General Discussion
Astrocytes are among the most abundant and versatile cell types in the central nervous system (CNS). We are only just beginning to understand the importance of this cell type in normal brain homeostasis and consequently little is known about its role in neurological diseases. In multiple sclerosis (MS), astrocytes may contribute to or even underlie the pathogenesis of the disease. Generally, MS is characterized by inflammatory lesions throughout the CNS, inducing widespread demyelination and neuronal degeneration. In order to halt MS progression, it is essential to limit chronic brain inflammation and associated neuro-axonal degeneration. In this thesis we elucidated novel mechanisms by which astrocytes can affect neuroinflammation and neuronal function. We demonstrated that astrocytes play a cardinal, but dual, role in both neurodegenerative and neuroinflammatory processes and as such represent an interesting target for therapeutic intervention. Here, our results will be summarized, discussed and put into perspective.

DUAL ROLE OF REACTIVE ASTROCYTES IN INFLAMMATORY MS LESIONS

Blood-brain barrier function and leukocyte infiltration

MS is characterized by the formation of inflammatory demyelinating lesions. In active MS lesions, leukocytes infiltrate the brain and phagocytose myelin, leading to oligodendrocyte and axonal loss. Although the initial trigger for leukocyte influx remains unknown, astrocytes may play a very important role in this process. Astrocyte dysfunction can initiate neurological diseases including Alexander disease and neuromyelitis optica (NMO). Alexander disease is caused by glial fibrillary acidic protein (GFAP) mutations and NMO has long been considered to be subtype of MS. Recent findings demonstrate that, in a majority of cases, NMO is characterized by an autoimmune reaction against aquaporin 4, a water channel expressed on astrocyte end-feet. Intriguingly, both NMO and Alexander disease result in demyelination and oligodendrocyte and axonal damage. It has been suggested that an autoimmune reaction against astrocytes may play a role in the pathogenesis of MS as well. Srivastava et al. found high amounts of antibodies against the astrocytic potassium-channel KIR4.1 in the cerebrospinal fluid (CSF) of 50% of the MS patients. However, the role of KIR4.1 antibodies in MS is heavily debated as a recent study could not repeat the findings of Srivastava et al. More convincing evidence for the involvement of astrocytes in early MS lesions formation comes from the fact that reactive astrocytes in MS are not only present within the lesion, but are typically also found in the NAWM adjacent to the lesions. Moreover, in experimental autoimmune encephalomyelitis (EAE), astrocyte reactivity is observed before immune cells infiltrate the brain. Taken together, these studies demonstrate that (reactive) astrocytes may underlie neuroinflammatory and degenerative processes and may play an important role in early MS lesion formation.

Astrocytes are the gatekeepers of the brain as they regulate and form an essential part of the blood-brain barrier (BBB). Secretion of sonic-hedgehog and retinoic acid by reactive astrocytes was shown to enhance endothelial barrier formation in active MS lesions. Although reactive astrocytes are generally protective they may cause unwanted damage. Astrocytes in MS lesions secrete a wide array of inflammatory and vasoactive molecules including; tumour necrosis factor-alpha (TNF-α), interleukin 1beta (II-1β), vascular endothelial growth factor (VEGF) interleukin-6 (IL-6), chemokine C-C motif ligand 2 (CCL2), and ATP. These molecules cause an
upregulation of vascular adhesion molecules and disrupt the BBB, thereby enhancing leukocyte migration and promoting oligodendrocyte and axonal loss. In chapter 2 we demonstrated that under inflammatory conditions, astrocytes produce and secrete increased amounts of acid sphingomyelinase (ASMase)-derived ceramide, a bioactive lipid which disrupts the BBB and enhances leukocyte migration \textit{in vitro} (Figure 1A, red arrow). Treatment of astrocytes with Gilenya®, a novel MS therapeutic which prevents T-cell egression from the lymph nodes, resulted in decreased production of astrocytic CCL2 and ceramide and reduced leukocyte migration. In addition, in chapter 3 we showed that the expression of peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) is increased in reactive astrocytes in active MS lesions. PGC-1α is a co-transcription factor involved in a variety of mitochondrial functions and reduces secretion of astrocytic IL-6 and CCL2 \textit{in vitro} (Figure 1A, green arrow). Mice devoid of astrocytic CCL2 exhibited reduced leukocyte infiltration and demyelination during EAE, illustrating the significance of astrocyte-derived inflammatory molecules for leukocyte migration and lesion formation.

