The effect of aging and senescence on the process of remyelination

By: Anne Gomes
Supervised by: Sjef Copray and Falak Sher

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Department of Neuroscience, University Medical Centre Groningen, University of Groningen, Groningen, the Netherlands

Abstract:

Aging has been shown to detrimentally affect oligodendrocyte function in axon remyelination. Examining what changes cause these detrimental effects can improve our understanding of the critical processes controlling this regenerative process. Since declined remyelination is a hallmark of demyelinating diseases like Multiple Sclerosis (MS), investigation of the aging process would be of interest in pursuit of future therapies. In addition, since MS is a disease that can last for several decades aging is a likely contributing factor in disease progression. Therefore, devising a means of combating the aging trend also seems to represent a promising approach to MS therapy itself. By reviewing the present data, a picture emerges in which oligodendrocyte differentiation is the most likely limiting step in the declined remyelination efficiency of the aged individual. During the differentiation stage aging has been shown to cause adverse changes in the epigenetic make-up of oligodendrocyte lineage cells, accompanied by changes in the expression of transcriptional regulators of remyelination. In addition, harmful changes have been reported in the environment of these cells - including changes in growth factor expression, immune factor response, and the activity of remyelination inhibiting proteins. As this evidence points to aging as multifactorial process, it is likely that therapies targeting normalization of only one of these factors would not improve remyelination. Instead, the only profitable approach will be to simultaneously modify a multitude of these components. Besides the enhancement of endogenous remyelination, strategies to increase the degree of remyelination could also aim to circumvent the endogenous repair process by transplanting myelinogenic cells. As knowledge about the remyelination process is growing, both strategies might be exploited in future MS therapy. Since endogenous remyelination declines with age the strategy that proves to be most beneficial is likely to depend on the individual patient.

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1. Introduction.

Glia, a supporting group of cells in the brain, play an important role in the functioning of neurons and their long axonal processes. For example, the class of glia called oligodendrocytes wrap with their cell membrane around axons in a multilayered spiral extension. The so formed insulating layer, called the myelin sheath, is required both for fast, saltatory conduction along a neuron as well as for the integrity and long-term survival of axons. These important functions make oligodendrocytes indispensable for virtually all motor, sensory and higher functions of the brain (Nave, 2008).

Problems arise when myelin is damaged, as occurs in various diseases, including multiple sclerosis (MS). MS is an inflammatory disease of the central nervous system (CNS) in which autoreactive immune cells repeatedly attack oligodendrocytes, myelin and axons (Chang et al., 2002). This leaves behind myelin debris and creates local areas of inflammation, which in turn cause a repair process to get started (Rist and Franklin, 2008;Sim et al., 2002). Repair of the lesion requires oligodendrocyte precursor cells (OPCs), an ubiquitous stem-like cell population in the developing and adult CNS, to migrate to the affected area. Subsequently, these cells need to differentiate into mature oligodendrocytes and reform the myelin sheath around the damaged axon (Nave, 2008). Usually this remyelination process results in complete healing, as this process can be highly efficient. Incomplete or failing remyelination on the other hand leaves axons demyelinated, which occurs in MS (Franklin, 2002;Shen et al., 2008a). The inadequate remyelination is thought to be one of the main contributors to the progressive decline in neural function typically seen in this demyelinating disease (Franklin, 2002). Therefore, besides modulating the immune system in a way so it does not cause further autoimmune attacks, and protecting neurons, stimulating repair of the damaged myelin sheath is one of the three main avenues of MS therapy approaches (Rist and Franklin, 2008). Since evidence from experimental models confirms that remyelination of a demyelinated lesion in the CNS is associated with functional improvement (Jeffery and Blakemore, 1997), stakes are high in this field.

Prolonged demyelination causes axonal atrophy, that is why some MS patients with extensive myelin sheath restoration still decline in neural function. Therefore, therapeutic promotion of remyelination represents an attractive option especially in the early stages of the disease. At this stage remyelination-enhancing therapies can restore axonal conduction and prevent secondary axonal loss, and thereby improve brain function (Dubois-Dalcq et al., 2005;Shen et al., 2008a). In order to realize this protection of brain function in therapies, it is helpful to examine whether features of the naturally occurring remyelination process can be exploited (Rist and Franklin, 2008). With resulting knowledge, therapies can be designed in which the endogenous remyelination is enhanced. On the other hand, therapies could aim to circumvent the endogenous repair process by transplanting myelinogenic cells (Chari et al., 2003). Whichever approach is aspired, the development of remyelination-enhancing therapies would be made much easier if the reason why remyelination fails is known, and in turn what aspects of the process need to be corrected.

One of the most important biological factors affecting remyelination efficiency is aging. As with many other regenerative processes in the body, remyelination becomes less efficient with increasing age (Shields et al., 1999;Sim et al., 2002). Indeed, studies on demyelination in animal models indicate that myelin repair is far less efficient in older animals compared to younger animals with initially the same degree of demyelination. So it seems that although the

mammalian brain is equipped with remarkable repair machinery, with age the efficiency of this remyelination process declines or is lost completely (Nave, 2008).

Determining the basis of declined remyelination efficiency with age is likely to shed light on the critical processes in remyelination. This, in turn, will inform future therapeutic developments for promoting remyelination in clinical demyelinating disease. Besides being indirectly valuable for enhancement of MS therapy, studying the effects of aging on OPC biology and remyelination is also directly valuable. If aging leads to a decrease in remyelination efficiency, this would have significant implications for the likelihood of recovery from new lesions in the disease, as MS can last for several decades (Rist and Franklin, 2008;Zhao et al., 2006). So an important question to ask is why the brain's ability to remyelinate lesions declines with age? To be able to answer this question we need to find out what factors in which stages in the process of myelin sheath restoration are modified in the aged individual. Since remyelination is a complicated process it is likely that a multifactorial and integrative explanation will prove to be the only satisfying one in the future. If it turns out that the progression of MS disease is indeed aggravated by aging effects, then it would be promising to devise therapies combating the aging trend (Rist and Franklin, 2008).

2. Cellular events in remyelination.

Before getting into the effects of senescence on remyelination, it is crucial to know how this regenerative process is organized and operates in the mammalian brain. The term "remyelination" implies some similarity with "myelination". This statement is covered in the recapitulation hypothesis, which claims that remyelination of demyelinated axons reactivates programs controlling the developmental process of myelination. But, regeneration in the adult may differ considerably from processes occurring during development (Arnett et al., 2004). For example, a distinctive feature of the environment for remyelination is the presence of myelin debris generated during demyelination. Even so, developmental studies have provided valuable insight (Hinks and Franklin, 1999;Kotter et al., 2006)

Additional research has been done specifically concerning remyelination. This research employs several different models of demyelination to gather information about the sequence of cellular events involved in the remyelination process. Models based on toxin-induced demyelination have proven useful, as they simplify matters. Demyelination is clearly separated from the regenerative phase as toxin exposure generally produces acute demyelination. But given that inflammation clearly contributes to demyelination in diseases like MS, viral and immune models are also exploited (Franklin, 2002;Zhao et al., 2006).

Studies using the above models show that the number of oligodendrocytes in a remyelinated area is greater than it was before demyelination (Prayoonwiwat and Rodriguez, 1993). Thus implying that oligodendrocytes are recruited and/or generated. In addition, even if oligodendrocytes are able to survive the demyelination assault, it seems unlikely that they are able to contribute to remyelination. Indeed, surviving oligodendrocytes are found fully-differentiated and therefore postmitotic, as they are unable to spontaneously divide in the presence of demyelinated axons (Franklin, 2002;Keirstead and Blakemore, 1997). Evidence supports the idea that most, and probably all, remyelinating cells come from OPCs (Blakemore and Keirstead, 1999;Gensert and Goldman, 2001). OPCs proliferate very slowly in the normal adult, but their proliferation rate is dramatically increased in response to demyelination (Sim et al., 2002). This increased proliferation rate is presumably due to increased expression of OPC mitogens in and around acute lesions (Hinks and Franklin, 1999;Hinks and Franklin, 2000). Some of these newly generated OPCs will subsequently differentiate into remyelinating oligodendrocytes and thereby restore myelin sheath (Woodruff et al., 2004).

