**Analysis of mediators of inflammation in Alzheimer's disease**

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Research question and background

Our research aim is to identify early changes in brain tissue that are associated with Alzheimer's disease (AD) pathogenesis. This may lead to the identification of therapeutic targets and of factors that can be used as biomarkers.

Methods and tissues used

We combine research at the tissue level (immunohistochemistry, as well as Western blotting and PCR to assess protein expression) with in vitro (cell culture) models. Microglia and astrocytes are isolated from post mortem brain specimens (periventricular white matter) collected in culture medium. Possible effects of factors that we find associated with amyloid deposits in the brain (amyloid associated proteins; AAP) on Aß-induced microglial and astrocyte activation, on Aß phagocytosis by microglia and possibly also astrocytes (fluorescence microscopy and FACS analysis) and on Aß-induced direct and indirect (glia-mediated) neurotoxicity are studied.

Results and conclusion

Initial immunohistochemical studies have identified AAPs associated with glial cell accumulation and activation in different types of Aß deposits. A large fraction of Aβ is N-terminally truncated rendering a glutamine that can subsequently be cyclized into pyroglutamate (pE). The pE-modified Aβ is more resistant to proteases, more prone to aggregation and increases its neurotoxicity. Expression of the enzyme glutaminyl cyclase (QC), which catalyzes conversion of glutamine to pE, and pE-modified Aβ both were found increased in the earliest stages of AD pathology, and may be an important event in the pathogenesis.

Our initial cell culture studies indicated that uptake of Aß by human microglial cells depends on Aß aggregation state, oligomers of Aß being taken up more readily than Aß fibrils. Different AAP (including Serum Amyloid P component (SAP), complement C1q, and apolipoproteins J and E) inhibited Aß uptake to some extent, and at the same time stimulated pro-inflammatory cytokine (IL-6 and TNF-$\alpha$) secretion by microglia. In further studies, also human astrocytes were found capable to ingest Aß, dependent on the degree of Aß aggregation and also, but sometimes with opposite effects, influenced by plaque associated proteins. Thus, a role for astrocytes in removal of Aß in early stages of aggregation was suggested, but the mechanism of uptake remained elusive. We now found that human astrocytes actively ingest Aβ via binding to the LRP-1 receptor, but not via scavenger receptor B1 or CD36. Studies to further characterize these and additional uptake routes are ongoing. In addition to Aβ uptake, we also identified the production of two of the main Aβ-degrading enzymes, neprilysin and insulin degrading enzyme (IDE) by human astrocytes. Protein and activity levels of these enzymes can be altered by the aggregation state of Aβ and the presence of AAPs, such as ApoE. The exact role of human astrocytes in Aβ clearance is subject of further studies.

We also seek for potential therapeutic approaches that reduce the neurotoxic neuro-inflammatory response elicited by microglia and astrocytes without affecting the ability of these cells to take up and remove Aβ from the brain. In earlier studies minocycline was found to hold promise. We now found that selective inhibition of IRAK-4, an essential kinase in the Toll-like receptor pathway, reduces the production of pro-inflammatory

cytokines. We found that IRAK-4 activity is increased in AD brain compared to non-demented control brain tissue. Selective inhibition of IRAK-4 does not affect the potential of microglia and astrocytes to take up Aβ.