Although reactive astrocytes are essential to limit inflammation, the data described in this thesis suggest, in line with current literature, that astrocytes in active MS lesions play a dual role by both enhancing and limiting leukocyte infiltration and inflammation. It is important to gain insight in these mechanisms in order to promote the intrinsic protective mechanisms and limit the detrimental processes in reactive astrocytes. Unfortunately, it remains difficult to determine whether astrocyte activation may precede and initiate leukocyte infiltration, since post-mortem obtained MS tissue only provides a snapshot of the pathology at the time of death. Although experimental animal models are valuable to mimic relevant disease processes in order to better understand and counteract such mechanisms, they are unsuitable to investigate the aetiology of MS. The last decade, several novel positron emission tomography (PET) tracers have been developed, which can monitor reactive astrocytes, activated microglia and infiltrating macrophages in patients. Promising astrocytic PET tracers include; aquaporin 4, monoamine oxidase-B (MAO-B), an enzyme located on the outer mitochondrial membrane of astrocytes and translocator protein 18 kDa (TSPO). TSPO is upregulated upon neuroinflammation, but may also visualize activated microglia. Finally, acetate is a selective energy substrate for astrocytes, it can be converted to acetyl-CoA and enter the tricarboxylic acid (TCA) cycle, therefore radioactive labelled acetate can provide insights on astrocytic TCA cycle activity. These new imaging techniques may prove a valuable tool to elucidate the time-frame of lesion formation and the role of astrocytes in this process.

**Glucose metabolism**

Golgi already postulated at the end of the 19th century that astrocytes provide metabolic support to the brain. To date, this is still recognized to be one of the most important functions of astrocytes, although, surprisingly little is known about metabolic alterations in disease. In chapter 4 we showed that reactive astrocytes in active MS lesions upregulate the expression of essential glucose and monocarboxylate transporters. Moreover, in chapter 5 we demonstrated increased expression of key glycolytic and tricarboxylic acid (TCA) cycle enzymes in active MS lesions (Figure 1A, blue arrow). Our data suggest that astrocytic glucose metabolism is
enhanced in active MS lesions. This is in line with the previous reported increase in the number of mitochondria in astrocytes in active MS lesions. In order to confirm our findings, both the activity and expression of additional glucose metabolic enzymes should be studied in MS tissue. We identified PGC-1α to be a key regulator of astrocyte metabolism, as PGC-1α regulates the transcription of nutrient transporters and TCA cycle enzymes. In contrast, PGC-1α has a negative impact on the expression of glycolytic enzymes, therefore other mechanisms are likely to be involved in the induction of glycolytic gene transcription. Hypoxia inducible factor 1α (HIF-1α) is one of the most important transcriptional regulators of glycolysis and was found to be increased in astrocytes in a subset of (early) active MS lesions. Inflammation and oxidative stress are prominent in active MS lesions and likely contribute to the increased astroglial metabolic activity as shown previously. In fact, in chapter 3, we showed that astroglial PGC-1α is upregulated by reactive oxygen species (ROS), whereas exposure of astrocytes to ROS also increases HIF-1α (unpublished data). In addition to cellular stress, glucose metabolism is also elevated when the energy demand rises. Astrocytes in MS lesions are essential to detoxify the environment by scavenging ROS, clearing excessive glutamate and maintaining ionic homeostasis, which are energy consuming processes, consequently increasing the need for energy. Thus, an increased demand for energy, ROS and inflammation increase the metabolic activity of astrocytes in MS lesions.