However, OPCs migrating to and remyelinating demyelinated lesions does not seem to be the whole picture. Gensert and Goldman (2001) have shown that the endogenous OPC population is more heterogeneous than once thought. Moreover, there is evidence that precursor cells at even earlier stages in the oligodendrocyte lineage, originating from the subventricular zone, are capable of remyelination (Nait-Oumesmar et al., 1999). Consequently, this heterogeneity might cause not all precursor cells to respond to environmental cues or contribute to remyelination in the same manner (Mason and Goldman, 2002).

<u>The process of remyelination</u>

A model can be proposed in which remyelination is divided into several key phases. The first phase involves the activation of sufficient OPCs, which rapidly proliferate and migrate into an area of demyelination. The second phase involves the differentiation of the recruited OPCs into myelin-sheath-forming oligodendrocytes. During this differentiation phase, the OPCs

first engage the demyelinated axon. Later, they extend their cell membrane to form spiral wraps and eventually form compact myelin sheath around the axon as mature remyelinating oligodendrocytes (Franklin et al., 2002;Franklin, 2002;Rist and Franklin, 2008).

What events after demyelination create a signaling environment that induce remyelination? Several studies have indicated that this process is initiated by the inflammatory response to demyelination. Indeed, remyelination efficiency has been shown to correlate with number and activity of a class of inflammatory cells called macrophages (Kotter et al., 2001) (Graca and Blakemore, 1986;Ludwin, 1980 as seen in Franklin 2002). However, the functions of macrophages in remyelination are not fully known yet. A possible important role is their clearance of myelin debris, as contact with myelin can inhibit OPC differentiation (Arnett et al., 2004;Kotter et al., 2006). Another possibility lies in the growth factors macrophages secrete, as OPC behavior during remyelination is regulated by growth factors (discussed in later sections) (Hinks and Franklin, 1999;Hinks and Franklin, 2000;Yao et al., 1995). Also, the production of factors that activate astrocytes might be a crucial role of macrophages, as astrocytes produce remyelination-associated growth factors too (Hinks and Franklin, 1999). Besides macrophages, the stages of remyelination associated with inflammation depend on pro-inflammatory cytokines as interleukin-1β (IL-1β) and tumor-necrosis factor-α (TNF-α). TNF-α has been shown important in the maintenance of proliferating OPC number (Arnett et al., 2001), and IL-1β provides an environmental signal that causes the differentiation of precursors into mature oligodendrocytes (Mason et al., 2001). Although these cytokines might not directly regulate the behavior of OPCs, they are important in the cascade of events that makes remyelination possible (Franklin, 2002).

A picture emerges in which the activation of both macrophages and astrocytes are important in the remyelination process. They are initially activated by the demyelination associated inflammation, but subsequently activate each other, and start a cascade of events that create an environment favorable for remyelination (Franklin, 2002).

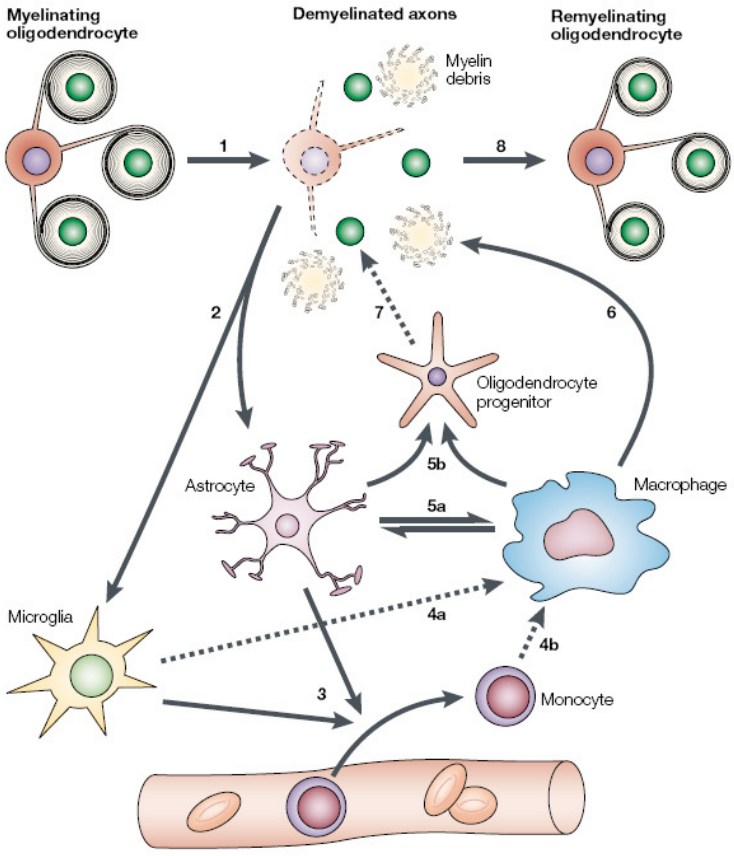


Figure 1: Franklin 2002. Creating a favourable environment for remyelination. Remyelination involves a sequence of orchestrated steps, the dysregulation of which will result in remyelination impairment. In response to a demyelinating insult, the myelinated axons undergo demyelination (1), a process that generates myelin debris. Demyelination causes the activation of resident astrocytes and mircoglia (2). The activated astrocytes and microglia produce factors that contribute to the recruitment of monocytes from blood vessels (3). Mircoglia (4a) and recruited monocytes (4b) differentiate into macrophages. Activated astrocytes and macrophages produce factors that activate each other (5a). As a result of this activation, both produce growth factors that act on oligodendrocyte precursors/ progenitors (5b). Macrophages remove myelin debris (6), a function that may be beneficial to remyelination. Under the influence of factors that are produced by astrocytes and macrophages, recruited oligodendrocyte precursors engage demyelinated exons (7) and differentiate into remyelinating oligodendrocytes (8).

