Chapter 7

Multiple sclerosis (MS) is the most well-known demyelinating disease in humans. Although its main cause is still not known, environmental (viral infections), autoimmunity and genetic factors have been implicated in the etiology of MS. Therefore, an effective therapy for MS is not available yet, but current treatments are directed toward preventing myelin breakdown via suppression of the immune system. However, promotion of myelin repair via remyelination-based strategies after or during treatment of the inflammatory response may become equally important. To promote remyelination in MS lesions via either endogenous or transplanted oligodendrocyte progenitors, it is necessary that oligodendrocyte progenitors migrate, proliferate and eventually differentiate to mature myelinating oligodendrocytes at the site of the MS lesion. Alternatively, remyelination by the remaining mature oligodendrocytes may be induced. To guide (re)myelination in MS lesions and to obtain an effective remyelination-based therapy, it is imperative to understand the key events in the process of myelination. Such events include the extracellular and subsequently activated intracellular events that are important during oligodendrocyte differentiation and the onset of myelination. The goal of the project, described in this thesis, was therefore to study the involvement of both extracellular and intracellular signals in oligodendrocyte lineage progression.

**Intracellular signaling: PKC signal transduction pathway**

In rats, oligodendrocyte progenitors migrate, proliferate and differentiate in vivo according to a precise schedule over a period of several weeks in order to myelinate axons at the appropriate time and place. This implies a tightly controlled mechanism in order to prevent premature and nonessential differentiation. The study described in chapter 2 showed that the protein kinase C (PKC) signaling route is an important negative modulator in the regulation of the switch to differentiation. We also demonstrated that activation of PKC prevents (premature) oligodendrocyte differentiation, whereas further differentiation toward myelinating oligodendrocytes is dependent on other signals, like elevation of cAMP levels, which may counteract the PKC signal transduction pathway. In chapter 3 it was revealed how activation of PKC keeps the oligodendrocyte progenitors arrested in the pro-oligodendrocyte stage. This is accomplished by regulation of the actin cytoskeleton structuring via phosphorylation of myristoylated alanine-rich C-kinase substrate (MARCKS). Upon PKC-mediated phosphorylation, MARCKS was translocated from the plasma membrane to the cytosol, and consequently no longer capable of regulating actin-membrane interactions. As a result, actin filaments are redistributed to the submembranous or cortical actin cytoskeleton, which caused an impediment of plasma membrane-directed traffic of ‘basolateral’-sorted proteins. On the other hand, under the same conditions, PKC activation stimulated the transport of ‘apical’-predestined proteins to specific sites just underneath the plasma membrane. Another PKC target is probably involved in this differential control of cognate polarized transport, but did not interfere with the plasma membrane-directed transport of oligodendrocyte progenitors.

**Extracellular signaling**

To establish an effective therapy for MS, it is not only necessary to block the basic fibroblast growth factor (FGF) signal transduction pathway, as described in chapter 2, but also cortical actin cytoskeleton structuring of oligodendrocyte progenitors establishes the proliferative potential. This was shown with PKC-specific knockout (KO) mice, where oligodendrocyte progenitors did not proliferate in vitro. This raises the question of whether pre-exposure to FGF and pp70 S6 kinase ever induced oligodendrocyte lineage progression in mature, mitogenically unresponsive, myelinating oligodendrocytes. If such a signal is involved in the differentiation of myelinating oligodendrocytes, then PLP was induced and expressed.
Although its genetic factors for MS is not breakdown via remyelination, become equally transplanted rate, proliferate become induced. To be induced. To induced therapy, it is important during project, described and intracellular polarized trafficking pathways, as direct perturbation of the actin cytoskeleton perturbation did not interfere with the translocation of ‘apical’-like proteins. Final incorporation into the plasma membrane might be hindered by a lack of appropriate docking devices in oligodendrocyte progenitors.

