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Model Systems to Define Remyelination Therapies

Robert H. Miller, Molly Karl, Reshmi Tognatta,
Ahdeah Pajooohesh-Ganji and Mohammad Abu-Rub

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Abstract

Demyelinating diseases of the central nervous system (CNS), such as multiple sclerosis (MS), are characterized by multiple focal *demyelinating* lesions, resulting in various functional deficits. The pathology of MS is defined by local loss of myelin sheaths in the brain and spinal cord associated with infiltration of peripheral immune cells. Classically, MS starts with a series of relapses and remissions, followed several years later by a more progressive form of the disease and a steady functional decline. Although the mechanism of disease initiation is poorly understood, disease progression is associated with immune system activation toward CNS antigens including myelin proteins. Animal models of MS have been critical in the development of MS therapies, with experimental allergic encephalitis (EAE) being the most common. This model has been instrumental in defining the role of T cells in disease progression and in the development of targeted therapies. Understanding the biology of myelin repair has, however, largely come from other model systems including local targeted demyelination *in vivo*, slice preparations, and *in vitro*. This has led to the identification of a diverse array of potential new targets to modulate disease progression. Development of these new avenues is the target of intensive ongoing research.

Keywords: remyelination, therapeutics, animal model, multiple sclerosis, oligodendrocytes, astrocytes, experimental allergic encephalitis (EAE)

1. Introduction

Myelin is the fatty insulation that surrounds axons, enhances axonal conduction rates, and protects axons from damage in the nervous system. In the central nervous system (CNS), the majority of myelin is a product of oligodendrocytes, and a single oligodendrocyte may

myelinate multiple segments of different axons. During development, oligodendrocytes are generated from precursors (OPCs—oligodendrocyte precursor cells) that arise in specific locations of the brain and spinal cord as a result of local inductive cues (discussed in more detail later). While oligodendrocytes and myelin are found throughout the CNS, the amount of myelin in white matter is substantially greater than that in gray matter. Indeed, the primary reason that white matter appears white is due to its high concentration of lipid-rich myelin. Because myelin plays a central role in modulating neuronal activity, its loss is frequently associated with functional deficits. Myelin loss or demyelination occurs in various different pathological conditions including developmental disorders such as the leukodystrophies, adult-onset insults such as stroke, and classical demyelinating diseases such as multiple sclerosis (MS) and related disorders. MS, the most common CNS demyelinating disease, was originally described over 100 years ago, and initial descriptions of the disease highlighted an illness of increasing functional deficits. Our understanding of the disease course and its progression has advanced over time, and it is now clear that MS is a more complex disorder in terms of clinical presentation and underlying pathogenesis [1–6].

In classic cases, MS initially presents as a sudden-onset neurological deficit that resolves over a period of time. Subsequent attacks (relapses) are followed by periods of remission; however, over time, remission fails to result in a return to normal functionality and deficits slowly accumulate. Following this relapsing-remitting phase (relapsing-remitting MS), the disease enters a more chronic phase in which deficits accumulate in a progressive manner (progressive MS) (Figure 1). Not all patients follow this disease trajectory. In a distinct subset of patients, the

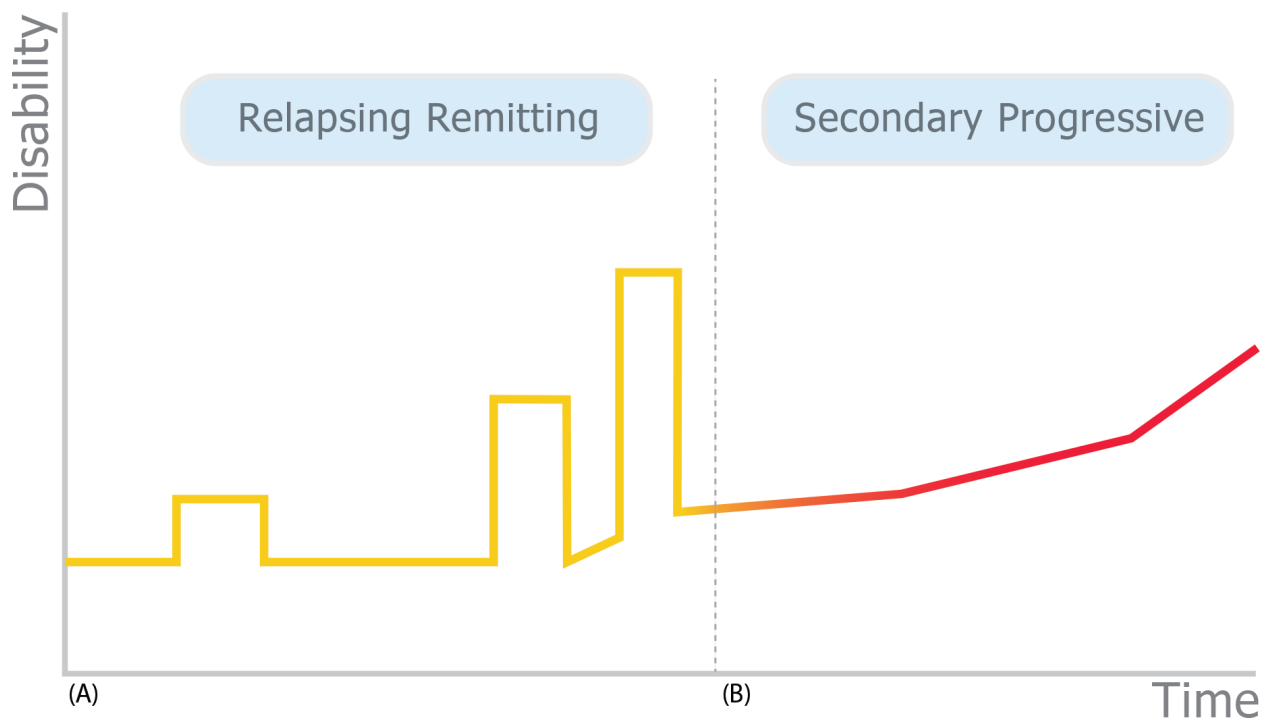


Figure 1. Graph showing typical disease activity in relapsing remitting MS, characterized by defined clinical attacks followed by full functional recovery (A) or incomplete recovery between attacks with residual deficits (B), eventually leading to worsening disability (C).

disease presents initially as progressive functional impairment without obvious remissions—a condition known as primary progressive MS. Alternatively, some patients never progress beyond the relapsing remitting phase, and others only experience a single attack. In MS and numerous MS-related conditions, myelin loss is localized and multiple areas of demyelination or plaques may be present in a single patient. Each of the plaques appears to progress or resolve independently during the progression of the disease.

