**Identification of a key role for complement in neurodegeneration in multiple sclerosis**

Huitinga[1], I., Michailidou[2], I., Van Strien[3,4], M.E., Van Eden[1], C., Fluiter[2], K., Neal[5], J.W., Giles[5], J., Morgan[5], B.P., Baas[2], F., Ramaglia[1,2,5] ,V.

[1]Department of Neuroimmunology, Netherlands Institute for Neuroscience, Meibergdreef 47, 1105BA Amsterdam, The Netherlands; [2]Department of Genome Analysis, Academic Medical Center, Meibergdreef 9, 1105AZ Amsterdam, The Netherlands; [3]Astrocyte Biology & Neurodegeneration, Netherlands Institute for Neuroscience, Meibergdreef 47, 1105BA Amsterdam, The Netherlands; [4]Department of Translational Neuroscience, Brain Center Rudolf Magnus, University Medical Center Utrecht, P.O box 85060, 3508AB Utrecht, The Netherlands; [5]Institute of Infection and Immunity, School of Medicine, Cardiff University, Cardiff CF14 4XN, United Kingdom.

Research question and background

Multiple sclerosis is a chronic inflammatory disease of the central nervous system, leading to neurodegeneration in progressive patients. The mechanisms responsible for neurodegeneration are unknown. We previously showed that activation of the complement system, part of innate immunity, accelerates neuroaxonal degeneration after nerve or brain trauma; these findings, and the recent identification of the association between complement genetic variants and classical neurodegenerative diseases, led us to test the localization and function of complement in post-mortem multiple sclerosis deep grey matter, thereby identifying a key role for complement in neuroaxonal pathology in progressive multiple sclerosis.

Methods and tissues used

In this study, we made use of the post-mortem multiple sclerosis brainstem tissue collection of the NBB. We analysed 154 brainstem lesions of different stages and lesion-free areas from 10 multiple sclerosis donors, control brainstem from 7 non-neurological donors and 7 donors with other neurological diseases. We first analysed the presence, localization and extent of complement activation products in relation to neuroaxonal pathology in reactive, active, chronic active and inactive lesions in post-mortem brainstem tissue from chronic (progressive) multiple sclerosis; and we compared the findings to brainstem tissue of non-neurological controls and donors with other neurological diseases. We then investigated *in vitro* and *in vivo* the implications of complement deposition, in particular the MAC, for inflammation and neuroaxonal integrity.

Results and conclusion

In lesioned donor brainstem tissue we found deposition of the classical pathway activator C1q at synapses, localization of the core complement activation product C3d on neurons and axons, whereas the terminal complement activation product MAC, was found only on astrocytes. Neurons showed reduced expression of *DAF* mRNA, a critical regulator of C3 activation, and up-regulated stress markers. Axons showed disturbed fast transport and lost synapses. Neurons were strongly positive for *CD59* mRNA, the regulator of the MAC, likely explaining their MAC resistance; in contrast, astrocytes expressed little *CD59*, were MAC-coated, and, although there was no evidence of cell death, were positive for IL-1β in the lesions, implying that sub-lethal MAC induced production of pro-inflammatory molecules occurred in astrocytes. In mice genetically deleted for *DAF* or *CD59a*, regulation of the terminal complement pathway was crucial to prevent MAC deposition on neurones, subsequent development of neuropathology and clinical disability in the experimental autoimmune encephalomyelitis model of multiple

sclerosis. Pharmacological inhibition of the MAC prevented astrocyte activation, IL-1β production and neuronal damage, and suppressed clinical disease and severity of pathology in acute experimental autoimmune encephalomyelitis. Our findings demonstrate that complement activation occurs in grey matter in multiple sclerosis and plays a key role in neurodegeneration in experimental models and patients.