**Extracellular signaling: PDGF and bFGF signal transduction pathways**

To establish the physiological relevance of the PKC-related effects, we investigated whether or not inhibition of oligodendrocyte differentiation by platelet-derived growth factor (PDGF) or basic fibroblast growth factor (bFGF) was related to PKC activation. In chapter 4, it is described that both PDGF and bFGF induce phosphorylation and translocation of the PKC substrate, MARCKS, and as a consequence induce redistribution of actin filaments to the cortical actin cytoskeleton. This indicates that PKC activity is instrumental in inhibiting oligodendrocyte differentiation by both growth factors. Yet, since the induction of proliferation was not PKC-mediated, differentiation was still inhibited after pre-treatment with PKC-specific inhibitors. Hence, to induce differentiation in PDGF- or bFGF-activated oligodendrocyte progenitors a simultaneous abrogation of the growth factor-induced proliferative activity is necessary. The mitogenic activity of PDGF and bFGF was blocked by pre-exposure to inhibitors of p42/p44 mitogen-activated protein kinase (MAPK), p38 MAPK, and pp70 S6 kinase, while the activity of these kinases was essential for the onset of oligodendrocyte differentiation as well. We suggest therefore, that oligodendrocyte lineage progression is regulated by independent signaling pathways, acting in parallel and steering mitogenic activity and/or differentiation arrest. As a consequence, oligodendrocyte maturation, and subsequently myelination, is accomplished by the action of two or more signals acting in concert that counteract PDGF- and/or bFGF-activated signaling pathways. Such a signal could be cAMP-dependent kinase (PKA)-mediated, as PKA activation was able to partly abolish the proliferative activity of bFGF.

**Signaling in an in vitro myelinating culture**

To study the significance of regulation of oligodendrocyte differentiation via modulation of signaling events with respect to (re)myelination in MS lesions, we introduced and optimized an in vitro myelinating system derived from fetal rat brain. In these cultures astrocytes, neurons and oligodendrocyte are present, and thereby mimicking the in vivo situation as closely as possible. Using this in vitro myelinating system, we describe in chapter 5 that when PKC or bFGF signaling routes were activated during development, oligodendrocyte differentiation was inhibited. The expression of the late myelin-specific proteins MBP and PLP was impeded, in contrast to the early myelin-specific proteins CNP and MAG that were expressed. Accordingly, myelin formation was absent in these cultures. Surprisingly, myelin...
formation was also absent upon continuous occupancy of PDGF receptors during development, even though CNP, MAG, MBP and PLP were expressed in normal amounts in these cultures. This indicates that differentiation per se does not inherently lead to myelination. We anticipate that this in vitro myelinating system will provide further insight into the regulation of differentiation and the onset of myelination. Preliminary experiments have also shown that it is possible to design a demyelinating model of this in vitro myelinating system, thus allowing a detailed study of the signaling events required for remyelination.

**Identification of functional caveolae in the oligodendrocyte lineage**

A mechanism to control asynchronous oligodendrocyte differentiation may imply a localized signaling via caveolae, which are known as organized signaling centers at the plasma membrane of mammalian cells. In contrast to previous claims, the results described in chapter 6 demonstrate that caveolin-1, the major ‘caveolar coat’ protein, is expressed in a oligodendrocytic cell line (OLN-93) and even in primary oligodendrocytes, albeit to a lesser extent. Furthermore, we demonstrated that functional caveolae were present in the oligodendrocyte lineage. However, electron microscopic analysis and cholera toxin subunit B (CTB) internalization assays showed that not every cell in the oligodendrocyte lineage assembled caveolae. Indeed, indirect immunofluorescence microscopy revealed that the majority of caveolin-1 was intracellularly located in oligodendrocytes. In this regard, the data indicate that functional assembly of caveolae is cell-cell contact-dependent. Therefore, it is possible that the critically timed regulation of oligodendrocyte differentiation is accomplished via local and organized signaling in caveolae. Furthermore, since caveolin-1 and caveolae has been implicated in polarized traffic events and given its intracellular localization, a role for caveolin-1 in vesicular trafficking in the oligodendrocyte lineage is suggested.