The variability in presentation and progression makes the accurate diagnosis of MS complicated [6]. This is further compounded by the lack of biomarkers that unambiguously identify MS, and consequently, a diagnosis of MS is dependent on several factors in an overall presentation rather than a single definitive test. Identifying factors include medical history and clinical examination, magnetic resonance imaging (MRI) imaging, and the presence of oligoclonal bands in CSF. While MRI and other imaging modalities are highly effective at identifying lesions in CNS white matter, they are not able to specifically characterize MS-associated demyelinated lesions, and other conditions such as inflammation may generate similar MRI findings. Recent advances in imaging modalities have enhanced the specificity of these approaches for demyelination, and it seems likely that more specific approaches will be implemented in the clinic in the near future. One such approach is using PET imaging to detect areas of myelin loss [7]. The development of selective tracers of myelin that can be visualized in a noninvasive manner is promising; however, the widespread application of this approach is likely to be limited by the short half-life of the probes and the necessity of a local cyclotron for their production.

While generally considered to be a disease of white matter, and myelin in particular, there is now strong data indicating that MS plaques also occur in gray matter, including synapses, and that altered synaptic transmission along with loss of gray matter may contribute to cognitive deficits and brain atrophy often associated with MS [8]. Wherever they occur, MS plaques are frequently associated with a core blood vessel and reactive astrocytes. The close association of blood vessels with MS plaques is indicative of the role of the immune system in the pathogenesis of MS, which is universally recognized as an autoimmune disease. Considerable evidence indicates that T cells that recognize myelin antigens enter the CNS and attack myelin and oligodendrocytes. This inflammatory insult recruits other cells including cells of the innate immune system such as macrophages and microglia that contribute to CNS damage. The majority of existing MS therapies are directed toward either suppressing the immune response or blocking the entry of T cells and other cells of the peripheral immune system into the CNS. While such approaches have been quite effective at modulating the severity and interval of relapses in relapsing remitting MS, it has become clear that they fail to block overall progression of the disease and brain atrophy, and neurodegeneration is only slightly improved. Such observations have led to a concerted effort to identify therapies for MS that are targeted toward promoting myelin repair or inhibiting damage within the CNS but have been somewhat hampered by the lack of understanding of the causes of MS.

The chronic nature of MS and the likelihood that the disease has been ongoing for an extended interval prior to becoming symptomatic make it extremely difficult to identify the initial pathogenic signal. One attractive hypothesis is that a potential trigger for MS is a response to

a prior infection or other environmental signal [9]. This notion is supported by the findings that MS patients have elevated immunological responses to various pathogens, which may account for some aspects of the epidemiology of MS. A wide range of pathogens including spirochetes, chlamydia, and a range of viruses have been linked to MS [1]. It seems likely that the role of such pathogens is to enhance susceptibility to MS rather than directly induce disease. One of the strongest links in MS is viral infection [10], and a number of different viruses have been implicated including Epstein-Barr, human herpes virus 6, and human endogenous retroviruses [10–12]. Precisely how the viral infection contributes to MS development has not been clarified, but it may reflect the initial stimulation of immune cells to viral antigens or the induction of oligodendrocyte death as a result of viral infection.

One aspect of MS that has been extensively studied is its genetic linkage [13], and instead of a gain or loss of function of one individual gene, there are a range of genetic associations linked to MS. In particular, MHC class II molecules such as HLA-DR and HLA-DQ alleles are considered risk factors for the disease [14]. Given the immunological nature of the disease, the association with immunomodulatory genes is expected; however, the mechanisms by which these genetic changes increase disease susceptibility are still unclear. For example, certain MHC molecules can promote the development of an autoimmune response following a sub-acute challenge from a structurally similar antigen. The contribution of genetic or epigenetic changes in cells of the oligodendrocyte lineage or myelin that contribute to MS susceptibility remains to be clarified.

To understand the biology of MS requires a clear understanding of the cellular and molecular mechanisms that mediate myelination and myelin maintenance, and much of our understanding of the control of myelination comes from studies in development. Oligodendrocytes, the myelinating cells of the CNS [15–18], are generated from precursor cells (OPC or oligodendrocyte precursor cells) that arise in distinct location of the embryonic CNS in response to selective inductive cues and subsequently disperse throughout the CNS [17, 19–21]. The early commitment of neural stem cells to the oligodendrocyte lineage depends on environmental cues that include sonic hedgehog and the subsequent induction of transcriptional signaling pathways that promote the appearance of OPCs, their proliferation, and subsequent migration. One of the major mitogens and potential growth factors that support the expansion of the oligodendrocyte lineage is platelet-derived growth factor alpha (PDGF α). The receptor PDGF α R is expressed predominantly by OPCs *in vivo* and allows for the unambiguous identification of OPCs in the setting of demyelination and repair. In the spinal cord, OPCs originate at the ventral midline during embryonic development and subsequently disperse widely through gray and white matter. This migration is guided by a number of different signals including Netrin 1 and Wnts and appears to track with the vasculature. Prior to myelination, OPCs differentiate to oligodendrocytes, a process that includes the cessation of proliferation and the induction of additional transcription factors including Myrf that are essential for oligodendrocyte maturation. The differentiation of oligodendrocytes is clearly environmentally regulated. For example, myelin debris has been shown to inhibit the differentiation of oligodendrocytes and may be an important factor in the control of remyelination where delayed myelin clearance may inhibit repair [22, 23]. Once oligodendrocytes mature, there is a defined time window during which they extend multiple processes to contact adjacent axons and initiate myelination. Less is known about the molecular interactions that orchestrate the initiation of

myelination. Several parameters such as axonal size and electrical activity have been implicated as important in the early stages of myelination. In addition, several factors have been suggested to inhibit the onset of myelination, and these include LINGO-1 and the expression of PSA-NCAM on axonal surfaces. Following differentiation and maturation, oligodendrocytes begin to generate myelin sheaths. An individual oligodendrocyte initially generates an excess number of myelin sheaths, some of which grow and are stabilized, while others shrink and are subsequently lost. What regulates the growth and retraction of myelin sheaths is not well understood, but recent studies suggest that it may be regulated by axonal activity.

