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The Power and Pitfalls of Strategies for Selective Ablation of Oligodendrocytes in the Vertebrate CNS

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Abstract

Normal functioning of the mammalian Central Nervous System (CNS) depends on a high degree of interaction between its constitutive cell types. Although neurons represent the fundamental units of information processing in the CNS, a number of different glial cell types dynamically coordinate and modulate the final neuronal outputs. As a result, it is difficult to identify the relative contributions of individual cell types in the execution of complex CNS functions. Novel experimental tools for eliminating selected populations of glial cells are shedding light on the mechanisms underlying complex cell-to-cell interactions that influence the ultimate functionality of the brain and spinal cord. In particular, *in vivo* models of targeted cell death are making it possible to determine changes in natural phenomena in the absence of distinct subsets of glial cells, providing clues about the roles of those cells in neurophysiology. Distinct but complementary models of oligodendrocyte ablation, the topic of this review, are not only uncovering novel physiological roles for this subclass of glia in maintaining tissue homeostasis but also in the pathogenesis of demyelinating diseases. The goal of the current review is two-fold: to discuss various strategies developed to ablate populations of oligodendrocytes from awake, behaving animals and to identify the key contributions as well as the limitations of each model moving forward. Topics discussed include 1) whether primary oligodendrocyte death is sufficient to drive an autoimmune response that resembles multiple sclerosis, 2) the concept of a homeostatic mechanism for regulating the number of myelinating oligodendrocytes in the adult CNS, and 3) the copper-dependency of oligodendrocytes and its potential implications for demyelinating diseases. In the future, analyses of the data being generated both between and within these various animal models will be key to discovering novel roles for oligodendrocytes in both normal physiology and disease.

Keywords: Central Nervous System, Diptheria Toxin, Inducible Caspase 9

Introduction

Early neuroanatomists conceived glial cells as merely a layer of connective tissue that provided structure to the more active, neuronal elements of the Central Nervous System (CNS). Accordingly, they were termed 'glia'—Greek for "glue". That glia were actually bona fide populations of cells was understood by the late 1800s but it wasn't until the mid-to-late 1970s that potential roles for glial cells in fundamental neurophysiological processes began to be revealed. Three major classes of CNS glia are now recognized: Oligodendrocytes whose primary role in the myelination of CNS axons is well established [1-3], astrocytes, whose primary role in maintenance of the extracellular ionic environment and formation of the blood brain barrier is well accepted, and Microglial cells that, although derived from the hematopoietic system have macrophage-like properties in the CNS.

Recent studies provide compelling evidence that the various subtypes of neural glia provide more than a supportive function in the CNS. Studies of mice in which mutations of various oligodendrocyte proteins underlie severe axonopathies in the absence of overt myelinopathies demonstrate that these myelin-forming glial cells are important not only for increasing the efficiency of neuronal signaling by way of myelin ensheathment but for long term axonal health and survival [4-7] by mechanisms that are currently under investigation. Astrocytes, among other functions, respond to synaptically released neurotransmitters with calcium-mediated exocytosis of gliotransmitters that modulate the final output of brain circuits in measurable and long-lasting ways. Such signaling is thought to play important roles in fundamental physiological processes such as sleep regulation and maintenance of basal inhibitory tone [8-10]. Microglia, in addition to their role in immune surveillance and defense against foreign pathogens, are turning out to be important for sculpting developmental neural circuits including, and probably not limited to, in the retinogeniculate system [11].

At the heart of these insights lie a number of advances in cell and molecular techniques. Among these, increasingly sophisticated methods for inducing targeted and controlled cell death in defined populations of glial cells are emerging as important tools to interrogate the functionality of distinct cohorts of glia. Changes in natural phenomena in behaving organisms in the absence of a particular glial cell type provide important clues about the function of each subclass of glial cells in neurophysiology. This approach has proven particularly successful in the study of myelin-producing oligodendrocytes. The attention being paid to oligodendrocyte ablation methodologies is in part due to its clinical relevance, considering the degradation and dysfunction of myelin observed in patients with demyelinating diseases such as Multiple Sclerosis [4] as well as in congenital, dysmyelinating diseases such as the leukodystrophies. In particular, models of experimentally induced oligodendrocyte death allow direct testing of the emerging possibility that dying oligodendrocytes may play a role in triggering the pathogenesis of MS. Whether or not this theory is correct remains to be seen but the necessary tools for testing such hypotheses now exist, as illustrated in the first section of this review.