3. Aging.

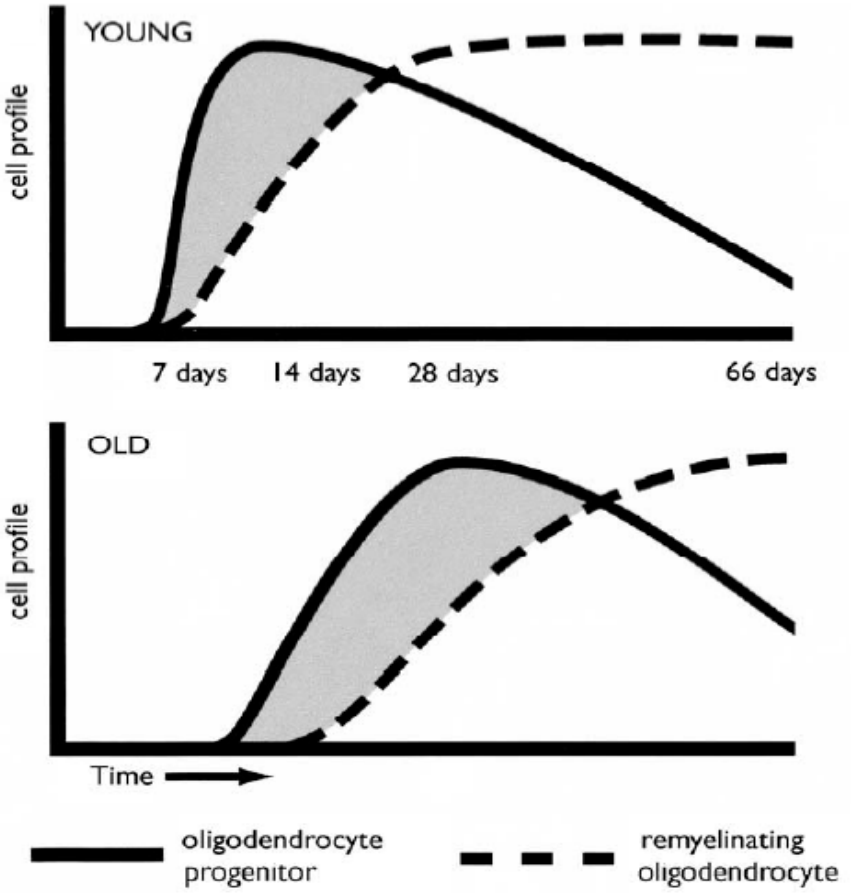
In order to get a better understanding of how aging affects remyelination, it is useful to consider the phenomenon of aging and its effect on stem and precursor cells on a more general level. Aging is a process that leads to the deterioration of many bodily functions over an individual's lifespan, for example there is a reduced capacity to regenerate injured tissues and organs (Hayflick, 1994 as seen in Rist and Franklin, 2008). This reduced regenerative capacity with age could partially be explained by impaired stem and precursor cell function. Indeed a correlation has been pointed out between the number and activity of stem cells and the potential for regeneration (Ho et al., 2005). Given the stem and precursor cells role in the impaired response to injury, the question arises what underlies their modified behavior. Is it intrinsic change or rather the modified environment in the aged tissue that causes them to behave differently (Rist and Franklin, 2008)?

3.1 How does aging affect remyelination?

Aged animal models remyelinate as extensively but significantly slower than young controls. So it seems that oligodendrocyte remyelination in old subjects is a more protracted process than it is in the CNS of young subjects, but nevertheless can still proceed to completion. This indicates that remyelination need not occur rapidly for it to be extensive (Franklin et al., 2002;Shields et al., 1999). Yet, problems arise when the rate of demyelination is higher than the rate of remyelination, leaving axons chronically demyelinated.

What changes cause this slowing of remyelination with age? As demonstrated for other regenerative processes, impaired OPC functionality explains an important part (Rist and Franklin, 2008). On the basis of the model of remyelination presented earlier, at least three possibilities should be taken into account: 1) the number OPCs declines with age, leading to a smaller pool of cells available for recruitment, 2) the rate of OPC recruitment decreases, 3) the rate of differentiation of recruited OPCs into remyelinating oligodendrocytes decreases (Franklin et al., 2002). Since delays at different stages require different approaches to correct the situation, it is important to find out where in the process bottlenecks occur in order to develop successful interventions.

Several lines of evidence support the idea that exhaustion of the OPC pool is not the limiting factor in the age-associated decline in remyelination rate. The number of OPCs in some white matter tracts is shown to differ between the old and young adult CNS (Sim et al., 2002). However, this decline in precursor cell number does not explain the impaired remyelination efficiency completely, since some tracts bear the same number of OPCs in both age groups (Shen et al., 2008b). In addition, repeated demyelination in rats does not lead to depletion of OPCs (Penderis et al., 2003), showing that their number is robustly maintained. Both the rate of OPC recruitment and their differentiation account for the decreased age-associated remyelination rate (Shields et al., 1999). Indeed, OPC recruitment to a demyelinated lesion is slower in older animals, and the same has been demonstrated for the rate at which OPCs then differentiate into myelinating oligodendrocytes (Sim et al., 2002).



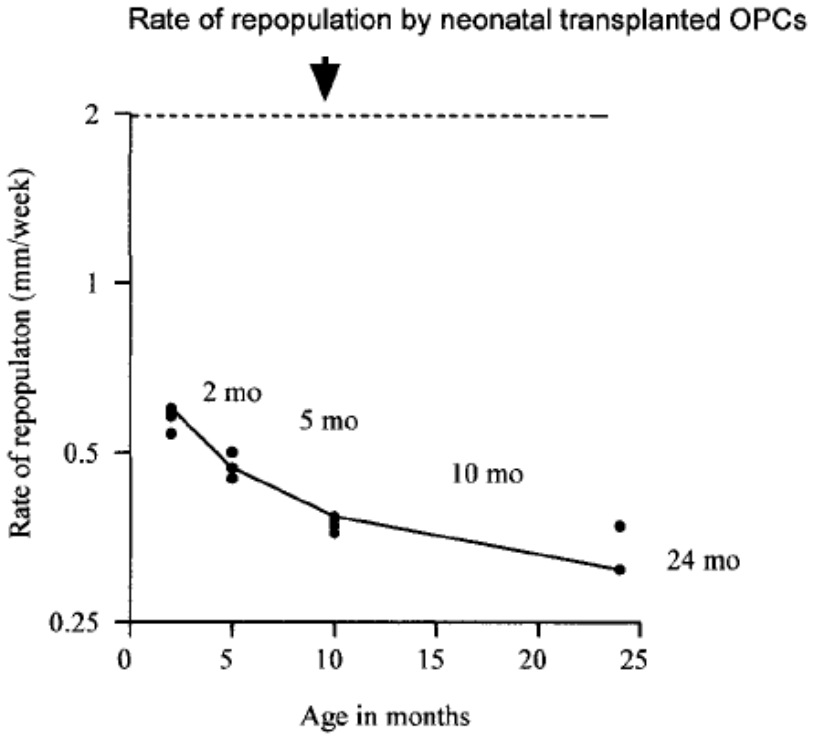
Figuur 2: Franklin et al., 2002. Profiles of OPC recruitment and the appearance of remyelinating oligodendrocytes during the remyelination that follows ethididum bromide (EB, toxin injected directly into or around CNS white matter causing oligodendrocyte cell death) induced demyelination of the caudal cerebellar peduncle (CCP) in young and old adult rats. The rate of OPC recruitment is slower in old animals, as is their differentiation into remyelinating oligodendrocytes (indicated by the larger shaded area in old animals compared to young).

Which of these two processes is the major contributor to the declined regenerative potential? If it were the recruitment phase, one expects that remyelination in old animals is enhanced when a lesion is supplemented with OPCs. However, when this is done by locally increasing platelet derived growth factor (PDGF), a growth factor shown to increase OPC density, no change is detected in remyelination efficiency (Woodruff et al., 2004). Losing this possibility as rate limiting step, leaves the decrease in differentiation efficiency of recruited OPCs the main determinant in the age-associated remyelination decline (Rist and Franklin, 2008;Shen et al., 2008a). This conclusion is supported by the finding that non-remyelinating lesions in humans with MS bear numerous oligodendrocyte-lineage cells that fail to differentiate into mature remyelinating cells (Chang et al., 2002;Shen et al., 2008a). Therefore, understanding the mechanisms which govern OPC differentiation will be crucial to explain the age-associated decline in remyelination, and will help in identifying potential therapeutic targets for diseases like MS.

3.2 Mechanisms governing the impaired functionality of OPCs.

3.2.1 Intrinsic factors.

Evidence shows that OPCs change intrinsically as they age. Tang and colleagues (2000) compared OPCs which were aged by long-term culture *in vitro* with OPCs aged *in vivo*. Postnatal day 7 OPCs were plated and kept proliferating to compare them later with freshly isolated adult OPCs at different time-points. Since the OPCs *in vitro* acquired properties highly similar to the phenotype of age-matched OPCs *in vivo*, it was demonstrated that perinatal OPCs have an intrinsic maturation program (Rist and Franklin, 2008). Further evidence is provided by Chari and colleagues 2003, who showed a significant reduction in the intrinsic recolonizing rate potential of OPCs with age. They induced depletion of OPCs in areas without demyelination and the associated inflammatory response, to make sure the cells operated independently of these environmental influences.