Myelin is a specialized plasma membrane that provides a fatty insulation around axons and allows the rapid conduction of electrical impulses by increasing conduction velocity, reducing the threshold for firing, and providing axonal protection [24]. Myelin sheaths are discontinuous and are linked by Nodes of Ranvier (**Figure 2**) that have a characteristic morphology and are areas of high concentration of ion channels that support electrical impulse propagation. Nodes of Ranvier appear to be particularly sensitive to damage, and their disruption results in perturbation of axonal conduction. The region between two nodes is known as the internode, and it is made up of a number of specific proteins [25], including the major proteins myelin basic protein (MBP) and proteolipid protein (PLP) as well as other minor proteins such as myelin-associated glycoprotein (MAG), myelin oligodendrocyte protein (MOG), 2'3' cyclic nucleotide 3'phosphodiesterase (CNP), and myelin-associated oligodendrocyte basic protein (MOBP) [26].

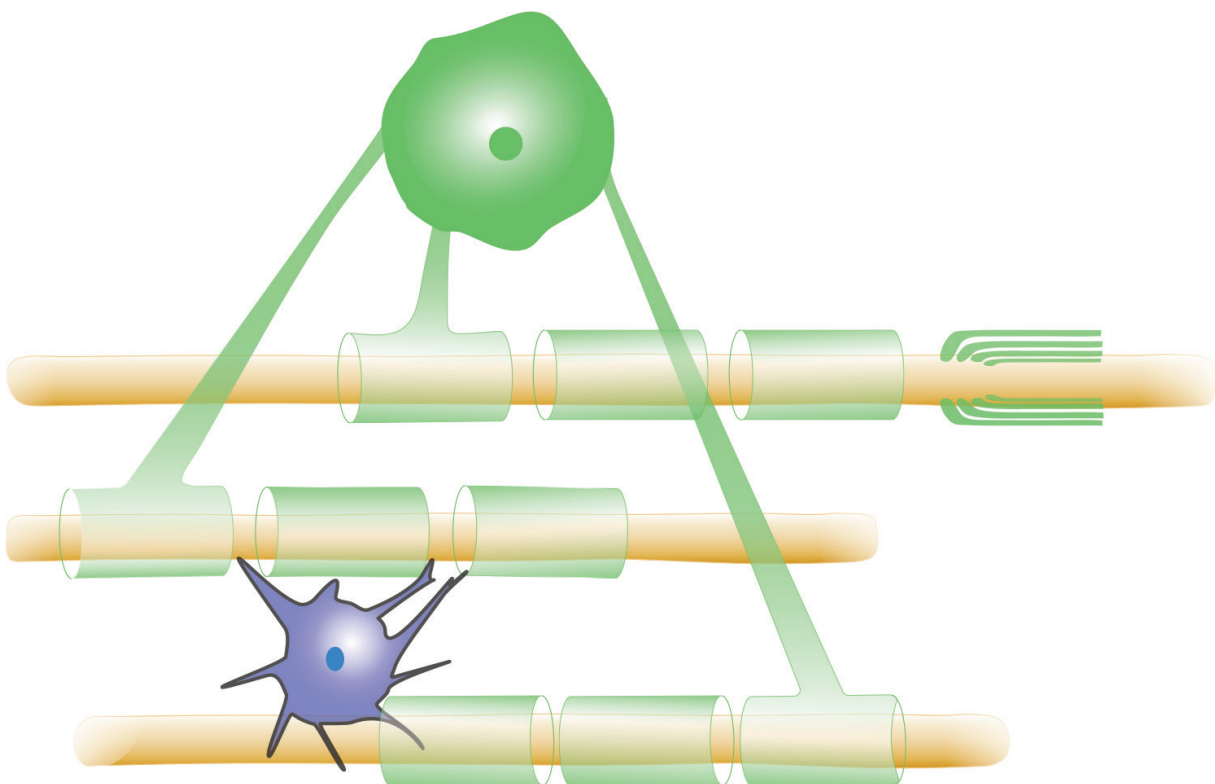


Figure 2. CNS myelin. Schematic representation of an oligodendrocyte ensheathing several axons in the CNS. Segments of myelin sheaths are separated by Nodes of Ranvier, which are contacted by astrocyte foot processes.

Each of these myelin components is presumably important for normal myelin function. For example, MBP mutant animals such as *shiverer* fail to form compact myelin and have limited life span [27], while animals lacking PLP develop normally but manifest axonal pathology later in life [28, 29].

The normal development of myelin is also dependent on additional CNS cell types: axonal processes are targets for myelination, and astrocytes are important in the development and survival of cells of the oligodendrocyte lineage [30]. Astrocytes are a heterogeneous cell population that have been proposed to perform multiple functions that support development and maintenance of the brain and spinal cord. During development, astrocytes guide the migration of neurons from their germinal zones to their final destination [31] and act as substrates for long-distance axonal growth to their targets. In the adult, astrocytes are important for the removal of neurotransmitters, control of the ionic environment, and maintenance of the blood-brain barrier as well as either supporting or inhibiting regeneration through the formation of glial scars [32–34] that are comprised of astrocyte processes and extracellular matrix. A similar glial scar is formed around chronic demyelinating lesions and has been suggested to block myelin repair [35], although recent studies indicate that the astrocyte response is beneficial in certain animal models.

Histological studies provide an evidence of neuronal damage in MS, including axonal loss in areas of demyelination [29, 36] or even frank brain atrophy due to widespread loss of neuronal cell bodies and their axons [37]. It is unclear whether the axonal loss is secondary to myelin loss or independent of it via direct antigenic targeting. The role of astrocytes in MS disease pathogenesis is less well defined. For example, in areas of demyelination, a reactive astrocytic response is commonly characterized by elevated expression of glial fibrillary acidic protein (GFAP) that may be either protective or pathogenic [38]. Disruption of the blood-brain barrier is also important in the formation of demyelinating lesions in MS, and astrocytes have been proposed to play an important role in the maintenance of the blood-brain barrier in the adult CNS. The best evidence for an astrocytic role in demyelination comes from the studies of the MS variant known as neuromyelitis optica (NMO) that preferentially presents in the optic nerve and spinal cord. In a significant subset of NMO patients, demyelination is thought to result from the binding of pathogenic antibodies against aquaporin 4, a molecule expressed on the end feet of astrocytes around blood vessels. Antibody binding results in astrocyte death and subsequent demyelination, although the molecular linkages in this cascade are unknown.

