Forum

Problems and Pitfalls of Identifying Remyelination in Multiple Sclerosis

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Regenerative medicines that promote remyelination in multiple sclerosis (MS) are making the transition from laboratory to clinical trials. While animal models provide the experimental flexibility to analyze mechanisms of remyelination, here we discuss the challenges in understanding where and how remyelination occurs in MS.

The modern era has seen a burgeoning interest in stem cells and their potential to transform regenerative medicine. The regeneration of new myelin sheaths (remyelination) in demyelinating-neurodegenerative diseases such as multiple sclerosis (MS) has emerged as one of the more promising directions for therapeutic intervention. Much of this optimism stems from an increased understanding of the process of remyelination and the identification of an abundant progenitor cell population, commonly referred to as oligodendrocyte progenitor cells or OPCs, in the adult mammalian CNS capable of generating new oligodendrocytes in experimental models of demyelination (Zawadzka et al., 2010). Based on these findings, most current research aimed at developing remyelination medicines has involved finding ways to pharmacologically induce OPCs to differentiate into oligodendrocytes (Deshmukh et al., 2013).

While it is now beyond doubt that in animal models of demyelination/remyelination new myelin sheaths can be made of oligodendrocytes newly generated from adult OPCs, there is also evidence from animal models that new myelin sheaths can be generated by existing oligodendrocytes without the need for de novo oligodendrogligenesis (Duncan et al., 2018). But what do we know of the generation of new myelin sheaths in MS, where the dynamics of a regenerative process are considerably harder to infer? The possibility that myelin regeneration in MS can occur without the need for the generation of new oligodendrocytes has come from a study that takes advantage of fluctuations in atmospheric 14C levels. These peaked during the 1950s and 1960s (when the testing of atomic bombs was at its most prevalent) and have subsequently declined toward pre-testing levels, making it possible to “birth date” cells based on their 14C content. This approach indicates that there is a minimal degree of de novo generation of oligodendrocytes in healthy adult human white matter (Yeung et al., 2014), and, surprisingly, in areas of the MS brain where remyelination was thought to have occurred (Yeung et al., 2019). Thus, the proposed model is that mature oligodendrocytes, once shorn of their myelin sheath-bearing processes, can generate new myelin sheaths that survive within an MS lesion. The possibility that this mode of remyelination might also exist in humans is intriguing and certainly requires further investigation, not least for its therapeutic potential—and in this regard, studies that aim to foster oligodendrocyte survival in the face of acute demyelinating insult are sure to be of great importance.

However, in studies that aim to elucidate the nature of remyelination in human disease, a critical issue is whether remyelination (which by definition will have been preceded by primary demyelination) can be reliably identified. The uncertainties that continue to exist in the unambiguous identification of remyelination by MRI or other imaging approaches are well recognized and are discussed elsewhere (Petiet et al., 2019). Identifying remyelination in tissue sections is felt to be more straightforward, and it is widely accepted that an area where the intensity of myelin stains is lower than expected—referred to in human neuropathology as a "shadow plaque"—indicates remyelination. But how valid is this assumption? In this commentary, we argue that not all shadow plaques are necessarily areas of remyelination, and that not all areas of remyelination are necessarily shadow plaques, and we suggest that a reappraisal of the relationship between remyelination and the shadow plaque is urgently needed to avoid making unjustified inferences about the nature of remyelination in the human CNS.

Why Are Shadow Plaques Thought to Represent Areas of Remyelination?

The basis for identifying remyelination in post-mortem MS tissue derives from animal experiments where it is possible to unambiguously show first the loss of myelin from axons and second the reinvestment of the denuded axons with new myelin sheaths. Some of the earliest
experimental studies of remyelination were conducted using the cuprizone model, in which a systemic toxin that preferentially kills oligodendrocytes is fed to mice, and involved examination of the mouse cerebellar peduncles that contain relatively large-diameter axons (Blakemore, 1973). These studies revealed that a cardinal feature of remyelination is that the new myelin sheath is thinner than expected for the axonal diameter. This characteristic of remyelination is convenient because the identification of thin myelin sheaths (often expressed as an increase in the ratio of the diameter of the myelinated axons to the axon alone, also known as the g-ratio) provides a very reliable means of inferring that remyelination has taken place (notwithstanding that there may be other causes for alterations in g-ratios, such as axonal swelling or thinning of the sheath). Indeed, demonstration of thin myelin sheaths has become the gold standard for identifying sites of remyelination. This means that because there is less total myelin in a remyelinated area it will appear paler when stained with a myelin marker, such as Luxol fast blue or Eriochrome cyanine. On this basis, pale staining areas of white matter in MS tissue (often referred to as shadow plaques) are generally assumed to be areas of remyelination. However, ultrastructural verification of thin myelin sheaths within these areas has been very limited, given the inherent difficulties in obtaining human post-mortem samples that are sufficiently well-preserved in patients where lesions have been tracked over time using in vivo MRI.

Will All Areas of Remyelination Necessarily Appear as Shadow Plaques?