In vivo modeling of oligodendrocyte ablation and demyelination is not a new topic. Various gliotoxin-based models, one of which is described in more detail in the third section of this review, have provided a knowledge base that can be further sculpted and refined using novel genetic tools that feature greater cell specificity and a mechanism of cell death that is well understood. These important

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advances allow for rigorous testing of old ideas and the generation of new ones. The purpose of this review is to provide an overview of the current strategies for achieving targeted oligodendrocyte ablation, outlining the relative strengths and weaknesses inherent in each model as they pertain to the discovery of novel functions of oligodendrocytes and to the genesis of MS and possibly other neurodegenerative diseases.

DT: Anti-CNS Immunity?

One tool for achieving targeted glial cell ablation involves the use of Diptheria Toxin (DT). The cytotoxicity of DT is conferred through its inhibition of elongation factor 2 and the subsequent halting of protein translation, which ultimately leads to death of the infected cell [12]. In its natural state, DT is composed of two subunits that execute distinct functions. Whereas the beta subunit interacting with the DT receptor is essential for the entry of the toxin into the cell, the alpha subunit (DT-a) is the cytotoxic component that acts intracellularly but is unable to cross cell membranes. Cell type-specific expression of the isolated alpha subunit is therefore sufficient to minimize non-specific induction of cell death in neighboring cells.

The specificity of the DT system for ablating glial cells comes from Cre/LoxP technology involving two distinct but complementary approaches. In a double-transgenic mouse model, an oligodendrocyte lineage-restricted promoter sequence (e.g. PLP or MBP) drives the expression of a tamoxifen-inducible Cre recombinase. Upon induction with tamoxifen, Cre excises a STOP signal designed to suppress uncontrolled expression of cytotoxic DT-a, resulting in the inducible expression of DT-a only in Cre-expressing oligodendrocytes (Figure 1A). A similar yet distinct model exists involving a single-transgenic mouse in which an oligodendrocyte-restricted Cre recombinase induces cell-specific expression of the DT Receptor (DTR), rendering an otherwise naturally DT-resistant mouse vulnerable to intraperitoneally injected DT (Figure 1B).