The cellular complexity and heterogeneity of MS-like diseases represent a significant challenge in developing effective animal models that accurately mimic disease progression, and this has led to the generation of a number of different models, each of which highlights distinct components of the disease [39]. Some of the most powerful and best-studied models of MS are those that utilize selective stimulation of the peripheral immune system as the major driver of CNS pathogenesis, and these are discussed in more detail later.

2. Animal models of demyelinating diseases: strengths and weaknesses

2.1. Immunological models for CNS demyelination

Multiple sclerosis is characterized by the engagement of the immune system, and this has been primarily modeled through approaches collectively known as experimental allergic encephalitis (EAE) [40, 41]. In general, EAE is an inflammation-mediated demyelinating disease that is induced in host animals through immunization with CNS tissue resulting in a host of functional deficits that correlate with immune cell infiltration into the CNS. The functional deficit is then scored on a 1–5 scale: 1 presents with a flaccid tail, 2 with hindlimb weakness, 3 with hindlimb paralysis, 4 with forelimb and hindlimb paralysis, and 5 death. In most studies, the scale is expanded to between 2.5 and 3.5, allowing for better definition of functional changes.

Initial development of EAE involved injection of spinal cord homogenates into rabbits resulting in hindlimb paralysis and other functional deficits. Subsequently, immunization of monkeys with spinal cord homogenate derived from rabbit CNS [42] showed the pathological accumulation of cells around blood vessels of the brain and spinal cord. Variability in individual animal responses limited initial studies; however, this has largely been resolved through the use of immune stimulants such as complete Freund's adjuvant (CFA) combined with pertussis toxin. This model has been refined through identification of effective protein antigens. These antigens are predominantly myelin-associated proteins including myelin basic protein (MBP), myelin-oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP) [41]. Minor myelin components are also capable of generating disease suggesting that most myelin components can act as effective priming antigens. The identification of specific myelin protein peptides that provoke a reproducible and consistent disease following immunization into genetically defined host populations has resulted in several major models of EAE that are now commonly used. These include the induction of EAE in the *S/JL* mouse genotype following immunization with the PLP₁₃₉₋₁₅₁ peptide, which generates a relapsing remitting disease mimicking some characteristics of relapsing remitting MS. An alternative model utilizes C57/Bl6 mice immunized with the MOG peptide₃₅₋₅₅. This model is often used to recapitulate more advanced stages of MS because it generates a more chronic disease course. Other less common models include the induction of EAE in PL/J mice following immunization with MBP or MOBP and immunization of Biozzi ABH mice with MOG protein that models selective aspects of MS.

Several major themes have emerged from studies on the mechanisms of disease in murine EAE. One common finding is a primary role for T cells in disease development. Adoptive transfer clearly demonstrated that T cells specific for MBP antigen were capable of transferring disease to naïve hosts [43]. The functional deficits in this model were transient, resolving within 1–2 weeks, and were not characterized by extensive demyelination suggesting the pathology in MS reflects multiple pathogenic processes. One strong candidate that contributes

to EAE and MS pathology is B cells [44]. B cells play multiple roles in immune-mediated pathology in the CNS. On the one hand, they facilitate activation and expansion of T cell populations within the CNS and enhance the recruitment of other immune cells into the CNS.

On the other hand, B cells produce antibodies directed against the different myelin antigens. For example, some MS lesions are characterized by an overexpression of anti-myelin antibodies, with MOG as a potential antigenic target [45]. Understanding the roles of B cells in the underlying pathogenesis of MS and other neuro-inflammatory diseases now seems to be at the forefront of research development after demonstrating that two B-cell inhibitors, Rituximab and Ocrelizumab, were shown to be highly effective in some MS patients, including those with primary progressive MS [46–48].

The role of the innate immune system in demyelinating pathologies is also an area of current focus. Microglial cells are also known to undergo reactive changes, whereby they aid in myelin clearance, but also could potentially participate in antigen presentation along with dendritic cells. One hypothesis is that pathological mechanisms vary by the stage of disease. Relapsing remitting disease, for example, may be largely driven by influx from the peripheral adaptive immune cells, whereas secondary and primary progressive forms of the disease are largely driven by the innate immune system.

Myelin components are not the only antigenic targets in MS. For example, axon-specific proteins, such as the neurofilament triplet, and node of Ranvier components, such as Contactin/TAG-1 and S100, have also been associated with EAE and MS [49]. It is unclear, however, whether the aforementioned proteins are primary disease targets or their involvement is secondary to myelin loss. It is likely, however, that as the disease progresses, the ongoing destruction of neural tissue expands the pathological basis of the disease resulting in more widespread damage and worsening functional deficits.

EAE models have been invaluable in elucidating critical aspects of MS biology and other demyelinating CNS diseases and have been reviewed in detail [40, 41]. One of the major advantages is that EAE utilizes well-defined antigenic targets and can be adaptable to numerous genetic animal models. This has allowed the identification of several well-defined networks resulting in T cell activation and trafficking, as well as shed light into the role of T cell subsets in disease progression. What is important is that these disease models still serve as primary tools not only for disease modeling but also for validating and identifying new therapeutic targets.

Another aspect of MS pathology that has started to gain ground is the effect on long-term synaptic plasticity, which is the physiological mechanism responsible for learning and memory and also is a key determinant of clinical recovery after cortical injury. It has now become clear that MS is frequently associated with cognitive and behavioral changes, which have been detected in the early stages of the disease, and are certainly more common than previously thought [50]. These changes are likely the result of synaptic impairment or altered synaptic plasticity. Among the different brain regions, the hippocampus is the most vulnerable. Despite its obvious importance, very few studies have been directed at understanding the hippocampal synaptic plasticity after EAE and not all are in agreement with what effect EAE has on hippocampal long-term synaptic plasticity. There is, however, sufficient evidence to indicate that

activated microglia are responsible, and that changes in synaptic plasticity are rather dynamic, effectively mirroring the stages of the disease and severity of inflammation. Along those lines, it has also been suggested that enhanced cortical plasticity is predictive of functional recovery after a relapse [51].