Subsequent studies in white matter areas containing smaller-diameter axons, such as the corpus callosum, revealed that the difference between the g-ratio of myelination and remyelination becomes much less clear the smaller the axon diameter (Stidworthy et al., 2003). This means that areas of remyelination are likely to look similar to surrounding intact white matter and are therefore very difficult to detect. Indeed, lesions detectable by MRI can subsequently appear normally myelinated when viewed by immunohistochemistry for myelin proteins (Trapp et al., 2018). A further factor that is likely to contribute to areas of remyelination being missed is that a significant degree of resolution (i.e., return to pre-lesion appearance) occurs in remyelinated areas over time. Although g-ratios may never return to normal, especially in areas containing large-diameter axons, there is some remodeling of the new myelin sheaths, and eventually the area of remyelination can very closely resemble the surrounding white matter. Figure 1 shows a lysolecithin-induced demyelinating lesion in the white matter of the adult mouse spinal cord ventral funiculus (an area containing many large-diameter axons) 150 days after induction. The lesion is nearly fully resolved, and it is a challenge to delineate where the remyelinated area ends and the normal white matter begins, even with optimal tissue fixation and processing that is rarely, if ever, achieved with human post-mortem tissue. Although this is a toxin model of demyelination whose cause is different from demyelination in MS, it illustrates why areas of remyelination may not resemble a classical shadow plaque and could easily be overlooked. Indeed, substantial generation of new oligodendrocytes was observed in so-called normal appearing white matter (NAWM) of some of the MS patients reported in the MS 14C dating study (Yeung et al., 2019). Specifically, in the NAWM in 10 out of 20 patients, the oligodendrocyte turnover was 20%–80%, which is significantly higher than the oligodendrocyte turnover in non-diseased tissue. Given that there is little oligodendrocyte exchange in healthy adult human white matter (Yeung et al., 2014), it is likely that these are areas of resolved OPC-mediated remyelination not easily distinguishable from NAWM.

Are All Shadow Plaques Areas of Remyelination?

Although thinner myelin sheaths may well result in paler myelin staining, there are several other processes that would have the same effect. These include any process that decreases axonal density, such as tissue expansion due to inflammatory infiltrates, edema, and astrocytosis following axonal loss (a well-recognized feature of both acute and chronic MS lesions and even of distant white matter via the process of Wallerian degeneration). Even a finding of thin myelin sheaths need not necessarily indicate that remyelination has taken place, as pathological axonal swelling can lead to a stretching and thinning of the original myelin sheath, which might in itself contribute to paler myelin staining. Thus, areas of myelin pallor described as shadow plaques may represent areas that have never undergone primary demyelination and therefore cannot be areas of remyelination.

What Can We Infer from Tissue Samples about Dynamic Biological Processes?

Modeling the dynamics of remyelination, independent of whether remyelination is OPC- or oligodendrocyte-derived, is difficult in MS tissue. Most importantly, it is usually unknown when a lesion occurred in MS patients. Dating the lesion age, regardless of when the disease first manifests clinically, is impossible to establish. Lesions may have occurred and resolved before the age of diagnosis, just as they may have occurred years, even decades, after diagnosis—especially in the era prior to highly effective disease-modifying therapy, which began in the mid-1990s. Moreover, in the 1950s and 1960s, a formal diagnosis of MS often occurred long after symptoms began, which is much more rarely the case today. The inability to define the lesion age makes the 14C dating of cells driving remyelination in MS lesions very challenging (an issue acknowledged in the study by Yeung et al., 2019). In this respect, it would be of interest to combine extended pre-mortem MRI follow-up with post-
mortem birth dating of oligodendrocytes in both lesions and NAWM (as has been done using primate models of MS). Being agnostic about the history of lesions is also problematic for approaches based on single-cell sequencing (Jäkel et al., 2019). A higher expression of myelin genes in a cell identified as a mature oligodendrocyte according to expression of key marker genes may indicate that a pre-existing oligodendrocyte engaged in remyelination. However, an increased expression of myelin genes is also a hallmark of newly formed oligodendrocytes that differentiated from OPCs. Pseudotime analysis is often used to circumvent this problem, and to establish a possible timeline of cellular fates. However, while this is a powerful tool to generate hypotheses about the dynamics of a biological process from a single snapshot in time, experimental validation is still needed to establish that distinct cellular states with functional heterogeneity exist and that differentiating cells actually follow the bioinformatically predicted trajectories.

Remyelination Therapies in Multiple Sclerosis: Too Soon to Give Up on Progenitors?

Thus, the data that currently exist, including those that use $^{14}$C dating, point to a picture of remyelination in humans where there is some remyelination mediated by OPCs, especially in lesions that formed early in the disease. Given that the degree of lesion resolution is likely to be much greater than currently recognized, the extent to which this occurs is very difficult to assess at present. With disease progression and aging, where the age-related decline in OPC function leads to a decline in remyelination efficiency, this mode of remyelination might be superseded by a different mode of remyelination mediated by surviving oligodendrocytes (Duncan et al., 2019). The possibility of novel and previously unrecognized means of remyelination is undoubtedly immensely invigorating for the field and warrants further experimental investigations. However, it would be unjustified and harmful for the development of much-needed regenerative therapies for progressive MS if we were to throw the OPC out with the bath water.

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