Alterations in astrocyte metabolism can have widespread effects for the whole CNS since astrocytes provide trophic support to surrounding cells. On the other hand, astrocytes may also adjust their metabolism to the needs of neighbouring cells. In order to provide metabolic support astrocytes are closely associated with oligodendrocytes via gap junctions. However, in MS lesions as well as the surrounding NAWM, gap junctions between astrocyte and oligodendrocytes are disrupted, which may have significant consequences for brain glucose metabolism and oligodendrocyte functioning. In turn, oligodendrocytes provide trophic support to the axon, but without adequate supply of nutrients by astrocytes, metabolic coupling could be hampered leading to axonal damage. Metabolic changes are not restricted to MS lesions but are found throughout the brain of MS patients. Moreover, even before patients are diagnosed with MS, the levels of several key metabolites was found to be altered in both the brain and CSF. Intriguingly, astrocytes cultured in medium containing high glucose levels become reactive, activate the key inflammatory molecule nuclear factor κ B (NFκB) and secrete pro-inflammatory mediators, illustrating that subtle metabolic changes may contribute to the ongoing inflammation in MS. In order to investigate the possible involvement of metabolic alterations in lesion formation, it is important to elucidate the metabolic alterations in the NAWM in more detail. Subsequently, these metabolic changes can be mimicked in an animal model to evaluate whether this may contribute to inflammation and lesion formation.

Axons are, together with oligodendrocytes, the main victims of the inflammatory attack in MS lesions. Although many demyelinated axons survive the initial attack, axonal degeneration continues slowly over time, which is thought to be one of the major contributors to MS progression. Therefore, it is important to elucidate mechanisms underlying axonal degeneration. Demyelinated axons require more energy to maintain proper conduction. Hence, it was not surprisingly that, in chapter 5, we found an evident increased expression of glycolytic
and a slight increase of several TCA cycle enzymes in axons, in line with the previously reported increased mitochondrial mass (Figure 1A, yellow arrow)\textsuperscript{18,34}. However, the expression levels of alpha-ketoglutarate dehydrogenase (αKGDH) were reduced in axonal mitochondria, which may impair mitochondrial metabolism. The role of αKGDH in MS will be discussed in more detail in the next section. Remarkably, we found no changes in the expression of axonal nutrient transporters in active MS lesions in chapter 4. Thus, astrocytes and, to a lesser extent, axons have increased expression of a variety of key metabolic genes, which are at least partly regulated by PGC-1α, to match the increased energy demand during inflammation and upon demyelination. Yet, increased metabolic activity may result in cellular stress, which will be discussed in the next section.

**Oxidative stress**

Oxidative damage is one of the key mechanisms leading to oligodendrocyte and axonal degeneration in MS as illustrated by the accumulation of oxidized DNA and lipids \textsuperscript{35}. Microglia and macrophages are the most prominent source of ROS in active MS lesions \textsuperscript{36}, whereas astrocytes are a major source of nitric oxide (NO) \textsuperscript{37}. Conversely, astrocytes are also the main source of antioxidants and are essential for maintaining the redox balance \textsuperscript{38}. Oxidative stress can be the result of increased extra- and intracellular levels of ROS. Intracellular ROS can be produced as a by-product of mitochondrial metabolism during oxidative phosphorylation (OxPhos) and by reactions catalyzed by alpha-ketoglutarate dehydrogenase (αKGDH). In order to control the redox balance, mitochondria are equipped with a specific antioxidant apparatus, which includes the mitochondrial antioxidants peroxiredoxin-3 (Prx3) and thioredoxin-2 (Trx2). Since mitochondrial content and activity is enhanced in MS lesions, this is accompanied by increased intracellular ROS production. When the antioxidant capacity is insufficient to maintain the redox balance, this may ultimately lead to mitochondrial dysfunction and apoptosis. In chapter 3, we showed that Prx3 and Trx2 are under the control of PGC-1α and are upregulated in reactive astrocytes in active MS lesions (Figure 1B). By increasing PGC-1α, Prx3 and Trx2 expression, astrocytes can increase mitochondrial metabolism without disturbing the redox balance. Overexpression of PGC-1α and Prx3 and Trx2 \textit{in vitro} protects astrocytes and also surrounding cells against exogenous ROS. Thus, elevated expression of PGC-1α and downstream mitochondrial antioxidants is an important mechanism to counteract both intracellular ROS produced by mitochondria and exogenous ROS derived from macrophages and activated microglia in active MS lesions.