An important variant of immune-mediated models of demyelination is the generation of local rather than systemic lesions [52]. This has been achieved by sensitizing host animals with sub-threshold levels of encephalogenic peptides and subsequently delivering a local injection of a pro-inflammatory cytokine to stimulate local demyelination. For example, injection of $_{1-125}$ MOG peptide and incomplete Freund's adjuvant into Lewis rats results in an immune response but no overt clinical symptoms. Subsequent local injection of tumor necrosis factor alpha (TNF- α) or interferon-gamma (INF- γ) results in localized infiltration of immune cells, local demyelination, and axonal damage. Such studies revealed a rapid local functional deficit reflecting immune-mediated damage. This was followed by some functional recovery, although axonal damage remained. There are several strengths to this model including the ability to assess long-term consequences of a localized immune response and the capability to develop novel therapies to modulate initial immunological insult and promote long-term functional recovery. Such a model has several weaknesses including the localized nature of the insult and the method of induction of inflammatory stimuli. Local injection of cytokines results in damage to the blood-brain barrier and the stimulation of a robust astroglial response making mechanistic interpretation of the outcome of these studies difficult. There are a number of important differences between MS and EAE. EAE is generated through injection of selected antigens, while the trigger for MS is unclear. To date, there has been no description of a spontaneously occurring form of MS in animals. Second, the inclusion of unrelated antigens when inducing EAE has led to developing disease mechanisms and therapies that have otherwise failed clinical trials

Many of the current therapies used in the treatment of MS have emerged from studies of EAE, and it is not surprising that they are targeted toward regulation of immune cell responses. Such recent treatments include Fingolimod (FTY720) directed against the sphingosine-1-phosphate receptor that regulates T and B cell responses appears to directly stimulate remyelination in the CNS [53, 54], and Natalizumab directed toward adhesion molecules on lymphocytes blocks the entrance of those cells in the parenchyma of the CNS [55]. Such therapies, while modulating relapse activity, have generated unexpected side effects in the setting of clinical applications that have in certain cases limited their utilization. Furthermore, long-term studies suggest that while such therapies are effective at modulating inflammatory responses, they are less effective at controlling the disease activity or promoting recovery in the CNS. Current studies are becoming increasingly focused on developing approaches to promote myelin repair in the CNS, and EAE is not particularly suited to identification of repair mechanism.

2.2. Demyelination induced by gliotoxins

One of the major drawbacks of the aforementioned immune-mediated models of demyelination is that both pathological and repair processes occur simultaneously, which complicates the interpretation of potential repair strategies. To define the pathways mediating myelin repair, a variety of alternative models are available, and these include both focal and systemic

glial toxin treatments. These models, while they do not recapitulate the complex etiology and pathogenesis of MS, have two major strengths. First, the onset of the insult can be tightly regulated in time and space; second, the epochs of demyelination and remyelination are largely separate, allowing for the characterization of molecular cues regulating each aspect of lesion generation and repair.

The most common model utilizes the generation of focal areas of demyelination induced by direct injection of chemicals that selectively ablate oligodendrocytes and their myelin. Many different demyelinating agents have been used, although the most common include lysolecithin, ethidium bromide, and antibodies against the major sphingolipid component of myelin, galactocerebroside.

Lysolecithin (L- α -Lysophosphatidylcholine or LPC) when injected into white matter as a 1% solution induces focal demyelination [56, 57]. Common locations for LPC-induced lesions include spinal cord white matter, the midline of the corpus callosum, and caudal cerebellar peduncle. Injection of LPC results in a rapid loss of myelin and oligodendrocytes. Compared to other models, LPC lacks absolute cellular specificity, and there is a reduction in astrocytes and some axonal loss in the lesion. One powerful feature of LPC lesions is their ability to recover. In general, demyelination occurs rapidly, and the lesion area is largely devoid of myelin 2–3 days after lesion generation. Oligodendrocyte precursor cells repopulate the lesion sites around 5 days and subsequently proliferate and differentiate into oligodendrocytes, with remyelination taking place between 7 and 14 days in rodents on average. The latter varies with the lesion site and animal age. By 30 days post-lesion, remyelination is essentially complete (**Figure 3**). These observations have led to the identification of several distinct molecular mechanisms, such as Notch and Wnt pathways, retinoid X receptor gamma signaling, growth factors such as hepatocyte growth factor and neuregulin, hormones including progesterone, cell cycle proteins such as cyclin-dependent kinases, chemokine receptors such as CXCR2, the NOGO receptor LINGO-1, and death receptor 6 (DR6) signaling. In white matter tracts containing large-caliber axons, the remyelinated axons have thinner myelin sheaths than the originals (**Figure 3**).

An alternative glial toxin, ethidium bromide results in cell loss due to its DNA-intercalating properties; therefore, all nucleated cells are affected in this model. Ethidium bromide is injected directly into white matter tracts, and the lesions tend to be larger than LPC lesions and have been utilized to assay the effects of age, sex, growth factors, and the role of microglia/macrophage activation on remyelination. As expected, ethidium bromide injections cause a more widespread loss of astrocytes, oligodendrocytes, and OPCs while sparing axons. This is followed by the influx of macrophages in and around the lesion and the development of reactive astrocytosis, which aims to seal off the lesion site [58]. In contrast to LPC-induced lesions, a significant amount of remyelination in ethidium bromide-induced lesions in the spinal cord is accomplished by Schwann cells. It was initially assumed that such Schwann cells were derived from peripheral nerves or spinal nerve roots adjacent to the lesion; however, fate mapping studies suggest that OPCs generate Schwann cells in the absence of astrocytes [59] raising the possibility that astrocytes regulate the fate of OPCs. Given the more widespread loss of neural cells, ethidium bromide lesions are less commonly used for the identification of remyelinating therapies.