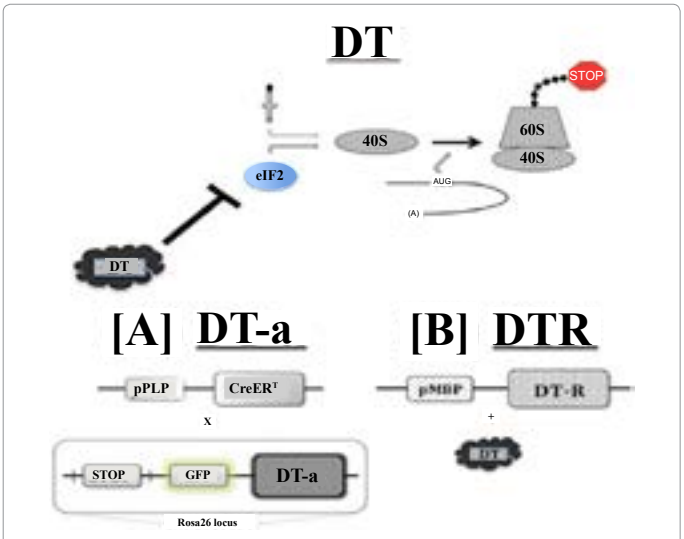

Figure 1: The Diptheria Toxin (DT) system is a genetic tool for ablating myelinating oligodendrocytes with the goal of understanding the role of these cells in neurophysiology and in various neurological diseases. (A) The cytotoxicity of DTresults from its inhibition of elongation initiation factor 2 (eIF2) which in turn suppresses protein translation. Oligodendrocyte specificity is achieved in one of two ways. In one case (A), an oligodendrocyte-restricted promoter sequence (e.g. pPLP) directs the expression of an inducible Cre recombinase, which upon experimental activation facilitates the expression of the alpha subunit of DT (DT-a) in addition to reporter GFP. The alpha subunit on its own is unable to cross cell membranes and therefore impacts only the cell in which it was induced. In a similar but distinct strategy (B), an oligodendrocyte-restricted promoter sequence (pMBP in this case) drives the expression of a transgenic DT receptor (DTR) in a mouse otherwise resistant to DT, such that treatment with exogenous DT affects only those cells expressing the cognate receptor.

Models of inducible oligodendrocyte death provide a platform upon which to test specific hypotheses regarding MS pathogenesis and progression. The pathogenesis of MS remains unknown and is relatively controversial [13-16]. Historically, MS has been presumed to be a disease of the immune system, in which misdirected, peripheral T-cells erroneously attack and destroy CNS myelin made by oligodendrocytes. Studies of the earliest detected MS lesions and in normal appearing white matter in a subset of patients, however, report the presence of dead or dying oligodendrocytes and myelin deterioration in the absence of peripheral immune cells. In these particular cases, damage to myelin sheaths or oligodendrocytes precedes the influx of cytotoxic T cells. Locatelli et al. utilized the oligodendrocyte-DTR model to ask whether primary oligodendrocyte death on its own was sufficient to initiate an autoimmune response against CNS myelin antigens in a manner resembling MS [17]. The authors initiated oligodendrocyte death by systemic injection of DT to eliminate DTR-expressing oligodendrocytes. They then examined various brain regions for signs of MS-like pathologies, particularly evidence of auto-reactive T-cells that might exacerbate the damage over time as occurs in MS. The authors examined animals beginning at one week after induction of oligodendrocyte death and up to six weeks later beyond which animal mortality predominated. Oligodendrocyte death was confirmed by immunolabelling with cell type-specific antibodies and a decrease in the mRNA of a number of oligodendrocyte-specific proteins. Significant myelin defects were apparent by three weeks and were manifested by delayed-onset neurological symptoms including paralysis and death. Myelin debris was found collecting in CNS-draining cervical lymph nodes.

Notwithstanding these changes, there was no evidence at any stage of pre-primed, auto-reactive T-cells in the brain as seen in MS, which could be explained neither by T-cell tolerance nor by disruption of regulatory T-cells. Even under some of the most biased pro-inflammatory conditions, there appeared to be no evidence that primary loss of oligodendrocytes generated an autoimmune response similar to what is seen in MS. It is unclear why widespread induction of oligodendrocyte death did not provide an immune response but one possibility is that the initial autoimmune trigger is exposed very early after oligodendrocyte death and the authors simply missed the relevant time window. In defense of this possibility, a recent publication using a different approach (discussed in more detail in the next section) documents rapid demyelination and clearance of myelin debris a mere 24 hours after apoptosis of approximately 25% of oligodendrocytes in the rat corpus callosum [18]. Another possibility is that the nature of cell death and the associated downstream signaling cascades are the critical determinants of resultant pathologies, including anti CNS-immunity. If an anti-myelin T-cell response requires the synthesis of de novo signaling molecules, then one might not expect to see such a response if DT is inhibiting the protein synthetic pathway. It is increasingly clear, for instance, that there are specific "find-me" and "eat-me" signals that are generated for clearing apoptotic cells [19,20] and that defects in apoptotic clearance have been associated with autoimmune diseases in a number of different organs [21,22]. Therefore the lack of anti-CNS immunity in the DTR mouse could reflect the absence of new proteins necessary for such a response. Although the findings in this particular DTR study argue against a pathogenetic mechanism of MS involving primary degeneration of oligodendroglia, side-by-side comparison of the results with other models of oligodendrocyte ablation will be necessary before a final verdict can be reached.