**General conclusions**

Taken together, the studies described in this thesis have provided insight into the complex regulation of oligodendrocyte lineage progression. The results obtained can be summarized in two fairly simplified models, describing (i) intracellular signaling events involved in oligodendrocyte differentiation (fig. 1A) and (ii) the complex interplay between extracellular and intracellular signaling events, leading to proliferation and/or differentiation (fig. 1B). Briefly, oligodendrocyte differentiation can be blocked by PKC activation or by p42/p44 MAPK, p38 MAPK or pp70 S6 kinase inhibition (fig. 1A). There seems to be no cross-talk between signaling pathways activated by the particular PKC signaling route and these other kinases. PKA activation can negatively interfere with PKC-mediated inhibition of oligodendrocyte differentiation. The PDGF- and bFGF-mediated inhibition of oligodendrocyte
Figure 1: Schematic model of signaling factors involved in oligodendrocyte differentiation and/or proliferation. Both models are based on results obtained in studies, described in this thesis. A) intracellular signaling events in oligodendrocyte differentiation; B) extracellular activation of intracellular signaling events in both oligodendrocyte differentiation and proliferation.
differentiation occurs at least in part via PKC activation (fig. 1B). Yet, differentiation has to be accomplished by PKC inhibition, and a simultaneous abrogation of growth factor-induced proliferation via inhibition of either p42/p44 MAPK, p38 MAPK, or pp70 S6 kinase. However, as these latter signaling molecules are also involved in the induction of oligodendrocyte differentiation (fig. 1A), the length of the period of activation of these kinases is probably important for determining whether proliferation or differentiation will take place. Transient versus sustained activation can be accomplished via cross-talk with the PKA signaling pathway, as PKA activation can induce oligodendrocyte differentiation to the next developmental stage in PDGF-treated progenitors, and significantly inhibits bFGF-mediated proliferation.

Insight has also been obtained with respect to possible approaches in the development of remyelination-based strategies. Although studies were performed with rat-derived cultures, the results obtained will certainly facilitate research in human. The absence of a significant degree of spontaneous remyelination in MS lesions may be due to either the presence of inhibitory factors preventing myelin repair or the absence of cells or factors necessary for de novo myelin synthesis. Possibly, a combination of signaling factors, likely including PKC-inhibitors, might be beneficial to the treatment of MS in the (near-)future. Still, extensive work is necessary to further elucidate the most promising set of signaling factors, capable of inducing (re)myelination in MS lesions. Not only the signaling factors involved in proliferation and differentiation of oligodendrocyte progenitors, but also those involved in migration and survival of oligodendrocytes are of crucial importance in this respect.

**Perspectives**

Future fundamental experimental research should be focused on the interaction between different signaling pathways, which provides specific and precisely timed regulation of oligodendrocyte differentiation. The essential asynchronous differentiation of oligodendrocytes is probably accomplished via integration of general and local signals, such as those exerted by astrocytes or neurons. Localized signaling, as a modulator of oligodendrocyte lineage progression, provides a means by which the intrinsic differentiation pathway can be modulated according to the need for oligodendrocytes at any particular location. Specifically, the cross-talk between growth factor- and integrin-mediated signaling in oligodendrocytes needs to be further explored, since the same signaling molecules as those in growth factor signal transduction pathways were activated upon extracellular matrix (ECM) binding and integrin clustering (Juliano, 1996). Furthermore, an indication that integrin-mediated signaling may play a significant role in modulating oligodendrocyte differentiation, is dictated by the switching of integrin β subunits during differentiation (Milner and ffrench-Constant; 1994). Moreover, there is some evidence that integrin- or ECM-mediated adhesion of oligodendrocyte progenitors may modulate, affect, or even arrest. Alternatively, integrins may modulate adhesion, hydrophobic interactions responsible for cell migration and arrest induced by extracellular matrix proteins and at intracellular levels, cell signaling pathways or signaling molecules modulating transcription. This will certainly provide a basis to examine the possible use of such combinations in the treatment of MS.

Another interesting new area of research is the significance of growth factor- and integrin-mediated signaling in oligodendrocyte development, and the identification of factors that contribute to the regulation of integrin expression, differentiation, and migration. Attention should also be directed towards the role of ECM in the differentiation and survival of oligodendrocytes. To elucidate the role of the ECM in the onset of myelination, Dubois-Dalcourt et al. (1994, Peshkov et al., 1994) have shown that the ECM affects, or even arrests, oligodendrocyte differentiation in vitro. The characterization of the ECM at the onset of myelination in MS lesions.
Summary and perspectives

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