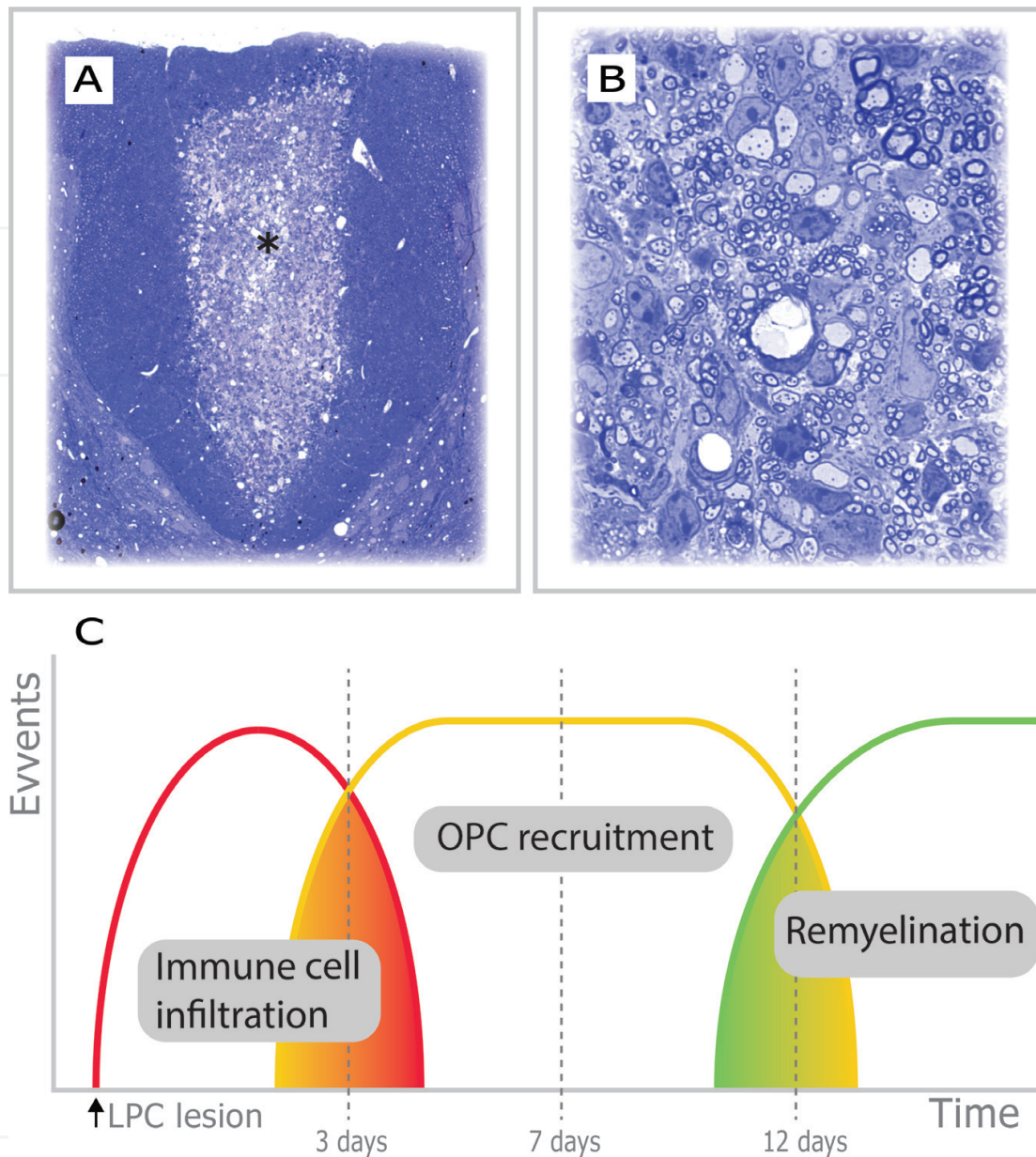


Figure 3. A) Representative image of dorsal spinal column cross section, stained with Toluidine blue, showing an LPC-induced demyelinating lesions denoted by the asterisk. B) Representative high magnification image of an LPC lesion during remyelination. C) Graph depicting typical disease progression in a characteristic LPC lesion; including immune cell infiltration around 3 days, followed by OPC recruitment peaking at 7 days, and then the onset of remyelination at approximately 12 days post injection.

To provide enhanced cellular specificity, cell type-specific surface antibodies have been used to target the complement cascade and induce selective cell lysis [60]. This model has been effective using antibodies to galactocerebroside (GalC), the major myelin sphingolipid to eliminate mature oligodendrocytes. Initial studies demonstrated that a single intraspinal injection of complement proteins plus anti-GalC resulted in demyelination and partial loss of oligodendrocytes. Analysis of the mechanism of myelin repair suggested that it was the result of recruitment of OPCs and not Schwann cells or mature oligodendrocytes [61].

A major strength of the local toxin models is that they provide a localized region of reproducible synchronized demyelination allowing for analysis of remyelination in the absence of concurrent demyelination. The timing of remyelination differs between the models, although all undergo spontaneous repair. Another advantage of using these models is their adaptability; lesions can be generated in animals of any age, at any accessible location, and from different genetic backgrounds. The disadvantage is that the mechanism of cell death is non-physiologic, and so whether this truly models naturally occurring lesion development, disease progression, and clinical phenotype is unclear. One particular aspect where these models have proven beneficial is the development of myelin-promoting therapies, as opposed to those modulating immune responses. For example, using an LPC-induced demyelination model, LINGO-1 was identified as a potential therapeutic target, whereby anti-LINGO-1 antibodies promoted OPC differentiation and subsequent remyelination [62, 63].

LINGO-1 knockout mice show precocious myelination, suggesting that LINGO-1 antagonists might be useful to accelerate myelin repair. Using both the LPC and cuprizone models (see below) of demyelination, anti-LINGO-1 antibody treatments significantly increase the speed of remyelination, suggesting a new therapeutic option for MS patients. The anti-LINGO-1 Li81 antibody is the first MS therapy directly targeting remyelination and is currently in MS clinical trials.

A second commonly used approach for glial toxin-induced demyelination is systemic oral delivery of toxins that preferentially target oligodendrocytes. Systemic delivery of a glial toxin in a noninvasive manner has a number of advantages. For example, it overcomes the complexity associated with direct injections into the CNS and provides a larger demyelinating area allowing for easier molecular analysis. The most frequently utilized systemic toxin is cuprizone.