In contrast, despite increased intra- and extracellular ROS levels, the expression of axonal cytosolic and mitochondrial antioxidants remains unaffected in active MS lesions \textsuperscript{39}. Consequently, demyelinated axons show signs of extensive oxidative damage, which can impair mitochondrial function and axonal viability \textsuperscript{35}. In addition to mitochondrial antioxidants, cytosolic antioxidants are also essential to maintain the redox balance. Glutathione is one of the most important cytosolic antioxidants and requires NADPH for its antioxidant activity. NADPH is predominantly generated in the pentose phosphate pathway (PPP). The PPP is a pathway parallel to glycolysis which metabolizes glucose to generate NADPH. Thus, glucose is needed to maintain both glycolysis and PPP activity. Under normal conditions, neurons have a low glycolytic flux, which allows glucose to be used to generate NADPH in the PPP. High neuronal glycolytic rates result in lower PPP activity and thus reduced antioxidant capacity, thereby increasing oxidative stress and
possibly contributing to neuronal degeneration \(^{40}\). Increased uptake of glucose could overcome this problem, however in **chapter 4** we found that demyelinated axons in MS lesions express reduced amounts of nutrient transporters. In order to evaluate the involvement of the PPP and related antioxidant capacity in MS associated neurodegeneration, the expression and activity of the enzymes involved in this metabolic pathway should be examined in MS tissue. Interestingly, the glycolytic enzyme HK2, may also play an important role in this process as cytoplasmic HK2 has been shown to promote PPP activity, whereas mitochondrial-bound HK2 directs glucose through glycolysis \(^{41}\). Therefore, we are currently determining the intracellular localization of HK2 in more detail.

We observed that axonal αKGDH expression was reduced in MS lesions, as previously described for other neurodegenerative disorders. αKGDH is one of the rate limiting enzymes of the TCA cycle because it has the lowest activity and its activity is regulated by many different factors. αKGDH is also highly sensitive to oxidative stress, which may explain why the expression of axonal mitochondrial αKGDH is reduced in MS lesions, whereas the expression of other TCA cycle enzymes remains unaffected\(^{42}\). The TCA cycle not only generates electron donors for the OxPhos but also provides the mitochondrial antioxidant machinery with the necessary reducing equivalents in order to regenerate their function upon oxidization. Reduced αKGDH results in lower TCA cycle activity, which may inhibit mitochondrial antioxidant capacity and oxidative phosphorylation since essential metabolites for these processes are produced in the TCA cycle. In sum, increased axonal oxidative stress due to inadequate antioxidant capacity could lead to reduced levels of αKGDH, which further impairs mitochondrial function. In order to investigate whether these processes are associated in MS, αKGDH immunostainings should be correlated to OxPhos activity, oxidative damage and mitochondrial antioxidant levels (Figure 1C) \(^{34}\).

Increased release of ceramide by astrocytes as described in **chapter 2**, may also contribute to mitochondrial dysfunction. Ceramide can readily cross the mitochondrial membrane inducing ROS production via complex I or by inducing cytochrome C translocation, leading to electron leakage, a drop in mitochondrial membrane potential and eventually inducing apoptosis \(^{43,44}\). Moreover, a recent study showed that exposure of neurons to ceramide induces oxidative damage and reduces neuronal respiratory capacity \(^{45}\). Interestingly, Gilenya® can prevent mitochondrial cytochrome c release and the related drop in membrane potential under different conditions \(^{46,47}\). As such, it would be interesting to evaluate the protective effects of Gilenya® on mitochondrial function in more detail.