Figuur 4: Chari et al., 2003. Graph illustrating the decline in rate of repopulation of OPC-depleted tissue by OPCs as a function of age (in months). The rates of repopulation calculated for individual animals are shown and a best-fit curve was applied to the data points. The endogeneous rates of repopulation calculated for adult rats were obtained from identical experiments (0-4 weeks) at the different ages using the focal (7 mm) OPC depletion model. The rate of repopulation calculated for neonatal cells (indicated by dotted line) is obtained from experiments where a suspension of neonatal OPCs was injected into OPC-depleted tissue (created by 40 Gy X-irradiation) in 5-month-old adult rat spinal cord (Blakemore et al., 2002).

These intrinsic changes are mediated by changes in the epigenome, and by the consequent changes in the expression of transcriptional regulators of remyelination.

<u>Change in epigenetic memory</u>

Epigenetic mechanisms reflect changes in gene expression that are not dictated by the DNA sequence itself. An important epigenetic mechanism is the modification of the chromatin at the level of nucleosomes, which consists of DNA wrapped around octamers of histone proteins. An example of this chromatin remodeling involves histone deacetylase (HDAC) enzymes, which comprise a family of proteins that remove acetyl groups from the lysine amino acids in the histone tails. The deacetylated histones favor a more condensed chromatin conformation, which is responsible for inactivation of transcription because it renders the DNA not accessible to specific transcription factors. HDACs can be further subdivided in four classes, each containing several isoforms. These different classes have been shown to be

involved in different patterns of gene repression (Copray et al., 2009;Nave, 2008;Shen et al., 2008b).

Besides histone acetylation, an important and closely associated histone modification is the methylation of lysine residues. In contrast to acetylation however, the position of the methylated histone and the number of methyl groups added determine the effect of the modulation. Transcription can be enhanced or inhibited (Copray et al., 2009).

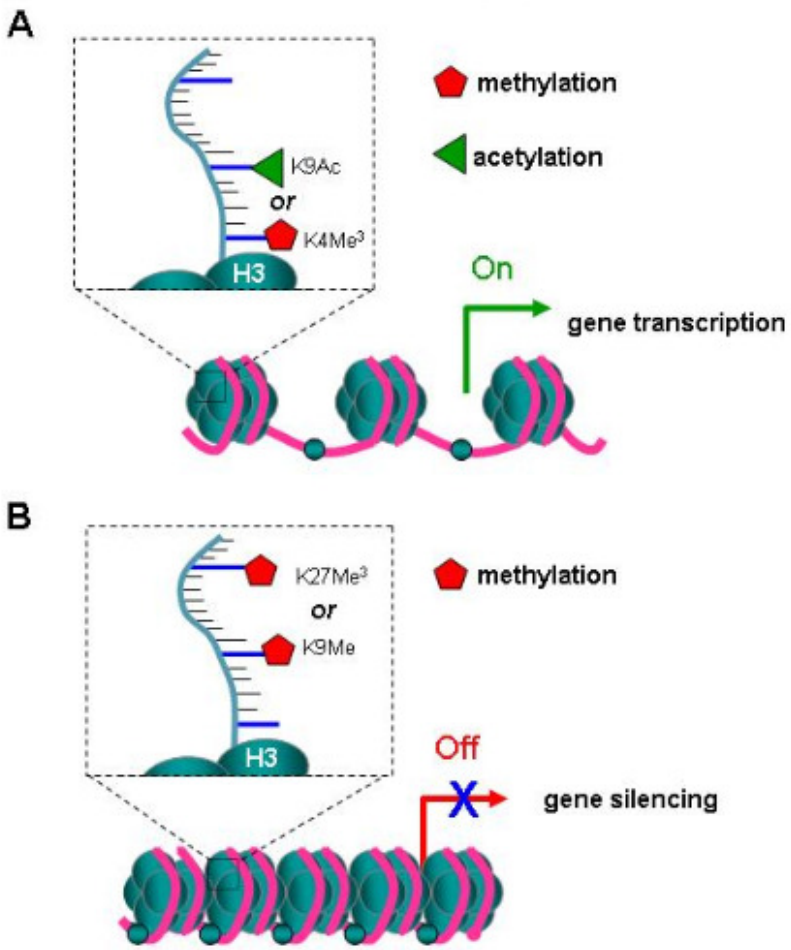


Figure 5: Modified from Copray et al. Histone modification. A. The scheme of 3 nucleosomes depicts a part of the chromatin with promotor and gene DNA that is open for transcription. The transcriptional active state is achieved by the acetylation of lysine 9 (K9) or trimethylation of lysine 4 (K4) respectively in the tail of histone 3 (H3). B. Transcriptional silencing can be caused by the histone modification resulting from the methylation of K9 or trimethylation of K27. Repressive histone methylation on K9 is often coupled with histone deacetylation.

The epigenome, and more specifically histone modification, is a critical component at the interface between environmental signals and transcriptional programs regulating cell fate decision and stem and precursor cell differentiation (Shen et al., 2008a). The importance of these epigenetic modifications have been confirmed for oligodendrocyte differentiation and myelin gene expression (Copray et al., 2009;Shen et al., 2008b). In addition, the importance of histone deacetylation for OPC differentiation has been supported by many experiments (He et al., 2007;Hsieh et al., 2004;Liu et al., 2009;Marin-Husstege et al., 2002;Shen et al., 2005;Shen et al., 2008b;Siebzehnrubl et al., 2007). The role of histone deacetylation starts at the early stages of neural stem cell (NSC) development into an oligodendrocyte. Here, HDACs mediate the critical processes of suppressing the development of alternative neural lineages, and repressing the expression of oligodendrocyte specific gene inhibitors (Copray et al., 2009).

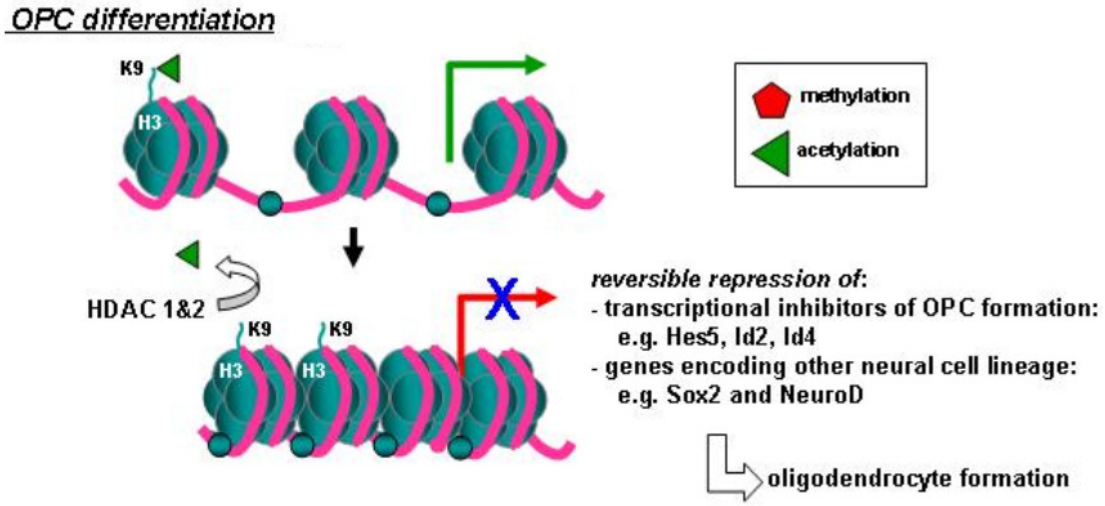


Figure 6: Modified from Copray et al. 2009. Histone modification and oligodendrocyte development. The differentiation of neural stem cells into oligodendrocyte precursor cells (OPCs) requires the recruitemnt of class one histone deaceylases (HDACs; in particular 1&2) at genes encoding transcriptional inhibitors of OPC formation (e.g. Hes5, Id2, Id4) and those encoding the fate towards neurons or astrocytes. The HDAC activity at lysine 9 (K9) of histone protein 3 (H3) leads to the silencing of the expression of these genes, enabling oligodendrocyte formation. The repression is still reversible, since some regulation is required to prevent premature myelination. Hes5= hairy and enhancer of split 5; Id2= inhibitor of DNA-binding 2; Id4= inhibitor of DNA-binding 4; Sox2; NeuroD= Neurogenic differentiationmarker.