Ingestion of the copper chelator cuprizone (biscyclohexanone oxaldihydrazone) results in demyelination of specific brain regions, which is thought to reflect mitochondrial stress and an innate immune response [64]. Cuprizone-induced demyelination results from loss of oligodendrocytes rather than direct insults to myelin sheaths, and mice aged 6–9 weeks given 0.2–0.3% cuprizone treatment for 5–6 weeks develop acute demyelination of the corpus callosum and other rostral white matter regions. Interestingly, the spinal cord is less susceptible, which could be in part due to a differential sensitivity by spinal oligodendrocytes to cuprizone, and/or nonuniform penetration in different CNS tissues. Oligodendrocyte apoptosis is also associated with extensive reactive astrogliosis and microglial activation. Acute demyelination is followed by spontaneous remyelination that occurs following removal of cuprizone from the diet. When cuprizone treatment is prolonged to 12 weeks or longer, remyelination is very sparse, resulting in a model of chronic demyelination.

The extended time course of disease induction and repair makes the cuprizone model useful for studying the biological processes related to both demyelination and remyelination in the CNS. The cuprizone model has been extensively used to examine the potential of various compounds to stimulate myelin repair [65, 66]. Because the time course of cuprizone treatment is so long, demyelination is progressive and remyelination begins while demyelination is still taking place. Combining cuprizone with rapamycin, which blocks mTor signaling,

decreases the efficiency of remyelination, making it easier to analyze and quantify repair processes. The cuprizone model is easier to use compared to other models in that the toxin is included in regular mouse chow that is fed to the animals each day. There are, however, a number of concerns with this model. First, cuprizone is generally limited to mice, and there is a clear genetic linkage to the susceptibility for cuprizone toxicity. Likewise, there are differences in susceptibility between gender and age that are poorly understood [67]; however, proof-of-principle studies demonstrate that signals known from *in vitro* studies to stimulate oligodendrocyte differentiation such as thyroid hormone (T3) promote remyelination in the cuprizone model, making it useful for therapeutic discovery.

2.3. Cell death models of demyelination

A number of studies have begun to suggest that demyelination may be a primary result of oligodendrocyte death, with activation of the immune system as a secondary event. Whether in the complex setting of disease damage to oligodendrocytes is direct or indirect likely depends on the immediate pathological conditions. An alternative cellular target that may trigger oligodendrocyte damage and demyelination is myelinated axons. Axonal damage and loss are frequently seen in MS lesions [36] and models of immune-mediated demyelination, although it is unclear whether axonal degeneration follows myelin loss or whether demyelination is a consequence of axonal degeneration. To distinguish between these possibilities, animal models in which oligodendrocytes are directly targeted for cell death are being developed to assess whether the loss of oligodendrocytes results in demyelination, how effectively and rapidly remyelination occurs, and whether localized demyelination results in axonal damage. Information from such studies will help define new mechanisms of CNS pathology and novel targets for therapeutic intervention. Currently, there are three major ways for selectively inducing oligodendrocyte cell death in the adult vertebrate CNS.

One approach to drive selective death of neural cells involves the selective expression of a toxic molecule targeted to specific cell types [68]. For example, extensive loss of oligodendrocytes has been achieved through the targeted expression of the alpha subunit of the diphtheria toxin (DT). Diphtheria toxin (DT) is composed of two subunits (alpha and beta), each having different functions. The beta subunit interacts with cell receptors to facilitate the entry of the toxin into the cell, whereas the alpha subunit is the cytotoxic component that acts intracellularly.

The cytotoxicity of DT results from inhibition of protein translation and cell death. In the absence of its beta subunit, DT is unable to penetrate cells, limiting the nonspecific induction of cell death in neighboring cells. Targeting the expression of the DT to oligodendrocytes is achieved using Cre/LoxP technology using a major myelin protein promoter, and its activation is through tamoxifen-induced removal of transcriptional stop sequences resulting in death of oligodendrocytes. One interesting finding from these studies is that extensive loss of oligodendrocyte cell bodies is not correlated with rapid myelin loss. After a post-treatment delay of approximately 3 weeks, the mice displayed progressive motor deficits associated with significant myelin degradation and vacuolization. A second unexpected outcome of these studies was that the widespread loss of oligodendrocytes did not trigger a rapid immune response.

While remyelination was extensive, and the animals appeared to recover completely with longer survival times, recovery was compromised and there was an infiltration of T cells into the CNS. Adoptive transplantation of these T cells into naïve hosts was sufficient to transfer disease. It is likely that the initial insult served to prime the immune system, which eventually led to an autoimmune response and subsequent CNS demyelination [69].

The DT model also differs from MS in a number of key ways. As discussed above, MS is a spontaneous disease, and the lesions develop in a variable manner in both time and space. MS is also not toxin-induced, although there might be a role for pathogens in initial disease stages. The cell death model, on the other hand, depends on the use of a toxin that effectively terminates protein translation, causing cell ablation, and subsequent recruitment of phagocytic cells. Another key difference between the two is that the clearance of myelin in MS following oligodendrocyte loss is rather rapid and is driven by both resident and peripheral immune cells. In contrast, myelin clearance is clearly delayed in the DT model, which would indicate that it is either inhibited or nonexistent. A major concern for the DT model is the complete nature of oligodendrocyte loss, which differs significantly from the focal loss of oligodendrocytes in MS.

In a related model, the specificity of the toxic insult is targeted through receptor expression in a null background [70]. For example, expression of the DT receptor (DTR) under the control of an oligodendrocyte-specific promoter results in cell type sensitivity to diphtheria toxin. Exposure to DT results in the induction of cell death by inhibiting protein synthesis. The clinical phenotype includes ataxia, limb paralysis, and tail spasticity that appear around 10 days post-injection and progressively develop. Perturbations in somatosensory evoked potentials together with histological markers of neurodegeneration, and abnormal Nodes of Ranvier indicate dysfunctional neural networks. The pathology differs between the models; while the DT mice display severe demyelination, the DTR mice show little demyelination. This may reflect that in the DTR model, there is a more extensive engagement of axonal damage leading to death before demyelination develops.

A potential strength of the DTR model is that it may provide a model system to examine the mechanisms and develop targeted therapies against axonal damage in demyelinating diseases since axonopathy is a frequent pathological finding in MS.