Taken together, in active MS lesions reactive astrocytes are actively involved in limiting inflammation and detoxification of the environment, resulting in increased glucose metabolism. Increased metabolic activity enhances ROS production, which is compensated in astrocytes by increased expression of cytosolic and mitochondrial antioxidant enzymes. However, in axons, enhanced ROS production does not coincide with increased antioxidant capacity, which might contribute to axonal oxidative stress, mitochondrial dysfunction and axonal degeneration. Thus, although astrocytes effectively scavenge free radicals and aim to provide trophic support, this is insufficient to protect the surrounding axons in active MS lesions.
REACTIVE ASTROCYTES IN CHRONIC INACTIVE MS LESIONS

Over time, some of the active MS lesions transit into chronic inactive lesions. In these chronic lesions, inflammation has abated and demyelinated axons are embedded in the astroglial scar. The astrocytic scar is made up of hypertrophic astrocytes with thick processes, which are tightly connected through gap junctions. Hypertrophic astrocytes produce extracellular matrix proteins thereby forming a physical barrier which limits further demyelination and inflammation. Conversely, this barrier also hampers remyelination by blocking oligodendrocyte precursor cell (OPC) migration, which is needed for remyelination. Furthermore, astrocytes in inactive MS lesions, express elevated levels of hyaluronan, which inhibits maturation of OPCs. On the other hand, astrocytes produce a wide range of growth factors which enhance OPC migration and proliferation. Astrocytes supply oligodendrocytes with nutrients and lipids needed for ATP production and (re)myelination. However, gap junctions between astrocytes and OPCs are reduced in MS which may hamper adequate OPC proliferation and remyelination. In inactive MS lesions, astrocytes produce less inflammatory molecules like CCL2 and ceramide, but secretion of proteoglycans inhibits neurite outgrowth and axonal regeneration. Nevertheless, demyelinated axons survive for many years in an environment consisting almost exclusively of astrocytes and their products. Moreover, experimental studies illustrate that inhibition of astrocyte proliferation or astrocyte depletion worsens the outcome of EAE and impairs proper repair. Thus, although astrocytes in chronic inactive MS lesions can have detrimental effects on oligodendrocyte repair and axonal function, astrocytes appear to be generally neuroprotective.

