Chapter 8

General discussion and conclusions
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As an intrinsic part of the normal operative CNS, glial cells perform a plethora of functions. They comprise a large fraction of the brain’s cell population and their presence provides structure support, helping to mold physiologically functional components, such as the blood-brain barrier and myelin sheaths. Glial expression of factors influencing cellular growth, differentiation and survival, together with factors regulating immunological processes are of great importance and overwhelmingly complex. Elucidating mechanisms involved in governing regulation of such processes is important for understanding cellular events and crucial in the case of pathologies, where there is often urgency for therapeutic interventions. The pathological situation known as multiple sclerosis is a demyelinating disease of the CNS. Therapeutic strategies attempt to intervene in a variety of mechanisms associated with MS. One possible strategy involves the application of growth factors aimed at repairing damage caused by immune invasion and replenishing precursor cell populations. Because IGF-1 has the potential to stimulate myelin production and proliferation of oligodendrocyte precursor cells this factor provides a promising tool in treating demyelinating diseases. However, the complex nature of IGF-1 regulation is not completely understood. In order to design a rational approach to obtain the desired effects, our knowledge of the IGF-system in the CNS must be extended. This thesis describes studies, aimed at elucidating the involvement of IGF and the regulatory IGFBPs on glial cell functions, with emphasis on astrocytes.

Knowledge of spatial expression patterns of IGFBPs within a given neuropathological condition may help to interpret the contribution of these molecules in regulating the actions of IGF-1 in different cells during in the course of disease. Characterization and localization should help to predict possible mechanisms of action for IGF-1-based therapeutic interventions. Results obtained from studies described in chapter 2 reveal an involvement of microglia in IGF-1 regulation. The finding that microglia/macrophages under normal conditions as well as in the active plaque in MS express the type-1 IGF receptor indicates that IGFs are mitogens for these cells. Although receptor levels in MS lesions are not elevated, we can not dismiss that IGFs may play a role in the activation of this cell type. Interestingly, we do observed
selective expression of IGFBP-2 in activated microglia/macrophages in MS plaques. Similarly, increased levels of IGFBP-2, together with IGFBP-4, were detected in astrocytes within MS lesions (chapter 3). The predominance of immunoreactivity for all IGFBPs in control astrocytes indicates a major role these cells play in regulating IGF-1 in the CNS. As described in chapter 3, functional in vitro studies demonstrate inhibition of astrocyte proliferation by exogenous supplementation of IGFBP-2 and IGFBP-4. Combined treatment of IGFBP-2 with IGF-1, however, resulted in an increase of proliferation, as compared to IGF-1 treatment alone. In contrast, this binding protein did completely inhibit IGF-2 stimulated proliferation. IGFBP-4, conversely, did not demonstrate this effect but rather inhibited IGF-1 stimulated proliferation. In contrast to treatment on astrocytes, combined treatment of IGFBP-2 with IGF-1 on oligodendrocytes resulted in a strong decrease of IGF-1 stimulated cell survival. The results obtained from these studies indicate that the upregulation of IGFBP-2 in MS lesions may facilitate astroglial activation, rather than providing support for oligodendroglial survival.

Results, described in chapters 2 and 3, offer an indication for distinctive roles of IGFBP-2 and –4 in glial cells, in the MS diseased brain. Further in vitro investigations were conducted on isolated primary rat astrocytes to broaden our understanding of regulatory mechanisms of the IGF-system on this individual cell type. Detailed in chapter 4 are investigations performed on an in vitro model of astrogliosis. Within this model resting cells (differentiated) were compared to cells with a high proliferative status (reactive). Alterations occurring in the reactive cell include morphological changes, upregulation of GFAP and vimentin and elevated proliferative capacity. All of these characteristics simulate the reactive astrocyte in vivo. Within this model, we demonstrated the upregulation of IGFBP-2 in the reactive cell. Further, we discovered that proliferating astrocytes secrete a protease that degrades IGFBP-2. The question arose as to why proliferating astrocytes would upregulate IGFBP-2 only to degrade it again. We concluded that IGFBP-2 might be upregulated to sequester IGFs, yet degraded in the vicinity of the astrocyte to release IGF-1 to act on type-1 IGF receptors of the astrocyte and support proliferation. We suggest that the upregulation of IGFBP-2 as documented in several injury and disease models, as well as in MS as reported here, indicates it is a crucial entity in IGF
modulation. To fully interpret the emerging hypothesis further research will be required.

A perplexing issue arises in chapter 5. Immunocytochemical staining for IGFBP-4 was performed on astrocytes and revealed a unique intracellular localization site for this IGFBP. A series of experiments confirmed an association of IGFBP-4 with the centrosomes and microtubules in these cells. Staining was also performed on oligodendrocytes and microglia, for which this association could not be observed. The data suggests cell specificity and a role of this binding protein in functions of microtubules and/or centrosomes. Lack of data indicative of a precise functional role for intracellular IGFBP-4 lets us only speculate about the purpose of this association. Since its discovery approximately 10 years ago, very little knowledge over this molecule has come to light. In reference to other studies, we discuss in chapter 5 possible roles of this association, such as involvement in microtubule dynamic instability and spindle formation. Morphological alteration is one characteristic of reactive astrogliosis and involves restructuring of the cytoskeleton, including microtubules. If IGFBP-4 is involved in stabilization of the microtubule cytoskeleton (as discussed in chapter 5) it may aid in processes molding astrocyte morphology. Similarly, microtubule stability is an important factor for cellular proliferation. IGFBP-4 may also influence cellular growth by playing a role in dynamic instability of microtubule structures. In this respect, functions of IGFBP-4 are possibly independent of IGFs.