During CNS development, many cell types including oligodendrocytes are produced in excess and the additional cells are eliminated through apoptosis-mediated cell death. Cell type-specific induction of apoptosis through activation of an inducible caspase 9 construct driven off a selective promoter has been used to specifically eliminate lymphocytes and oligodendrocytes [71]. Induction of oligodendrocyte apoptosis in the adult CNS results in rapid demyelination and local activation of microglia in the absence of T cell infiltration [72, 73]. During development, activation of oligodendrocyte apoptosis in the first postnatal week inhibits myelination, which subsequently recovers but has increased susceptibility to adult insults [72]. The role of oligodendrocyte apoptosis in early stages of MS is not well defined; however, apoptotic oligodendrocytes have been reported in the early lesions [74], suggesting this may contribute to MS plaque formation. Similarly, activated microglia but an absence of peripheral immune cells has been described in some early lesions.

Overall, while models of selective oligodendrocyte death have provided important insights into the response of the neural cells and the pathway of myelin loss, they have not yet been used to identify new pathways of pathology or illuminate new targets for therapeutic interventions. Whether they will provide a useful platform for the development of therapies for distinct subsets of MS awaits further refinement and analysis.

2.4. In vitro discovery platforms for therapeutic development

Over the past decade, there has been significant development of new platforms for remyelination drug discovery. These include the use of isolated purified cell preparations, rodent IPS cells that provide an unlimited supply of cells, human cell line-derived neural cells, human IPS cells, and in silico model systems. Each of these platforms has its own advantages and disadvantages. In general, such in vitro approaches have been relatively powerful in identifying pathways that regulate myelin formation from mature oligodendrocytes but have been less effective at identifying signaling pathways that regulate the proliferation and survival of oligodendrocytes and their precursors.

With the development of culture models for CNS neural cells and the ability to unambiguously identify distinct cell populations, the ability to identify molecular signaling that promoted the development of oligodendrocytes was feasible. Early studies utilized mixed cultures derived from either white matter such as the optic nerve, mixed gray and white matter such as the spinal cord or predominantly grey matter such as cerebral cortex. Addition of selected growth factors or other signaling molecules that resulted in an increase in mature oligodendrocytes was considered potential therapy. There are two major concerns with this approach. First, the cellular target(s) of the added molecules is unclear, since the culture contains not only cells of the oligodendrocyte lineage but also astrocytes, neurons, and innate immune cells of the CNS, any of which might mediate the response. The second concern is that increased numbers of mature oligodendrocyte may result from either enhanced progenitor proliferation, reduced cell death, or increased cell differentiation, and distinguishing between these mechanisms has proven challenging. To refine the cellular target(s) of potential therapeutics, purified cell cultures have been utilized. Purification of rodent or murine OPCs either through differential antibody binding (panning) or FACS sorting allows for assessment of the direct response of the cell population to therapeutic exposure. Such approaches have been used recently to identify signaling mechanisms that promote the appearance of mature oligodendrocytes [75–78]. One concept that has gained significant support in recent years is the notion that the rate-limiting step in remyelination is the differentiation and maturation of oligodendrocytes to myelinating cells. Several more refined approaches have been developed to identify factors that directly regulate oligodendrocyte maturation. These include the use of purified OPCs initially derived from human material. The emergence of IPS technology combined with identification of molecular environments that promote the survival of human cells has facilitated the identification of several small molecules that mediate oligodendrocyte maturation such as retinoic receptors, benzotropine and miconazole. In the majority of such screens, the readout has been enhanced by expression of myelin proteins such as MBP. While this has proven useful, the ultimate goal of remyelinating therapies is the generation of new

myelin. Recent studies have used a biophysical approach to identify signals that promote the formation of myelin on artificial substrates. When grown in the presence of inert fibers of the appropriate dimensions, oligodendrocytes will begin to enwrap them as if they were immature axons. Molecules that enhance that process are considered strong candidate to promote remyelination in the CNS, and molecules including Clemastine an anti-histamine drug have been identified in similar assays.

While the reductionist approaches provide important insights into isolated cellular responses of the oligodendrocyte lineage, they lack any physiological setting. As a result, it is unclear whether signals that modulate oligodendrocyte maturation in isolation will promote myelin repair in the developing or diseased CNS. One model to address this concern is the use of slice cultures. Slices of the CNS grown on the air/medium interface develop robust myelination. The most successful slices are those derived from cerebellum and coronal sections through the corpus callosum. Treatment of such slices with LPC results in rapid demyelination and allows for analysis of drug-induced repair in an efficient and physiological environment. In most studies, multiple different models are used to determine the efficacy individual compounds to promote remyelination.

3. Conclusions and comments

There is a broad range of animal models that address distinct aspects of multiple sclerosis and other demyelinating diseases. Each of the models has specific strengths and weaknesses in furthering our understanding of the pathogenic processes that mediate demyelination and in identifying new opportunities for the effective promotion of myelin repair. EAE models have led to the development of many therapeutic targets aimed at halting disease progression. More recently, other models such as those targeting oligodendrocyte cell death have been instrumental in fine-tuning our understanding of the pathology of demyelination/remyelination in MS and other similar diseases. Each of the model systems discussed in this review deserves particular credit, as it has helped solve a different piece of the puzzle. For example, while EAE models have unraveled many of the immunological bases of the CNS demyelination, particularly the role of T cells in MS, the use of glial toxins such as LPC or ethidium bromide has emerged as extremely useful in reshaping our understanding of the environmental and cell-based mechanisms of remyelination, and the models of oligodendrocyte death provide insights into factors driving the pathology. It seems likely that new models will be forthcoming that more effectively address the role of cells other than those of the immune and oligodendrocyte lineage. Understanding the role of microglia and astrocytes, as well as further clarity around the mechanism of vascular components in disease progression, will allow new therapeutic avenues to be developed in future studies.

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Author details

Robert H. Miller^{1*}, Molly Karl¹, Reshmi Tognatta², Ahdeah Pajooresh-Ganji¹ and Mohammad Abu-Rub¹

*Address all correspondence to: rhm3@gwu.edu

¹ School of Medicine and Health Sciences, George Washington University, Washington, DC, USA

² Gladstone Institute, San Francisco, CA, USA

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