Our immunohistochemical analysis described in chapter 4 and 5 suggests that astrocytes become more glycolytic in inactive MS lesions. Mitochondrial metabolism, as indicated by expression levels of TCA cycle enzymes, PGC-1α and mitochondrial antioxidant enzymes is reduced, whereas expression of glycolytic enzymes is increased in inactive compared to active lesions (Figure 2B). Increased glycolytic rates commonly coincide with enhanced production of lactate, also known as the Warburg effect. Lactate can be produced from pyruvate by lactate dehydrogenase (LDH), which forms a multienzyme complex consisting of LDHA and LDHB. LDHA converts pyruvate into lactate, whereas LDHB is involved in the generation of pyruvate. We observed an increased ratio of LDHA/LDHB and enhanced expression of the lactate transporter MCT1 in astrocytes, suggesting that astrocytic lactate production and secretion is increased in inactive MS lesions (Figure 2A). Lactate is an important metabolite for axons, which can be metabolized into pyruvate to fuel the TCA cycle and is under normal conditions supplied by oligodendrocytes. However, in the absence of oligodendrocytes astrocytes may take over this role. In chapter 5 we described that astrocytes and axons are more closely associated in MS lesions compared to the surrounding NAWM. Whereas astrocytic production and secretion of lactate is increased, the LDHA/LDHB ratio is reduced in demyelinated axons, which may lead to enhanced axonal lactate utilization (Figure 2A). In chapter 4 we showed that the axonal expression of GLUT3 and MCT1 was reduced in inactive MS lesions. However, extracellular levels of lactate are elevated in inactive MS lesions, suggesting that despite the downregulation of axonal MCT2 expression axons are still able to take up sufficient amounts of lactate. In order to prove the functionality of the hypothetical lactate shuttle between astrocytes and demyelinated axons more experimental studies are needed. Intravital microscopy of chronically demyelinated lesions induced in an experimental animal
Figure 1 (previous page). Schematic overview of reactive astrocytes in active MS lesions. A) In active MS lesions macrophages produce large amounts of TNF-α, IFN-γ and ROS. TNF-α induces ASM expression, resulting in increased production of ceramide, which disrupts the blood-brain barrier and enhances leukocyte infiltration. Ceramide may also affect mitochondrial ROS production (red arrow). Macrophage-derived TNF-α and IFN-γ increases IL-6 and CCL2 release by reactive astrocytes, which can be attenuated by overexpression of PGC-1α. Upregulation of PGC-1α enhances levels of glucose and lactate transporters and reduce mitochondrial ROS production (green arrow). Expression levels of nutrient transporters and key glycolytic and TCA cycle enzymes are increased in reactive astrocytes (blue arrow). In demyelinated axons glycolysis and mitochondrial content is increased, but mitochondrial function is impaired resulting in increased ROS production (yellow arrow). B) Astrocytic mitochondria in active MS lesions have increased expression of TCA cycle enzymes, oxidative phosphorylation and the mitochondrial antioxidants Prx3 and Trx2. C) Axonal mitochondria in active MS lesions have increased expression of pyruvate dehydrogenase and malate dehydrogenase but decreased expression of α-ketoglutarate dehydrogenase. Expression of axonal mitochondrial anti-oxidants is unaffected in active MS lesions and oxidative phosphorylation activity is reduced in a subset of axons, leading to increased levels of ROS.

model can be used to analyze lactate production and utilization visualized with a fret-based lactate nano-sensor. Additional experiments are also required to demonstrate the astrocyte-axon interactions in MS lesions more detail. The GFAP stainings we performed to illustrate increased association between astrocytes and axons, are not fully representative since GFAP expression levels are increased in MS lesions. Therefore, additional astrocyte markers including, glutamine synthetase, glutamate transporters, S100 calcium binding protein B (S100B) and vimentin need to be included. Alternatively, astrocytes can be loaded with a fluorescent dye to visualize the complete astrocyte including its processes, in a demyelinating animal model. Furthermore, EM analysis is required to examine the astrocyte-axon interactions in more detail. To date, very little is known about astrocyte-axon interactions in both health and disease. It is known that astrocytes contact axons at the node of Ranvier, but it is not completely understood how these structures connect and what the functional role of these interactions is. Instead, most research has focussed on astrocyte-neuron interactions and the involvement of astrocytes in the tripartite synapse.

Lactate taken up by axons is metabolised into pyruvate which can enter the TCA cycle to generate electron donors for OxPhos. Since axonal aKGDH and OxPhos activity are reduced in inactive MS lesions it remains indefinite whether lactate can be effectively utilized as an energy source in demyelinated axons (Figure 2C). Alternatively, lactate utilization could also be an important mechanism to restore the neuronal redox balance, since metabolising lactate into pyruvate gains NADH. However, more research is needed to elucidate the functions of lactate in more detail. The expression of axonal glycolytic enzymes is even higher in inactive compared to active lesions, perhaps to compensate for the increased energy demand and mitochondrial dysfunction. Axons are not well equipped to handle high glycolytic rates, as they heavily depend on the PPP for their antioxidant capacity, therefore increased glycolytic flux may also contribute to the ongoing degeneration. Thus, mitochondrial metabolism is less evidently increased in inactive lesions compared to active lesions, which may be compensated by increased glycolytic rates. Astrocytes reside in close proximity of the demyelinated axons and play an important role in maintaining axonal function possibly by supplying lactate.