Evidence pointing to HDAC enzymes as critical players in oligodendrocyte development is provided by the use of pharmacological HDAC inhibitors. HDAC inhibition (HDACi) induces developmental plasticity in OPCs, so that they regain the potential to differentiate into alternative neural lineages. This developmental conversion in oligodendrocytes is found to be partly mediated through reactivation of SRY-box 2 (Sox2), a transcription factor involved in stem cell pluripotency. In addition, other genes important in the maintenance of the NSC state that simultaneously inhibit oligodendrocyte differentiation are shown to be activated. These results demonstrate that histone acetylation, induced by HDACi, can reverse the lineage restriction of OPCs and induce developmental plasticity (Lyssiotis et al., 2007). As HDACi has also been shown to increase the number of NSCs that differentiate into astrocytes and neurons at the expense of oligodendrocytes (Balasubramaniyan et al., 2006;Siebzehnrubl et al., 2007), a strong case can be made for HDAC activity to be critical for oligodendrocyte identity (Shen et al., 2008a).

In young oligodendrocytes several HDAC isoforms have been identified at the promoter region of genes inhibiting oligodendrocyte differentiation and stem cell marker genes. Therefore multiple isoforms are probably involved in the early stages of oligodendrocyte development. Levels of HDAC1 and HDAC2 isoforms are now identified to be critically involved in the execution of an oligodendrocyte differentiation program (Shen et al., 2005;Shen et al., 2008a). Until the onset of myelination, these HDACs perform the job of forming repressive complexes with transcriptional inhibitors so that oligodendrocyte specific genes can be transcribed (Copray et al., 2009). However, after this time point, the reversible repression mediated by HDAC enzymes is made more stable by the addition of inhibitory methyl groups (Shen et al., 2005). Both deacetylation mediated by HDACs, as well as the repressive methylation serve the purpose of establishing an “epigenetic memory”, which is stored in the chromatin of differentiated oligodendrocytes (Marin-Husstege et al., 2006).

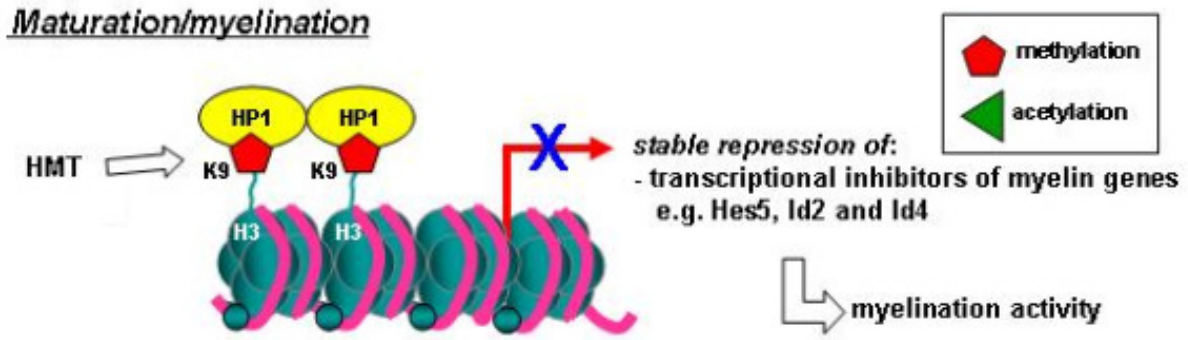


Figure 7: Modified from Copray et al. Histone modification and oligodendrocyte development. Essential stable repression of inhibitors of myelin genes at the mature stage of the oligodendrocyte is established via the action of histone methyl transfereases (HMTs). This results in the methylation of H3K9 (lysine 9 of histone protein 3) and is followed by the binding of HP1 (histone protein 1) causing compaction of the nucleosome. The stable silencing of the myelin gene inhibitors promote myelination activity.

Recent evidence shows that there is an age-related decline of this histone deacetylation and repressive methylation (Shen et al., 2008a;Shen et al., 2008b). Therefore, the progressive loss of an "epigenetic memory" is now defined as important mechanism in the age-dependent decline in remyelination efficiency (Shen et al., 2008a). Since the loss of this memory results in a decrease of repressive complex formation, myelin gene transcriptional inhibitors and NSC marker genes (i.e. Sox2) become transcriptionally active. Impaired oligodendrocyte function is due.

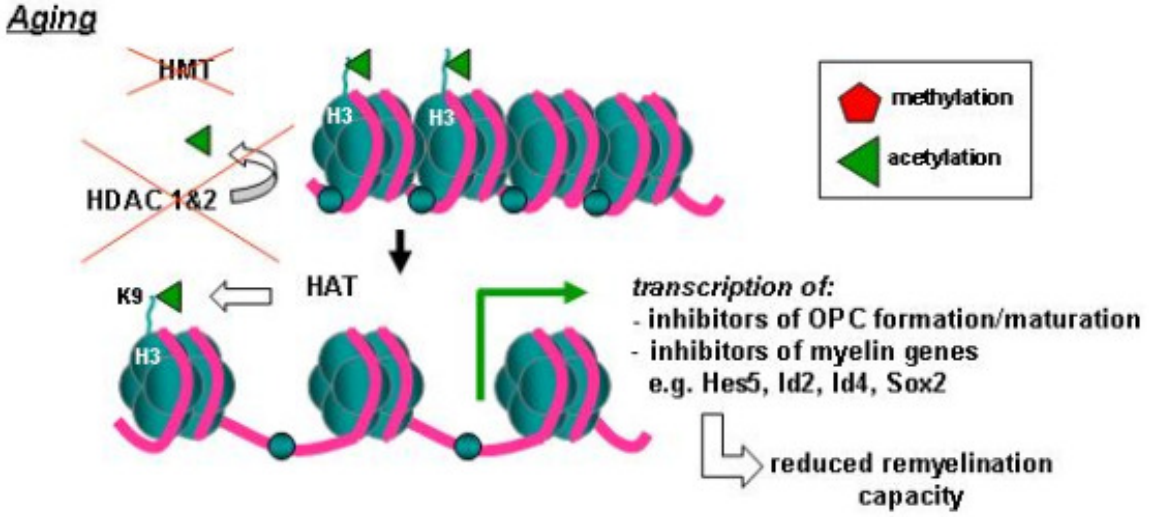


Figure 8: Modified from Copray et al. Histone modification and oligodendrocyte development. The remyelination capacity of oligodendrocyte precursor cells (OPCs) in aged animals is reduced due to diminished recruitment of histone deacetylases (HDACs) and histone methyltransferases (HMTs), resulting in histone acetyl transferase (HAT) activity to prevail causing an acetylated, transcriptional activated, state of genes blocking proper OPC development, maturation and myelination.