A notable limitation of the DT model is inconsistent findings across research groups. Whereas Locatelli et al. published data showing significant myelin loss, another group using the same DTR mouse claimed no evidence of overt demyelination despite notable damage to axons [23]. Results generated in experiments involving the DT-a mouse are equally mixed, with some groups reporting axon preservation despite significant myelin loss and decreased motor and sensory performance [24] whereas others identified axon loss secondary to significant myelin loss [25]. Whether the disparate research findings between and within DT models reflect differences in age, sex, strain, or otherwise remains uncertain but these data highlight the complexity of the induction of CNS demyelinating pathologies. Future studies should elucidate the circumstances or conditions favoring certain pathologies over others. Given the heterogeneity across MS patients with regard to clinical disease course, underlying histological defects, and response to treatment, such information will be extremely useful from a clinical perspective.

iCP9: Lineage-Specific Homeostatic Control of Cell Numbers

A recently developed tool for ablating myelinating oligodendrocytes involves a system of experimentally induced apoptosis. In this model, an oligodendrocyte-specific promoter sequence drives the expression of a transgenic Caspase-9 protein (Figure 2A) that has been modified to activate exclusively in response to a synthetic compound termed CID, for chemical inducer of dimerization [26]. It is based on the notion that endogenous Caspase-9 is activated by an induced proximity mechanism [27-30] in which the dimerization and auto-activation of Caspase-9 is necessary and sufficient to initiate cell apoptosis. Accordingly, the CID is a membrane-permeable, non-toxic molecule that binds with high affinity to the transgenic Caspase-9 (iCP9) and in doing so, causes dimerization and activation of downstream effector molecules (Figure 2B). Chemically induced dimerization in cells expressing iCP9 is sufficient to initiate cell apoptosis.

The iCP9 approach offers certain advantages over other models of oligodendrocyte ablation. Foremost among these is the fact that the mechanism of death is physiological, well understood, and disturbances in this pathway are tied to autoimmune disease [31-36]. Cell apoptosis is a well-conserved, genetically encoded form of controlled cell dismantling that occurs when either the cell is no longer needed or has undergone damage that poses a threat to survival of the organism [37]. Importantly, there is not an inflammatory response associated with this mode of cell death [38], which eliminates a potentially confounding variable when induced experimentally. The iCP9 system represents a relatively straightforward method for characterizing the effects of oligodendrocytes death in living tissues.

An experimental model of inducible apoptosis holds great promise for elucidating mechanisms of homeostatic control of oligodendrocyte cell number either during development or in the adult. Lineage-tracing studies have shown that >20% of all oligodendrocytes in the adult mouse corpus callosum are generated after 7 weeks of age and are derived from brain-resident, mitotic, stem-like cells characterized by the cell-surface proteoglycan NG2 [39]. Although the cell cycle time becomes prolonged with mouse age, the vast majority of NG2 cells are actively diving throughout life [40,41]. This suggests a degree of cell turnover that was previously unappreciated in oligodendrocyte-lineage cells.

A study examining the effects of CID-induced oligodendrocyte apoptosis in the adult rat corpus callosum demonstrated spatially