ASTROCYTES AS A THERAPEUTIC TARGET

Astrocytes are involved in all stages of MS pathology and have a great impact on the function of neighbouring cells, making astrocytes an interesting therapeutic target. Although reactive astrocytes are generally protective, they are involved in several detrimental processes in MS.
Hence, it is important to limit cytotoxic and simulate intrinsic protective properties of astrocytes. Hereto, production of ceramide and other pro-inflammatory mediators should be limited (1), whereas the production of (mitochondrial) antioxidants should be increased (2). In order to limit axonal degeneration metabolic function of demyelinated axons should be restored (3). Finally, this section will briefly discuss the potential of astrocytes and astrocyte-derived products as a biomarker to monitor disease progression (4).

(1) Here, we discovered that Gilenya® reduced astrocyte-derived ceramide production. Although we mainly focussed on astrocyte-endothelial interactions, reducing ceramide production with Gilenya® could also be neuroprotective since ceramide can induce neuronal mitochondrial dysfunction and cell death. Currently, Gilenya® treatment is primarily aimed at suppressing T-cell egression from the lymph nodes and is accordingly approved for RRMS only. However, our data suggests it may be beneficial to continue treatment in progressive MS patients because Gilenya® may also suppress local inflammation in the brain. Several other drugs which can modulate the inflammatory profile of astrocytes include: laquinomod, interferon-beta (IFN-β), glucocorticoids and tacrolimus (macrolide). Laquinomod has been shown to reduce NFκB activation in astrocytes resulting in reduced production of inflammatory cytokines and reduced demyelination in a MS animal model. IFN-β, glucocorticoids and tacrolimus are all immunosuppressive drugs that have been show to reduce astrocytic production of inflammatory molecules although the underlying mechanism are not completely understood.

(2) In order to boost the brain's antioxidant capacity, there are several interesting therapeutics available. The recently introduced drug Tecfidera® can activate nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a transcription factor which activates genes with an antioxidant redox element (ARE) thereby increasing the cellular antioxidant capacity. Therefore, it will be interesting to monitor the long-term effects of Tecfidera® and study its therapeutic potential for progressive MS. Moreover, MitoQ an antioxidant molecule which targets mitochondria has been shown to be effective in EAE animals and other neurodegenerative models but has not been tested in MS patients yet.

(3) In order to counteract the ongoing neuronal degeneration it is vital to boost brain metabolism. Since ROS likely contribute to the observed mitochondrial dysfunction, antioxidant treatment may already improve glucose metabolism. We identified PGC-1α as an interesting new therapeutic target to limit inflammation, improve mitochondrial function and reduce ROS production. The remaining obstacle to overcome is to specifically target PGC-1α. Several studies have shown that resveratrol and progesterone, both known to activate PGC-1α, are neuroprotective and reduce clinical symptoms in EAE. However, these molecules do not target PGC-1α specifically making the actually effect on PGC-1α difficult to assess. We also found that αKGDH levels are decreased in MS patients, similar to other neurodegenerative diseases like Alzheimer disease (AD). Boosting αKGDH function may represent an interesting neuroprotective approach due to its cardinal role in ROS handling and metabolism. αKGDH activity can be increased by the vitamin thiamine, which is an essential cofactor of αKGDH. Thiamine or derivatives of thiamine have beneficial effects in AD patients, although larger studies should be performed to determine its neuroprotective potential.

In order to target astrocytes or other brain cells, drugs need to be delivered into the CNS specially.
This remains a major obstacle to overcome, although recent years progress has been booked, for example, glutathione labelled liposomes were shown to enhance drug delivery to the brain. Notably, glucocorticoids packed in these liposomes were shown to suppress neuroinflammation and are currently in phase I clinical trials in MS patients and healthy controls\textsuperscript{63}.

