



Anti-GM1 Antibodies in Acquired Demyelinating Neuropathies

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The thesis is based on studies carried out during my employment in the Departments of Neurology and Clinical Chemistry at Aarhus University Hospital as a research fellow financed by the Danish Multiple Sclerosis Society. The studies were performed during 1994 to 1997 and were presented in monograph form. Parts of the data included in the monograph have been published (three papers).

Elevated levels of IgM antibodies against ganglioside GM1 have been reported in Multifocal Motor Neuropathy (MMN), motor forms of Chronic Immune-mediated Demyelinating Polyradiculoneuropathy (CIDP) and Guillain Barré syndrome (GBS). Because antibodies are also reported in patients without neuropathy and in healthy controls, the pathogenic significance of these antibodies has been controversial and remains unknown. However, the methods for measuring anti-GM1 antibodies have been characterised by a lack of data on reproducibility.

The aim of the PhD study was to develop a method for the measurement of circulating IgM-type antibodies directed against the GM1 structure and to investigate the significance of anti-GM1 in CIDP, MMN and GBS.

The first part of the thesis describes the development and optimisation of an ELISA method for measurement of IgM anti-GM1 antibodies. The ELISA uses a high titre patient serum as internal calibrator to which patient sera are correlated. Controls at high, medium and low levels are also included, and adsorption of the GM1 antigen is also controlled by inclusion of the GM1-specific peroxidase labelled B-subunit of Cholera Toxin. With this technique it is possible to achieve intra-assay and inter-assay coefficients of variation of 4-8% and 6-16% respectively. CIDP and Multiple Sclerosis patients had significantly higher levels of IgM-type anti-GM1 than healthy blood donors and controls ($p < 0.002$ and $p < 0.01$ respectively).

Reference: Bech et al. *Clinical Chemistry* 40(7): 1331-1334(1994).

The second part of the thesis describes a longitudinal study of patients with Guillain Barré syndrome, where the association of anti-GM1 and clinical course was investigated. No statistically significant correlation was found between the clinical severity of neuropathy and the level of antibodies. In contrast, I did find a significant association of the changes in the levels of anti-GM1 with neuromuscular condition, as the decline in anti-GM1 and clinical remission were correlated ($r = 0.9$, $p = 0.006$). The decline of anti-GM1 was detected two weeks (mean) before patients remitted clinically.

Reference: Bech et al. *Journal of Neuroimmunology* 72: 59-66(1997)

The third part of the thesis describes a longitudinal study of patients with Chronic Acquired Demyelinating Polyradiculoneuropathy (CADP), where the association of anti-GM1 with clinical parameters was investigated. I found a statistically significant relationship between changes in isokinetic muscle strength and the change in anti-GM1 level ($r = -0.79$; $p = 0.036$). A statistically significant correlation was not found between clinical measures of muscle strength evaluated by manual muscle strength testing and the change in anti-GM1. In spite of the association with anti-GM1 in CADP of the MMN and motor CIDP types, some of these patients do not have elevated levels of anti-GM1. It is possible that these patients have elevated levels of antibodies directed against GM1-like structures on glycoproteins rather than on glycolipids.

Reference; Bech et al. *Annals of Neurology*, 43(1): 72-8 (1998).

The fourth part of the thesis describes a study of the specificity of Gal β 1-3GalNAc antibodies in CADP patients, where in particular the alpha/beta anomeric specificity and significance of neuraminic acid in the epitope was investigated. Alpha anomers of Gal β 1-3GalNAc are protein-bound and beta anomers of Gal β 1-3GalNAc are lipid-bound. The study showed that the number of neuraminic acids on the epitope was inversely correlated with the binding of antibodies, and that binding was better to alpha compared with beta anomeric forms of Gal β 1-3GalNAc. The specificity of patient sera was, however, heterogeneous. Some patient sera had affinity primarily for GM1 (5/8), others for glycophorin (5/8), which presents Gal β 1-3GalNAc disaccharide in clusters. A smaller group (3/8) reacted with GM1 and glycophorin. It was shown by immunoblotting of human nerves with lectin and serum that the Gal β 1-3GalNAc structure is present in human nerves. Consequently, it is possible that new pathogenic mechanisms involving the epitope Gal β 1-3GalNAc in nerve tissue glycoproteins play a role in some CADP patients.

Reference: Bech et al. *Journal of Neuroimmunology*, submitted (1997).

In conclusion, the study has shown a correlation between changes in anti-GM1 and the clinical neuromuscular condition in patients with immune-mediated polyneuropathies of the GBS and CADP types. The role and significance of anti-GM1 antibodies in the pathogenesis of CADP and GBS remain unknown. Anti-GM1 might well be an epiphenomenon secondary to the inflammation. However, the study indicates that anti-GM1 is closely related to the pathological process in immune-mediated neuropathy of GBS and CADP types.