Glia in Alzheimer's Disease and Aging. Molecular Mechanisms Underlying Astrocyte and Microglia Reactivity

A.M. Orre

English Summary

Alzheimer's disease (AD) is the most prevalent form of dementia in our society and with the elderly population rapidly expanding the number of AD patients is expected to rise dramatically in the coming decades. The pathological hallmarks of AD are the formation of extracellular deposits of aggregated amyloid-β (Aβ) and the intraneuronal accumulation of hyper-phosphorylated tau, known as plaques and tangles, respectively. In addition, Aβ plaques are surrounded by reactive astrocytes and activated microglia, which are part of a neuroinflammatory response considered to be the main feature in AD pathogenesis. Reactive glia are believed to contribute to the disease progression through the release of proinflammatory molecules and reactive oxygen species, leading to neuronal damage and increased Aβ production. These data have mainly been obtained by histological analyses and *in vitro* experiments. However, it has been a challenge to unravel the exact role of astrocyte and microglia functions from histological analysis, since this method is always biased towards a specific question. Therefore, is still a great deal we do not yet know about their molecular and functional changes in relation to the pathogenesis and progression of AD. In this thesis, by using an unbiased genome-wide approach, we have set out to characterize the molecular phenotype of reactive astrocytes and activated microglia in relation to AD. This has been done by isolation and characterization of these cell populations *ex vivo* by cell-type-specific isolation and genome-wide characterization, in combination with histology and *in vitro* experiments. Using this approach we have obtained a more detailed insight into astrocyte and microglia functions and contribution to AD pathogenesis in the APPswePS1dE9 mouse model and in AD patients.

In the introductory chapter 1, we briefly describe the phenotypic and functional alterations in astrocytes and microglia in relation to Aβ stimulation and Aβ plaques in the context of AD. Furthermore, we introduce and discuss the role of the proteasome, particularly the immunoproteasome (iPS), in relation to astrocyte and microglia reactivity and neuroinflammation in AD. The ubiquitin proteasome system is the major protein degradation system within the cell and is a natural target for investigation in AD, as accumulation of ubiquitinated proteins is a prominent feature of AD. Over the last decade several studies have focused on the role of the proteasome system and found this system to be impaired in AD. However, until recently, little attention has been paid to the role of the iPS in neurodegenerative diseases; our understanding

of the iPS function is mainly based on studies of peripheral blood cells and tissues. In this chapter, we summarize what is known about the iPS in the brain and in relation to AD. In addition, we discuss the possibilities of targeting its activity to reduce neuroinflammation in AD.

In chapter 2, we aimed to elucidate the role of the proteasome in relation to glia reactivity in AD with a focus on Aβ (plaques). For this, we used novel methods including cell-permeable proteasome-activity probes – enabling readout of proteasome activity in living cells, and new proteasome subunit-specific activity assays. These new methods allowed us to get more detailed information on proteasome activity than the methods we used in previous studies. For the first time, we could analyze the different activities of both types of proteasome present in the cells: the more abundant constitutive proteasome and the immune-induced iPS. In strong contrast to previous studies, we found an increase in proteasome activity, specifically of the iPS, both in human AD tissue and in the APPswePS1dE9 AD mouse model. By means of immunohistochemistry and gene expression analysis we showed that plaque-associated reactive astrocytes and microglia were the main cell types expressing elevated levels of iPS subunits. Moreover, we showed that specific inhibition of the increased β5i iPS activity led to a strong reduction in the expression of proinflammatory molecules, such as Il1b, Tnf in LPS-stimulated microglia from aged AD mice. In this chapter, we thus provide a new insight in the function of the iPS in plaque-related glial cell reactivity and neuroinflammation in AD. We also suggest that the iPS could be a target for reducing neuroinflammation in AD.