In chapter 6, we characterize mRNA expression of IGFBP-1-6 in primary rat microglia. In vitro activation of microglia was achieved by supplementing cultures with lipopolysaccharide. This model simulates inflammatory conditions and provides us with a means to compare resting with activated microglia cells. Within this model, we demonstrated basal expression of all IGFBPs except IGFBP-1. In the activated state we observed a down regulation of IGFBP-4 and IGFBP-6, whereas IGFBP-5 could no longer be detected. Contrasting findings of microglial expression of IGFBPs shown here, as compared to chapter 2, emphasize the difficulty of extrapolation of data derived from studies on rodent cells to human cells. Studies will be necessary to determine IGFBP expression at protein level in rat microglia cells and provide clarity
for these conflicting findings. Further examination will be necessary to evaluate precise mechanisms of IGF regulation in microglia.

Reactive Astrocyte

**IGFBP-2 upregulation in reactive astrocytes.**

Upon activation, astrocytes express higher levels of IGFBP-2, which sequesters IGF-1. In the vicinity of the astrocyte, proteases cleave IGFBP-2 and release IGF-1 to interact with type-1 IGF receptors on astrocytes.

In the investigations described in *chapter 7*, we initially intended to examine the effects of IGF-1 on oligodendrocyte survival. In an attempt to neutralize the type-1 IGF receptor on oligodendrocytes, we unexpectedly found an antibody, which enhances receptor activation on these cells, yet effectively neutralizes the receptor on other cell types including astrocytes. This finding is the first indication of a functional variance of this receptor expressed in oligodendrocytes. This intriguing finding offers a possible approach to specifically target IGF effects on oligodendrocytes. Further investigations must determine the mechanism of this unique receptor stimulation and elucidate whether or not this functionally diverging receptor characteristic is specific only for oligodendrocytes.
Role of astrogliosis: inhibiting or supporting remyelination

Astrogliosis occurs following injury and in several diseases of the CNS, such as MS. The gliotic scar has, until recently, been considered the major cause of regenerative failure in the CNS preventing remyelination by repressing oligodendrocyte development. Yet, it has become an increasingly popular idea that astrocytes may be a source of growth factors needed to promote oligodendrocyte survival and stimulate remyelination in the early lesion. In vitro, it is known that astrocyte monolayers support the survival and proliferation of oligodendrocyte progenitors. It has been suggested that reactive astrocytes serve to repair damage in MS by creating a milieu and providing growth factors for oligodendrocyte survival. In this sense, astrocytes boast a supportive role in remyelination processes in vivo. After induction of a lesion with ethidium bromide axons are denuding of myelin and oligodendrocytes and astrocytes are destroyed. Analysis of repaired lesions has shown that where remyelination occurs astrocytes create a fine network of their processes providing an environment, in which remyelination can take place.

The development of strategies to reverse mechanisms that induce reactive gliosis should be designed to mild the negative effects of an astrogliotic scar that impairs remyelination processes. An attempt to restrict astrocyte proliferation should be careful not to restrict proliferation of other cells involved in the repair processes, e.g. oligodendrocyte precursor cells. Furthermore, positive repair processes of the astrocyte population, such as providing a physical structure for remyelination and providing factors for neuronal survival should not be impaired. It seems likely, that astrogliosis and the presence of astrocytes in MS lesions may at some crucial point provide support for remyelination processes, yet at later stages of lesion development the glial scar may indeed impair repair processes. Therapeutic interventions must therefore find a balance in the dual effects of astrogliosis by alleviating glial scarring whilst not eliminating supportive functions of astrocytes.

Possible implications of IGF-1 axis in therapeutic strategies

Doubts on the effectiveness of growth factors as a therapy have arisen due to conflicting hypothesis of the role of astrogliosis in MS. Whether the induction of astrogliosis in MS is a response that facilitates or hinders the repair process remains a central question. It is, therefore, difficult to state whether or not therapies with growth
factor application may cause further damage by promoting astrogliosis and thereby aggravating the situation in a MS lesion. However, investigations with chronic relapsing EAE in mice where IGF-1 was applied to animals showed many positive effects of this treatment. BBB defects were reduced and the number and sizes of inflammatory and demyelinated lesions declined. In these studies, administration of IGF-1 was not targeted to any particular site or cell type suggesting that astrogliosis was not relevantly enhanced by this growth factor or at least did not impede oligodendrocyte development.

One obstacle preventing treatment with IGF-1 in man remains the BBB that impedes passage of this molecule into the CNS. It is known that IGF-1 poorly crosses the BBB in man. It has been suggested that this molecule can be transported into the CNS as a complex with IGFBPs capable of transcytosis through endothelial cells, such as IGFBP-1. It was also speculated that IGFBP-1 may not be synthesised at all within the human brain but is capable of crossing the endothelial cell border. This molecule may function to transport IGF-1 from the circulation across the BBB in form of an IGF-1/IGFBP-1 complex.

The characterization of the IGF-axis in the CNS of the normal and diseased or injured brain is beginning to reveal expression patterns that occur under these circumstances. Cell type specificity of the expression of the IGFBPs and their distribution in tissues during recovery from injury suggests complex interactions between specific cell types, IGFBPs, IGF-1 and the receptors. Strategies for transporting and targeting of IGF-1 to particular cell types or damaged regions remain to be established. A clearer understanding of the mechanisms of these molecules in the damaged brain must be obtained to devise effective therapies which selectively repair demyelinated regions and effectively treat diseases such as multiple sclerosis.