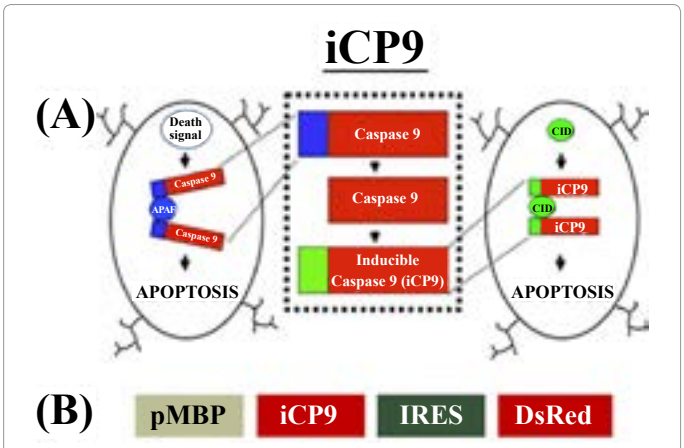

Figure 2: Inducible Caspase 9 (iCP9), a modified version of the endogenous, pro-death Caspase 9, enables experimental control of the cell's apoptotic program. (A) Activation of iCP9 is controlled in space and time by separate administration of a synthetic compound (termed 'chemical inducer of dimerization' or CID), which was designed to activate iCP9 and ultimately the apoptotic death cascade. CID is a small, hydrophobic FK506 analog that crosses cell membranes and binds to the FKBP domain (green) fused to the catalytic domain of Caspase 9 (red). (B) As with other genetic models, an oligodendrocyte-specific promoter such as myelin basic protein (pMBP) confers specificity of programmed cell death.

restricted proliferation of oligodendrocyte progenitors from the Sub Ventricular Zone (SVZ) following induced oligodendrocyte apoptosis [18]. This contrasts with what Rivers et al. showed in fate-mapping experiments using Pdgfra-creER^{T2}/R26-YFP double-transgenic mice in which oligodendrocytes in the adult corpus callosum were replaced not by cells originating in the SVZ but by pre-existing parenchymal NG2^{+} cells that were actively dividing. Future work should aim to reconcile the differences between the two studies. It could be that there are two distinct pools of progenitors cells—one for regular maintenance and a separate one for repair—such that inducing apoptosis in cells that otherwise would be spared stimulates the system to draw from a different pool of progenitors than would occur under baseline tissue sculpting and maintenance. Along similar lines, it may be that the needle trauma from injecting the lentivirus weeks before the CID somehow conditioned or primed the tissue for an abnormally rapid injury response and in the process recruited replacement cells from a pool of SVZ-derived progenitors rather than resident NG2 cells. Whatever the ultimate outcome, the iCP9 model stands to provoke further discussion surrounding the number and source of new oligodendrocytes in the adult CNS.

Cuprizone: Linking Animal Findings To Human Myelinopathies

In contrast to both the DT and iCP9 models of oligodendrocyte ablation, the cuprizone model of oligodendrocyte ablation and demyelination is well characterized and has been extensively used since its inception in the late 1960s. Cuprizone is a copper-chelating agent that induces oligodendrocyte death by mechanisms not entirely understood. Both cell- and non-cell-autonomous mechanisms have been suggested [42]. Towards the latter, profound effects of cuprizone on cell mitochondrial enlargement and dysfunction in the liver have been reported, owing to significant matrix expansion and a reduction in key mitochondrial enzymes such as monoamine oxidase and cytochrome oxidase [43]. If this is the predominant mechanism then demyelination in the brain could be secondary to liver damage, especially given the pathological link between the liver and brain such as in Wilson's disease

		Myelin Loss?	Axon Loss?	Microglia Activation?	Peripheral Immune Response?	Anti-CNS Immunity?	Progressive Disease phenotype?	References
OL DEATH MODEL	DTR	Y/N	Y/N	Y	N	N	Y	[1,2]
	DT-a	Y	N	Y	N	?	Y	[3,4]
	iCP9	Y	N	Y	N	?	N	[5]
	Cuprizone	Y	N	Y	N	?	Y	[6-10]

Table 1: A comparison of the development of pathologies and potential outcomes in each model is likely to shed light on both novel roles for oligodendrocytes in neurophysiology as well as in the pathogenesis of demyelinating diseases.

[44,45]. Cell autonomous explanations for the demyelinating effects of cuprizone include reductions in the enzyme carbonic anhydrase II, important in brain pH regulation, that may generate noxious fluxes in pH and selectively render the oligodendrocytes susceptible to cell death [46-48]. Whether or not copper chelation per se is responsible for primary oligodendrocyte death also remains subject to debate. In support of this possibility, there are clinical reports of copper deficiencies accompanied by extensive CNS demyelination (described below), although a causal link remains as yet unproven. Further, simple copper deficiencies do not necessarily generate the same pathological sequelae as observed in cuprizone treatment, arguing against the stereotyped pathologies being explained by copper chelation alone.