Biomarkers that monitor disease progression are warranted to improve assessment and understanding of the effectiveness of therapeutics in clinical trials, which is now mainly based on lesion load and clinical score. MS is a heterogeneous disease, therefore additional information could help to predict disease progression and develop individual treatment strategies. Since the astrogial phenotype is subjected to changes in the different stages of MS, astrocytes may be useful for the development of novel biomarkers. Interestingly, astrocyte-derived GFAP is increased in the CSF of MS patients and correlates with disease progression\textsuperscript{64,65}. Moreover, new PET tracers
have been developed that can monitor astrocyte reactivity in vivo, but remain to be tested in a large cohort of MS patients. Imaging studies have indicated that glucose and lactate levels are related to disease progression. Serum lactate levels are also increased and correlate with the clinical score of MS patients\textsuperscript{66}. However it is not clear which cellular source is responsible for the increased amount of lactate in the serum of MS patients. Muscles are likely a major source of serum lactate levels, though, the muscle mass of MS patients declines and exercise does not lead to altered serum levels of lactate in MS patients\textsuperscript{67,68}. Therefore, brain derived-lactate may also contribute to enhanced serum levels. Since we identified astrocytes as the main producer of lactate in MS lesions, peripheral lactate levels may partly reflect astrocyte activity. However, increased understanding of the functional role of glucose and lactate metabolism in MS patients is crucial to interpret these findings.

**CONCLUDING REMARKS**

In this thesis we have demonstrated an important role for astrocytes in inflammation, glucose metabolism and neuroprotection in MS. As such, astrocytes are involved in the initial steps of lesion formation, but also lesion resolution and MS progression. Current therapies for MS are aimed at repressing the immune system, which is effective to limit lesion formation and resulting clinical relapses, but do not affect the ongoing neurodegeneration and MS progression. Thus, these treatments mainly aid in reducing symptoms, but don’t stop or delay disease progression, which requires additional therapeutic strategies. Therefore, it is pivotal to gain more understanding about the aetiology of the disease. A new era of high resolution imaging techniques may shed new light on lesion formation and disease onset as this will allow to monitor cell and protein specific alterations (i.e. astrocyte activation) and drug efficacy in living patients. Such information can also be used to develop a proper animal model which better mimics MS. EAE only reflects the inflammatory phase of the disease but lacks the chronic and neurodegenerative aspects of MS. Without an appropriate animal model it is very difficult to develop potential new drugs which do not target the immune system directly. The other major challenge the MS community faces is to limit disease progression. MS progression is likely caused by ongoing local inflammation, associated mitochondrial dysfunctional and neurodegeneration\textsuperscript{69}. Therefore, new therapeutic approaches should aim to limit CNS inflammation and improve metabolic function and repair. We and others have shown that reactive astrocytes are star players in these processes and primarily aim to remove harmful triggers and limit inflammation, although the astroglial production of pro-inflammatory molecules can also be detrimental. Limiting the detrimental processes in astrocytes, for example with Gilenya®, or boosting the protective mechanism like PGC-1α function, is crucial to halt the slow burning inflammation and related degeneration in MS patients. Leukocyte infiltration can effectively be prevented with current therapeutics, leaving burnt-out demyelinated lesions throughout the brain, which mainly consist of astrocytes and axons. Here, we provide evidence that altered interactions of astrocytes with demyelinated axons could be essential for long-term axonal survival by scavenging ROS and supplying lactate. Thus, in order to maintain axonal function therapies should be aimed at improving the function and cross-talk of astrocytes and axons.

In conclusion, astrocytes are star players in metabolic regulation, redox handling and controlling
local inflammation. Given that inflammation induced ROS production and metabolic alterations play an important role in driving neurodegeneration, astrocytes represent an interesting therapeutic target to limit both inflammation and neurodegeneration in MS.

REFERENCES