Genes do not reactivate randomly, but rather are activated in reverse fashion from the order is which they were silenced during the initial establishment of their "epigenetic memory". In accordance with this observation, it is hypothesized that with aging the enzymatic activity responsible for the "epigenetic memory" in mature oligodendrocytes progressively declines. Based on a study in zebrafish (Cunliffe and Casaccia-Bonnefil, 2006), this role has in part

been assigned to HDAC enzymes. The pattern of gene expression observed in the aging brain is namely shown to be comparable to the pattern detected in neural precursors lacking HDAC1 activity (isoform of class 1 HDACs). In agreement with this hypothesis, differentiating primary oligodendrocytes treated with HDAC inhibitors show a pattern of gene expression that is similar to the one observed in the aging brain (Shen et al., 2008b). These results provide evidence for a role of HDAC activity in the oligodendrocyte identity change within the aging brain.

Change in expression of transcriptional regulators of remyelination

Transcriptional regulators affect remyelination efficiency (Arnett et al., 2004;Gokhan et al., 2005). Indeed, they are shown to regulate stem cell marker genes, oligodendrocyte differentiation inhibitory genes and myelin specific genes during spontaneous remyelination. The controlled pattern of gene expression regulating this regenerative process starts with the downregulation of stem cell marker Sox2, and with the oligodendrocyte differentiation inhibitors hairy and enhancer of split 5 (Hes5), Hes1, inhibitor of DNA-binding 2 (Id2) and Id4. An upregulation of the oligodendrocyte transcriptionfactor 1 (Olig1) follows, and myelin gene expression increases accordingly. The coordinated decrease of multiple inhibitory transcripts is detected only in young animals. In contrast, old animals transcribe genes encoding the inhibitors of differentiation for much longer than normally seen in remyelination (Shen et al., 2008a;Shen et al., 2008b). This indicates that the age-associated changes in "epigenetic memory" are accompanied by changes in gene expression. In turn, changes in gene expression will influence remyelination efficiency.

Out of all of the above mentioned genes, Sox2 is emerging as the most likely candidate target gene for the improvement of remyelination (Shen et al., 2008a). Although it has not been examined whether Sox2 directly inhibits oligodendrocyte differentiation and myelination, it is likely to do so. This hypothesis is based on the evidence that Sox2 directly inhibits Schwann cell differentiation and myelination, and on the fact that oligodendrocytes perform a comparable function in the CNS as Schwann cells perform in the peripheral nervous system (Le et al., 2005).

3.2.2 Extrinsic factors.

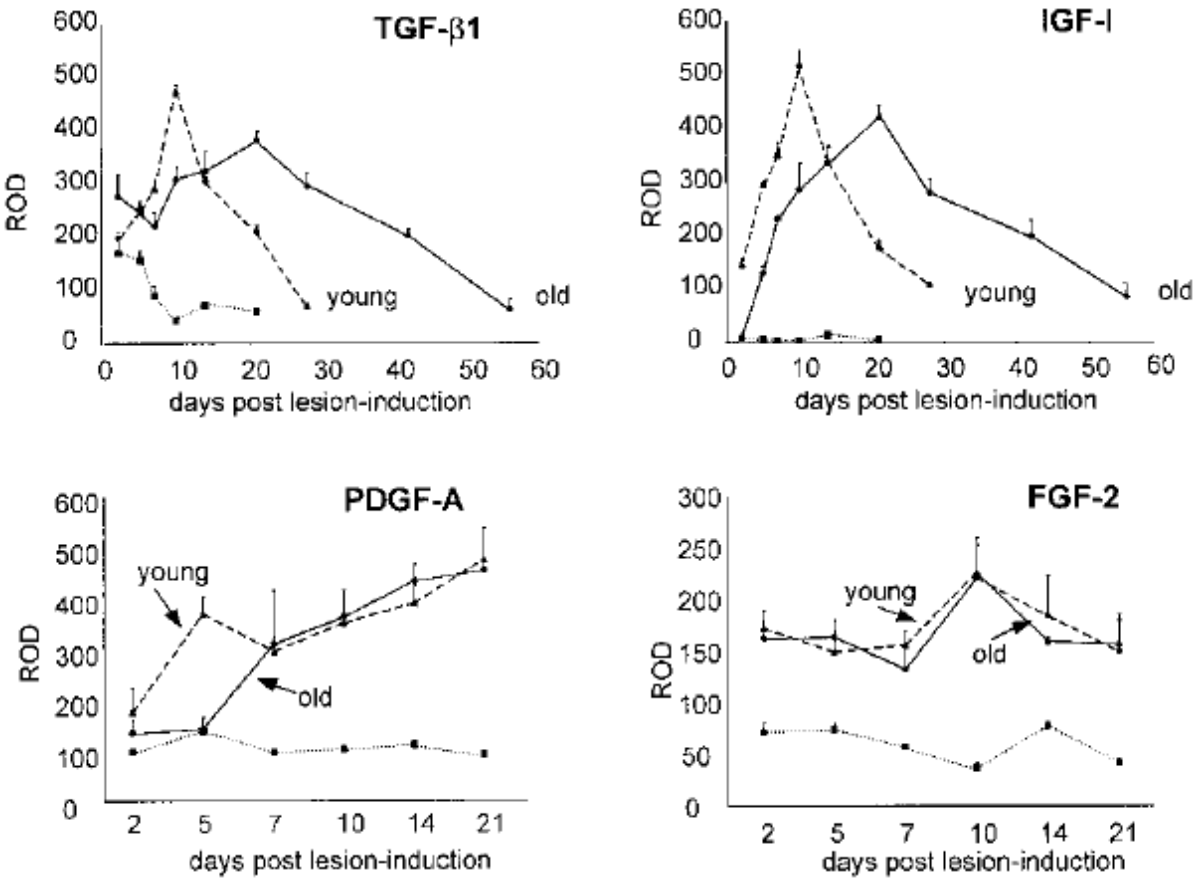
Evidence is growing that aging intrinsically affects the stem and precursor cells that mediate remyelination, making it less responsive to environmental signals. In addition, there is now strong evidence that the signaling environment is changed and dysregulated in the aged organism. This evidence includes regulation of growth factors, immune factors and remyelination inhibitory proteins.

Growth factors

The growth factor response to demyelination changes with increasing age. Growth factors affect the proliferation, migration and differentiation of OPCs. Indeed, the expression profiles of different growth factors that are secreted by astrocytes (e.g. PDGF and fibroblast growth factor-2 (FGF-2)) and inflammatory cells in the lesion are shown to be delayed in aged animals (Hinks and Franklin, 2000;Rist and Franklin, 2008).

As previously described, the repair of demyelinated lesions is divided into an initial OPC recruitment phase that gives way to a differentiation phase. The two growth factors PDGF and FGF-2 both enhance OPC proliferation and motility in vitro, and so are likely to be associated with the recruitment phase of remyelination. Indeed, they have increased levels of expression early in the repair process. The levels of PDGF remain high throughout remyelination. The levels of FGF-2, in contrast, decline at transition day, when the appearance of new myelin sheaths within the lesion signals the advancement of recruitment to differentiation phase. FGF-2 is shown to inhibit OPC differentiation. Furthermore, the growth factors insulin-like growth factor 1 (IGF-I) and transforming growth factor-beta 1 (TGF-β1) show peak expressions at transition day. On the basis of these expression patterns a model can be proposed in which PDGF and FGF-2 mediate the recruitment phase, while peak expression of IGF-I and TGF-β1 initiate the differentiation phase (Franklin and Hinks, 1999).