Previous data from our lab show that inhibition of the proteasome reduces the expression of GFAP in astrocytes. In chapter 3 we therefore set out to test the hypothesis that proteasome activity is an important regulator of GFAP expression. We also investigated molecular mechanisms linking the proteasome to GFAP regulation. We treated astrocytoma cells with molecules known to increase proteasome activity in other cells and found two molecules that substantially increased proteasome activity in the astrocytes; one agonist for an opioid receptor, and one antagonist for a purinergic receptor. The induction of proteasome activity by these compounds was paralleled by an increase in GFAP, in line with the hypothesis. Both compounds also increased expression of iPS subunits and molecules involved in Notch signaling.

By using specific inhibitors for the iPS (β5i activity) or Notch signaling, we showed that both these pathways are vital for proteasome-mediated regulation of GFAP expression, probably via STAT3 activation. Interestingly, we showed that proteasome activation resulted in a more anti-inflammatory astrocyte phenotype, in contrast with the proinflammatory phenotype of reactive astrocytes isolated from aged AD mice (chapter 5). This highlights the heterogeneity of astrocyte reactivity, a point further discussed in chapter 6.

Research on functional changes of astrocytes and microglia in age-related, neurodegenerative diseases has been held back by the absence of a good method to isolate these glial cell types from the brains of aged mice. In chapter 4, we describe the set-up and optimization of a cell-isolation protocol to obtain viable and pure astrocytes and microglia from the aged mouse cortex based on cell surface markers. Using this optimized method we isolated cortical astrocytes and microglia from young adult (2.5 months) and aging (15-18 months) mice that were later subjected to genome-wide microarray analysis to generate expression profiles. These expression profiles provided valuable information on astrocytes and microglia functions and complemented the transcriptome from the currently available post-natal and young astrocytes. With this information, we could substantiate the concept of astrocytes as an important part of the tri-partite synapse, expressing many genes involved in neuronal signaling, and microglia as the main immune player in the brain. Furthermore, the comparison between young adult and aging populations gave an insight into the molecular alterations of microglia and astrocytes in relation to normal aging, and showed that aged astrocytes have a more pronounced inflammatory phenotype compared to young astrocytes. The inflammatory differences were more ambiguous in the microglia comparison; aging microglia had higher expression of genes within the TNF-ligand family and younger microglia showed higher transcript levels of CC-chemokines.

In chapter 5, we isolated astrocytes and microglia from wild type (WT) controls and AD mice of 15-18 months - an age where the plaque load and glia reactivity are extensive in the AD mice. To analyze the molecular alterations associated with this Aβ plaque-induced astrocyte and microglia reactivity, we performed a genome-wide expression analysis of the isolated WT and AD glia populations. Both

cell types showed an increased expression of proinflammatory genes, together with a parallel reduction of highly cell-type-enriched genes - vital for normal astrocyte and microglia functions, such as genes involved in glutamate uptake and conversion in astrocytes, and genes involved in endocytosis/phagocytosis, in microglia. These data suggest that reactive glia shift their efforts towards defense and repair tasks at the expense of their normal supportive functions, which may further exacerbate neuronal dysfunction and AD pathology. To confirm the mouse data with the expressional changes observed in human Alzheimer's disease, we used a clustering approach to compare the regulated gene clusters obtained from the mouse astrocyte and microglia populations with a gene expression dataset from the prefrontal cortex of human Alzheimer's disease and control donors. This comparison showed that the plaque induced inflammatory changes in the mouse astrocytes were remarkably similar to the inflammatory changes present in human AD. These expression data revealed strong indications for neuroinflammation and glia dysfunction, both likely to be contributing factors to the neuronal dysfunction and memory impairment observed both in the AD mouse model used here and in human AD pathology, as more extensively discussed in chapter 6.

In chapter 6, we discuss the benefits of cell-type specific analysis in relation to pathological changes observed in AD and the neurological phenotype of the APPswePS1dE9 mouse model, linking it to the change in glial phenotype induced by plaque pathology, as described in this thesis. Finally, we also discuss the role of neuroinflammation and its consequences for the initiation and progression of AD, and its potential as a treatment target for AD.