Nevertheless, the clinical literature contains several provocative cases in which copper deficiencies are accompanied by myelin damage. X-linked Menkes Disease, a neurodevelopmental disease that primarily affects male infants, is caused by a dysregulation of copper metabolism in the body, which in turn causes deficiencies in key enzymes such as cytochrome oxidase and dopamine beta-hydroxylase [49]. Myelin loss is one of several pathological findings, which also includes grey matter degeneration, ventriculomegaly, tortuous intracranial vasculature, and epipletogenesis [50,51]. Given the diversity of perturbed cellular processes together with the complexity of neural development, it is difficult to infer a causal relationship between copper deficiency and demyelination in Menkes Disease. It is worth noting, however, that early diagnosis and treatment of affected infants with copper injection treatment and/or gene therapy to replace a defective copper transporter are demonstrating higher child survival rates and improved neurodevelopment [52,53]. Furthermore, it has been shown that copper ions potently desensitize NMDA receptor currents [54,55] and that these receptors are expressed on oligodendrocytes [56] as well as neurons. It could be that copper chelation results in toxic calcium overloads in myelinating oligodendrocytes, resulting in myelin destruction.

The picture is more straightforward in case reports of adults in their mid-40s who present with typical hematologic manifestations of copper deficiencies accompanied by a demyelinating process of the CNS. Patients present with neurological symptoms including paresthesiae of the upper and lower limbs, hyperactive reflexes, and ataxic gait [57,58]. MRI images indicate multifocal lesions of myelin loss either in the brain or spinal cord, although rarely both tissues at once. These case studies lend further credence to the use of a copper chelator for experimental modeling of oligodendrocyte loss and demyelination.

There are two cuprizone treatment paradigms described in the literature. A so-called acute demyelination regimen involves feeding cuprizone to mice for six weeks after which they are switched to normal chow. The goal in this case is to model reversible demyelination with predictable repair and remyelination. On the other hand, a paradigm of chronic demyelination involves feeding mice cuprizone for an additional six weeks on top of the acute six-week period. In this case, myelin loss is thought to be chronic given that the repair process is delayed if not entirely impaired. Chronic progressive neurodegeneration turns out not to be restricted to the chronic treatment regimen. Mice analyzed between 6 and 20 weeks following the withdrawal of six-week cuprizone treatment demonstrated evidence of a progressive motor dysfunction with underlying axonopathies in callosal axons [59], even in axons with an intact myelin sheath. Such a finding underscores the bi-directionality of the axo-glial relationship and suggests a rethinking of the assumptions made when interpreting data from either cuprizone treatment paradigm.

A major limitation when using Cuprizone, as when analyzing patient data is the potentially correlative relationship between copper deficiency and demyelination. A potential link between the two does not establish causality. That the mechanism of cell death following Cuprizone treatment remains unknown limits its utility moving forward since it is unclear if regimes developed to enhance recovery in this model will be applicable to MS. Until knowledge of the role of copper in the pathogenesis of demyelination emerges, interpretation of data generated in this model, at least compared to the more specific, genetic models of oligodendrocyte ablation, remains precarious.

Conclusions and Future Directions

The output of the central nervous system is dictated by complex interactions between its constituent cells; specifically between neurons and glial cells including oligodendrocytes. A number of emerging technologies are shedding light on the details of these interactions, building on the knowledge previously generated in better characterized, if less specific models. Oligodendrocyte-specific expression of Diptheria Toxin, for example, can address the role of oligodendrocyte death in the development of myelin autoimmunity in ways that the Cuprizone model, with its off-target effects on peripheral organs and other copper-dependent cells, cannot. These data, in turn, are supplemented by data using the iCP9 model of inducible oligodendrocyte apoptosis in which cell death by a genetically encoded program is induced. Although each of these models come with its own set of caveats and limitations, a side-by-side comparison (Table 1) of the development of pathologies and potential outcomes in each model is likely to shed light on both novel roles for oligodendrocytes in neurophysiology as well as in the pathogenesis of demyelinating diseases.

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