If a causal relationship exists between growth factor gene expression and remyelination then one would predict that slower rates of remyelination should have correspondingly altered patters of growth factor expression. This prediction can be tested by comparing expression in old versus young animals. In general, the changes in growth factor expression support the proposed model. The same four growth factor mRNAs are upregulated in both age groups. But, there is a short delay in onset of PDGF mRNA expression in old animals, which may account for the impairment of OPC recruitment. The peak expression of both IGF-I and TGF-β1 occurs later, which could account for the differentiation delay. In contrast, FGF-2 shows no change in expression between groups, implying that this factor does not underlie the changes in remyelination efficiency induced by senescence (Franklin et al., 2002;Hinks and Franklin, 2000).



Figuur 3: Franklin et al. 2002. The expression of remyelination-associated growth factors, TGF-β1, IGF-I, PDGF-A, and FGF-2, has been examined during remyelination of lysolecithin (toxin injected directly into or around CNS white matter causing oligodendrocyte cell death) induced demyelination in the dorsal and ventral funiculi in the spinal cord of adult rats. The levels of expression are estimated by measuring the relative optical density (ROD) of autoradiographs following radioisotope in situ hybridization. Results are expessed as meand ± SEM. In young animals (dashed line) the onset of increased PDGF-A mRNA expression occurs before that in old animals (solid line), although from 7 days onwards the levels are similar. In young animals there is a peak of IGI-I mRNA expression at day 10, the time at which differentiation of recruited OPCs into remyelinating oligodendrocytes begins (in this toxin-induced demyelination-remyelination model). In old animals the peak of this putative differentiation trigger is delayed providing a possible explanation for the differentiation delay associated with remyelination in old animals. Similar patterns of expression to those of IGF-I mRNA are also seen for TGF-β1 mRNA, another putative trigger for OPC differentiation. (The dotted lines in the graphs represent the control saline injected animals (Hinks and Franklin, 1999)).

If it would be true that the slowed remyelination in old animals is a consequence of changes in growth factor expression, then creation of a growth factor profile from young animals should increase their rate of remyelination. Indeed, systemic delivery of IGF-I is associated with remyelination enhancement in some autoimmune experimental models (Yao et al., 1995). Nevertheless, this induced enhancement is not due to direct effects on oligodendrocyte-lineage cells. By the use of IGF-I expressing adenoviral vectors, it was shown that when the IGF-I peak is brought forward within areas of demyelination in old rats the remyelination rate is not changed (O'Leary et al., 2002). This observation could be interpreted as IGF-I not being a critical differentiation signal for OPCs. However, it could also indicate that multiple factors induce OPC differentiation and that changing only one them is insufficient to significantly alter the process (Franklin et al., 2002). The results of this study provide insights into the possibility of influencing remyelination in MS by delivering growth factors directly into areas of demyelination (O'Leary et al., 2002). While administering growth factors will be beneficial for remyelinating cells in general, there is a clear need for more knowledge before the

therapeutic use of remyelination promoting growth factors can be applied. Before proceeding to a clinical application, caution must also be taken as it is likely that the inappropriate administration of growth factors has adverse consequences. The aspired outcome will only be produced if the appropriate local concentrations are achieved (Hinks and Franklin, 2000).

Immune factors

A second extrinsic factor shown to be delayed in aged animals is the innate immune response. Indeed, the inflammatory response shows a delay with aging (Hinks and Franklin, 2000;Zhao et al., 2006), as evidenced by a more rapid macrophage recruitment and activation in young compared with old animals. Differences in the expression of inflammatory mediators may account for this difference in macrophage responsiveness. One would predict the slower macrophage recruitment in older animals to be associated with impaired cytokine expression after demyelination. However, IL-1β, IL-6 and TNF-α are expressed for a longer period in older animals (Zhao et al., 2006). Therefore, the impaired macrophage responsiveness may reflect intrinsic age-associated changes as well.

Whichever reason, the altered age-associated macrophage responsiveness is likely to be one of the key determinants of the changed efficiency in the remyelination process. The difference in macrophage response correlates most closely with the previously mentioned difference in IGF-I and TGF-β1 mRNA expression during remyelination in young and old animals (Hinks and Franklin, 2000). From this observation it can be concluded that these growth factors are mainly produced by macrophages. But besides expressing growth factors (Hinks and Franklin, 1999) as remyelination enhancing activity, macrophages activate astrocytes and clear the lesion of myelin debris (Kotter et al., 2006). If the macrophage response is indeed that important in the remyelination process then reducing it during the rapid remyelination in young animals should result in an impairment of remyelination efficiency. Kotter and colleagues (2001) tested this hypothesis and found that a reduced macrophage response leads to a significant reduction in remyelination. However, whether it is possible to modify the inflammatory response in the older age group so that it contributes to more efficient remyelination remains a future challenge (Zhao et al., 2006). Nevertheless, these results provide further confirmation of a pro-remyelinating role of the macrophage. This contradicts the widely recognized opinion that macrophages fulfill a role in the demyelinating process. Therefore, the macrophage must have several functional roles in demyelinating disease depending on the environmental context (Franklin et al., 2002).

Remyelination inhibitory proteins

An alternative extrinsic factor explanation for senescence might be that remyelination failure results from the presence of remyelination inhibitory proteins in the lesion region. Proposals for such proteins are based on evidence from MS research and include the polysialylated form of the neural cell adhesion molecule (PSA-NCAM), hyaluronan, leucine rich repeat and Ig domain containing 1 (LINGO-1) and Notch ligands.

During development, PSA-NCAM is expressed at the axonal surface. Here it inhibits myelination, presumably by preventing the attachment of oligodendrocytes to the axon. Consequently, in order for developmental myelination to get started removal of PSA-NCAM from the axonal surface is required. Normally this molecule is absent from the adult brain. However, PSA-NCAM is shown to be re-expressed on demyelinated axons located within MS plaques (Charles et al., 2002), showing that PSA-NCAM is a crucial factor for remyelination.

A high molecular weight form of the glycosaminoglycan hyaluronan, synthesized by astrocytes and a component of the ECM, is shown to accumulate in chronic demyelinated MS lesions. This molecular form causes the OPC number to increase in lesions but prevents them from maturing into myelin-forming cells. In addition, this form of glycosaminoglycan hyaluronan is shown to inhibit remyelination in experimental models (Back et al., 2005).

LINGO-1 is another important negative regulator of CNS myelination that is expressed by oligodendrocytes. Here it inhibits oligodendrocyte differentiation and axon myelination (Mi et al., 2005). Inhibitory mechanisms of myelin debris also seem to work via LINGO receptors (Mi et al., 2004). As pointed out by the critical role of LINGO-1 in myelination, it is likely to be an important mediator of the remyelination failure seen in senescence and diseases like MS.

During mammalian CNS development, activation of Notch1 receptors on OPCs by the ligand Jagged1 induce Hes5, which inhibits OPC maturation in oligodendrocytes. Moreover, TGF-β1, a cytokine upregulated in MS, specifically re-induces Jagged1 in human astrocytes. Within and around MS lesions lacking remyelination, TGF-β1 is present, Jagged1 is expressed at high levels by astrocytes, and Notch1 and Hes5 are expressed by OPCs. In contrast there is negligible Jagged1 expression in remyelinated lesions. These date implicate the Notch pathway in the limited remyelination in MS. Therefore modification of Notch may represent a potential therapy in this disease (John et al., 2002).

Originally the explanation of remyelination inhibitory proteins in remyelination failure was proposed to explain the process of failing remyelination in the brain of MS patients (Nave, 2008;Shen et al., 2008a). But one can hypothesize that these inhibitory proteins might also be an important determining mechanism in the remyelination failure of senescence. Clearly, more research is needed, not only to determine the exact roles these inhibitory proteins play, but also to unravel why these molecules are expressed despite their detrimental effects (Nave, 2008).

