreased disability measurements (Minagar et al. 2008).

In a combination study of the two most commonly prescribed treatments for MS, IFNβ, and glatiramer acetate (GA), the safety of the combined treatment and efficacy was explored in RRMS patients (Lublin et al. 2013). The use of both agents was theorised to be more efficacious than either alone, as they both have been proven to be safe in humans and act through different mechanisms of action. However, it was found that although there were some improvements to MRI measurements including reductions in new lesion activity, there was no overall significant clinical benefit gained from combining the two treatments (Lublin et al. 2013). Though the plan to attack disease processes from multiple angles is a well-founded one, this proves that there are other features of clinical trials that need to be addressed.

4.1.2 Ignoring rehabilitative constraints

Another point that previous clinical trials have failed to recognise is the overwhelming evidence that non-pharmaceutical interventions have significant effects on disease progression and overall symptomology (Pan and Chan 2021; Ploughman et al. 2022). This includes interventions such as exercise or occupational therapies to promote rehabilitation and plasticity. Previous articles have summarised the significant benefits of exercise and environmental enrichment on animal studies, such as by Ploughman and colleagues (2022), and how this might be applied to future clinical trials.

Others have also highlighted the role of encouraging neuroplasticity to create a biochemical environment that fosters remyelination. Not only can physical activity promote remyelination, but myelin plasticity can also be influenced by experience based complex motor or cognitive training (Pan and Chan 2021). This has been seen in animal and human experiments regarding the positive effects of cognitive or physical training on myelin plasticity. Examples given in a review by Pan and Chan (2021) including six weeks of juggling training (Schoz et al. 2009), or utilising car racing video games (Hofstetter et al. 2013). This creates a stimulating environment not only for the patient’s wellbeing but for their brains, allowing for an environment capable of plastic activity for myelin remodelling to occur. Similar results were found in a study that used transcranial direct stimulation, such that 15 m of excitation significantly improved fatigue symptoms (Ferrucci et al. 2014). This was attributed to an increase in neuronal and axonal excitability, as direct stimulation assisted in axonal conduction along demyelinated axons (Ferrucci et al. 2014).

4.2 A potential way forward

Although these extra steps have been shown to provide benefits towards remyelination and/or further neuroprotection, most clinical trials have not utilised this advice. A possible reason is that the age and corresponding degree of disease severity may inhibit participants from being able to participate in physical exercise at the extent required to reveal this effect. However, Ploughman and colleagues (2022) have further addressed the tendency for clinical trials to enroll participants in advanced disease stages and may no longer have the capacity for repair by the targeted remyelinating pathways. As the possibility for OLG maturation and myelin repair is relatively inhibited with age, there is a chance that older participants in these clinical trials have not been adequately screened for inclusion. Therefore, the combination of appropriate age recruitment and implementing exercise or other cognitive activities to promote plasticity may be the key to fully invoking repair in demyelinating diseases (Pan and Chan 2021; Ploughman et al. 2022). In cases where age dependent recruitment is not a viable option, such as in diseases that predominantly affect the elderly, the use of cognitively stimulating exercises or learning should be implemented. This is relevant not only to common diseases targeted for remyelinating such as MS, but also diseases where myelin dysfunction is highly implicated, including glaucoma and AD.

5 Conclusions

The formation of myelin sheaths across axons creates many benefits for neurons, including reduced energy consumption and increased synaptic speeds. This is accomplished by reducing ATP load required for ion gradient restoration and maintaining the localisation of electrical connections at the ends of the node of Ranvier. As beneficial as myelin formation can be, the degradation of myelin has deleterious effects on the axon and connecting neurons. These effects of demyelination are seen in a variety of diseases, not only in those classically associated with demyelination, such as MS, but also in diseases such as AD and glaucoma. It also plays a role in secondary processes of degeneration, including TSD, which further exacerbates the role of demyelination in neurodegeneration. Therefore, the need for remyelination products has become a necessity in research for treatments in these diseases, as none have been successfully approved.
following clinical trials. This is despite overwhelming evidence from pre-clinical animal trials that show the encouraging effects of different remyelinating pathways. This includes treatments such as antioxidants to inhibit inflammation, as well as targets of the RXR–RAR pathways to encourage OPC differentiation and OLG maturation. There have also been significant findings from animal studies and in patient clinical trials that utilise different, already approved drugs that have remyelinating effects outside of MS, such as antipsychotic use in schizophrenic patients. Despite this, clinical trials testing these therapeutic pathways had little to no effect, and sometimes came with serious side effects. This calls into question not only the current modalities of designing clinical trials, but the need for other concomitant therapies. This does not have to be reduced to combination drug treatments, although some have proven to be effective, but also implementing exercise or non-invasive therapies to coax a neuroplastic environment in patients. Therefore, future studies could ameliorate the failures of their predecessors by designing clinical trials that embody a holistic approach to remyelination: by targeting multiple pathways of remyelination as well as ensuring a rehabilitative environment in these patients.

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