3.3 Hypotheses for remyelination failure with age.

Dysregulation hypothesis

In young animals a sequence of events is initiated by the demyelination associated inflammation, in which multiple signaling molecules are produced in a coordinated pattern to create appropriate conditions for efficient remyelination. However, as the adult CNS ages, this complex signaling network becomes controlled with less precision. Synchronization of the signaling network is lost and remyelination efficiency declines. Clearly, the creation of an environment favorable for successful remyelination requires precise regulation of multiple factors. Remyelination of a lesion might fail because of insufficient OPC recruitment. For example, an environment favoring recruitment of OPCs is not maintained long enough. Alternatively, an environment favoring differentiation develops too soon, so that recruitment becomes arrested before enough OPCs can repopulate the lesion. On the other hand, remyelination might fail because of insufficient OPC differentiation. Differentiation could fail because the proper promoting signals are missing or because inhibitory signals are present. Since signals from the recruitment phase inhibit differentiation, a prolonged recruitment phase might reduce differentiation efficiency (Franklin et al., 2002;Franklin, 2002;Rist and Franklin, 2008).

According to the dysregulation hypothesis, there are no individual culprits that are responsible for remyelination failure. Instead, the process fails because the regulating mechanism loses its precise coordination (Franklin, 2002). For example, neither macrophages, nor astrocytes are responsible for remyelination on their own, instead their reciprocal activation create an environment conductive for the process to occur (Franklin et al., 2002).

An important prediction of the dysregulation hypothesis is that manipulating the signaling environment can potentially improve suboptimal remyelination. Furthermore, it is predicted that although redundancy might exist in the system, some factors will be non-redundant. The regulation of these factors will be crucial to remyelination efficacy (Franklin, 2002).

Temporal mismatch hypothesis

The temporal mismatch hypothesis is closely related to the dysregulation hypothesis and is based on the intrinsic factor changes that occur as an OPC ages. For complete remyelination to occur, an environment favoring OPC recruitment must be maintained long enough in order for sufficient OPCs to migrate to the lesion. And only after sufficient OPCs repopulate the demyelinated area, should the environment become optimal for differentiation (Franklin, 2002). Since senescence induces a delay in OPC recruitment, this hypothesis predicts that large lesions in older MS patients will not be completely remyelinated. Problems arise because by the time the aged OPCs arrive in the centre of lesions, the optimal pro-remyelination conditions associated with acute injury are subsided (Chari and Blakemore, 2002). Support for this concept is provided by the demonstration that the extent of remyelination achieved by OPCs decreases if their entry into an area of demyelination is delayed (Blakemore et al., 2002). In addition, the concept could explain why repair often takes place around the edges of lesions while the centre remains demyelinated (Chari and Blakemore, 2002).

So it seems that although OPC differentiation has been shown to be the most important step limiting remyelination efficiency with age, the efficacy of differentiation is clearly dependant on the preceding OPC recruitment stage. Therefore, means of improving OPC recruitment

might still be beneficial in MS therapy. In addition, the present conclusions are based mostly on animal experiments. Yet, there is a discrepancy between the small size of demyelinated lesions in animal models compared to the size of lesions in human MS patients. The combination of large lesions and the extent of OPC loss found in MS might present greater demand on OPC recruitment (Blakemore et al., 2002;Franklin, 2002).

If functional OPC depletion is a determining feature of MS lesions, then therapies might aim to increase OPC recruitment. This can be accomplished by increasing migration rate of endogenous OPCs or exploiting cell transplantation therapy with neonatal cells. As the recolonizing rate of endogenous cells decreases with age, exploiting cell transplantation therapy becomes more attractive in older patients (Chari et al., 2003;Chari and Blakemore, 2002;Rist and Franklin, 2008).

4. Conclusion/Discussion.

From all the reviewed literature, a picture emerges in which OPCs are the main class of cells contributing to axonal remyelination in the mammalian brain. These cells require optimal conditions for their recruitment into the lesion and subsequent differentiation in mature oligodendrocytes for complete remyelination of the demyelinated axon. The reduced capacity of the remyelination process in the aged individual can at least partially be explained by impaired OPC function. Both the OPC recruitment phase as well as the differentiation phase have been implicated in this impaired process. Oligodendrocyte differentiation is the most likely limiting step in the declined remyelination efficiency of the aged individual. It is becoming clear that multiple factors are responsible for this decline in differentiation capability. Indeed, multiple intrinsic factors as well as multiple extrinsic factors are adversely modified by senescence.

Several lines of evidence indicate that HDAC activity is crucial for appropriate oligodendrocyte function, as they play a role in establishing their epigenetic memory. This intrinsic process is shown to become dysregulated with increasing age causing the epigenetic memory characterizing young animals to be lost progressively. Another intrinsic factor change involves the expression of transcription factors regulating remyelination. Indeed, genes of other neural lineages as well as genes that inhibit oligodendrocyte development are progressively expressed with increasing age. Concerning OPC environmental factors, age-associated changes are reported in growth factor expression, immune factor response, and the activity of remyelination inhibiting proteins.

Two highly interrelated hypotheses, the dysregulation hypothesis and the temporal mismatch hypothesis, are now proposed to explain the behavior of OPCs in the impaired remyelination process of the aged individual. Both argue that OPC behavior becomes suboptimal as a result of losing the precise coordination characterizing efficient remyelination. These punctual orchestrations are required for complete remyelination.

As it is becoming clear that aging is a likely contributing factor in disease progression, devising means of combating the aging trend represents a legitimate approach for designing remyelination-enhancing strategies. However, there are some challenges to be met. For example, the age-associated intrinsic changes in OPC behavior make them partly independent from extrinsic factors, including therapy adjustments. The finding that transplanted neonatal OPCs are able to remyelinate areas of demyelination with much greater efficiency than endogenous OPCs, make this cell population an attractive alternative for regenerative therapies. Indeed, cell transplantation might bear the potential to restore myelination in situations where the endogenous repair process is failing. Nonetheless, challenges remain, as it is still necessary for transplanted OPCs to be introduced into an environment that allows them to differentiate in mature oligodendrocytes (Chari and Blakemore, 2002).

Regarding the enhancement of endogenous remyelination, as aging is a multifactorial process, it is likely that therapies targeting only one of these factors would probably not induce significant improvement of remyelination efficiency. Instead, the only profitable approach will be to simultaneously modify a multitude of these components. In order to realize such therapies more knowledge is needed about which dysregulated events call for correction. Therefore, a more complete picture of the aging process and its effects on oligodendrocyte function in efficient remyelination is needed. Generally, this includes further examination of the remyelination control processes that are shown to be modulated in the aged individual. How do they become dysregulated? For example, what causes HDACs in the aged individual

to change activity? More specific aspects can also be mentioned to increase knowlegde further. In addition to the suggestions made in previous sections we propose three new aspects. First, although evidence is pointing towards an important role of HDAC activity in remyelination rate, the development of clinical therapies requires the identification of HDAC isoforms. This would make it possible to adjust more specific molecular targets and thereby make therapies more effective (Shen et al., 2008a). Second, besides histone modification other epigenetic mechanisms might be modulated by the aging process. Therefore, research is needed to examine the effects of aging on such epigenetic mechanisms as DNA methylation and microRNA activity in the oligodendrocyte lineage. Third, on the basis of the finding that the presence of myelin debris inhibits OPC function in the remyelination process, new strategies should be explored in pursuit of regenerative therapies. For example, the possibility of stimulating macrophages to remove myelin debris could promote myelin regeneration. Moreover, if OPCs could be made less responsive to the inhibitory effects of myelin debris, the debris would not pose a problem in lesion healing (Kotter et al., 2006). Finally, we need to keep in mind that results obtained in animal experiments cannot simply be extrapolated to human beings. Evidence is needed to ensure that the same principles are at work in humankind.

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