Gangliosides and anti-ganglioside antibodies in neuromuscular synaptic function

Zitman, F.M.P.

CHAPTER 3.1

The role of complement and complement regulators in mediating motor nerve terminal injury in murine models of Guillain-Barré syndrome

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Published in Journal of Neuroimmunology 201 - 202 (2008) 172 - 182

Abstract

Recent research into the Guillain-Barré syndromes (GBS) has focused on anti-ganglioside antibodies that correlate with specific clinical phenotypes. Our increasing understanding of the role of antibodies in mediating GBS has naturally focused our attention on complement involvement in the pathological procession. We have studied the axonal and glial components of the murine motor nerve terminal as a model site of antibody and complement mediated injury. Such studies are providing us with clear information on the molecular components underlying our clinicopathological model for GBS and have led us to the testing emerging complement therapeutics that are potentially suitable for human use.

Acknowledgements

Financial support was provided by the Wellcome Trust, Guillain-Barré Syndrome Support Group UK, Guillain-Barré Syndrome Foundation International and the Prinses Beatrix Fonds.

Introduction

Recent developments in our understanding of the Guillain-Barré syndromes (GBS) and variants including Miller Fisher syndrome (MFS) have divided them into distinct phenotypes with defining clinical, serological, electrophysiological and pathological features (Hughes and Cornblath, 2005). The major current research direction strongly suggests that antibody and complement mediated mechanisms are important in pathogenesis (Willison and Yuki, 2002; Yuki, 2001). It is clear that many different glycolipids act as autoantibody targets in peripheral nerve where their epitopes are spatially distributed in sites that reflect the clinical distribution of disease. Where antibody (of appropriate class and subclass) is found, complement follows, and there is also now extensive evidence that complement activation through the classical pathway is an important factor in driving pathology in animal models of GBS, supported by human pathological studies (Hafer-Macko et al., 1996a; Lu et al., 2000; Putzu et al., 2000; Wanschitz et al., 2003). This emphasis on humoral mechanisms sits in contrast to extensive past research based on predominantly T cell-mediated models of GBS, namely experimental allergic neuritis, in which clinical correlates have not been widely found to date, and in which complement would be expected to play a lesser role (Kieseier and Hartung, 2003). In this review and primary data report, we will focus on an area we have extensively researched in recent years, namely the emerging relationship between complement mediated distal motor nerve and motor nerve terminal injury, anti-GD1a, -GM1 and -GQ1b ganglioside antibodies, and motor axonal forms of GBS and MFS.

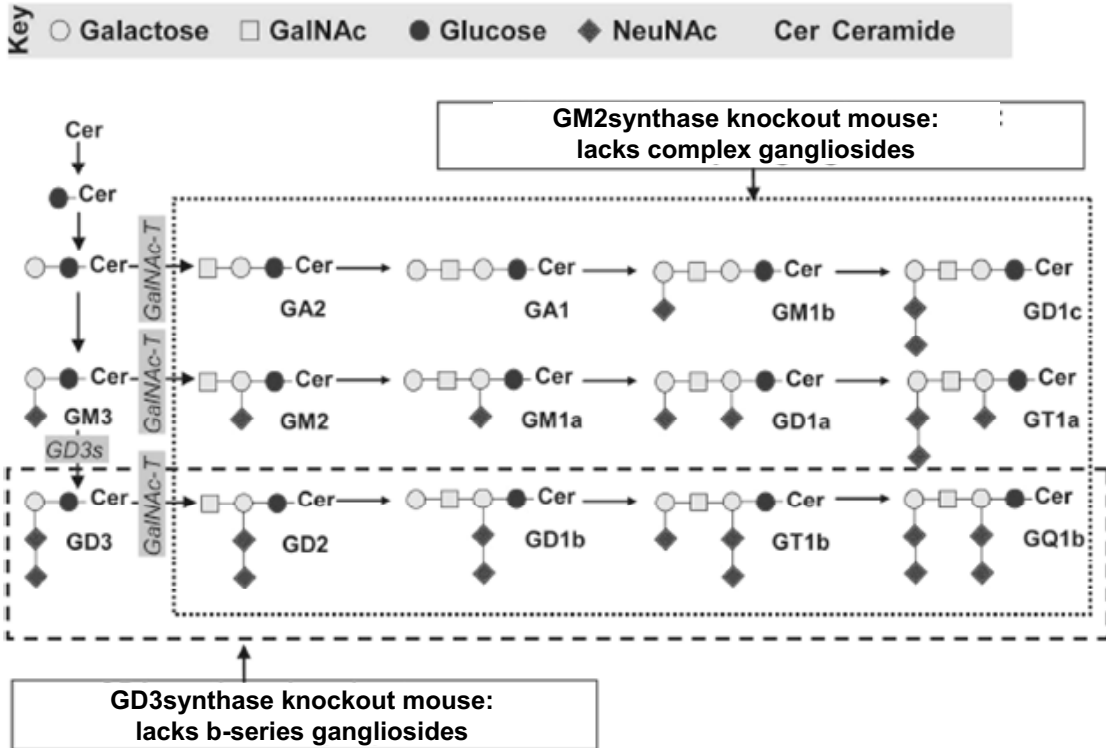

Figure 3.1. Schematic overview of the synthesis and composition of the ganglioside family
Indicated are the synthesis steps that are catalyzed by GM2synthase and GD3synthase and the ganglioside subgroups that are lacking in the respective knockout mice strains.

Our interest in the motor nerve terminal first arose through the knowledge that the pattern of weakness seen in MFS is similar to that seen in human botulism, a known disorder of presynaptic transmitter release at the neuromuscular junction (NMJ) (Lange et al., 2006). MFS comprises the clinical triad of ataxia, areflexia and ophthalmoplegia (Fisher, 1956). Interest in MFS was revived when Chiba and colleagues identified anti-GQ1b antibodies in the acute phase serum of MFS cases (Chiba et al., 1992). From this seminal observation, the measurement of anti-GQ1b antibodies in suspected cases of MFS is now widely used in clinical practice, with >90% sensitivity and specificity, akin to routine measurement of acetylcholine (ACh) receptor antibodies in myasthenia gravis.

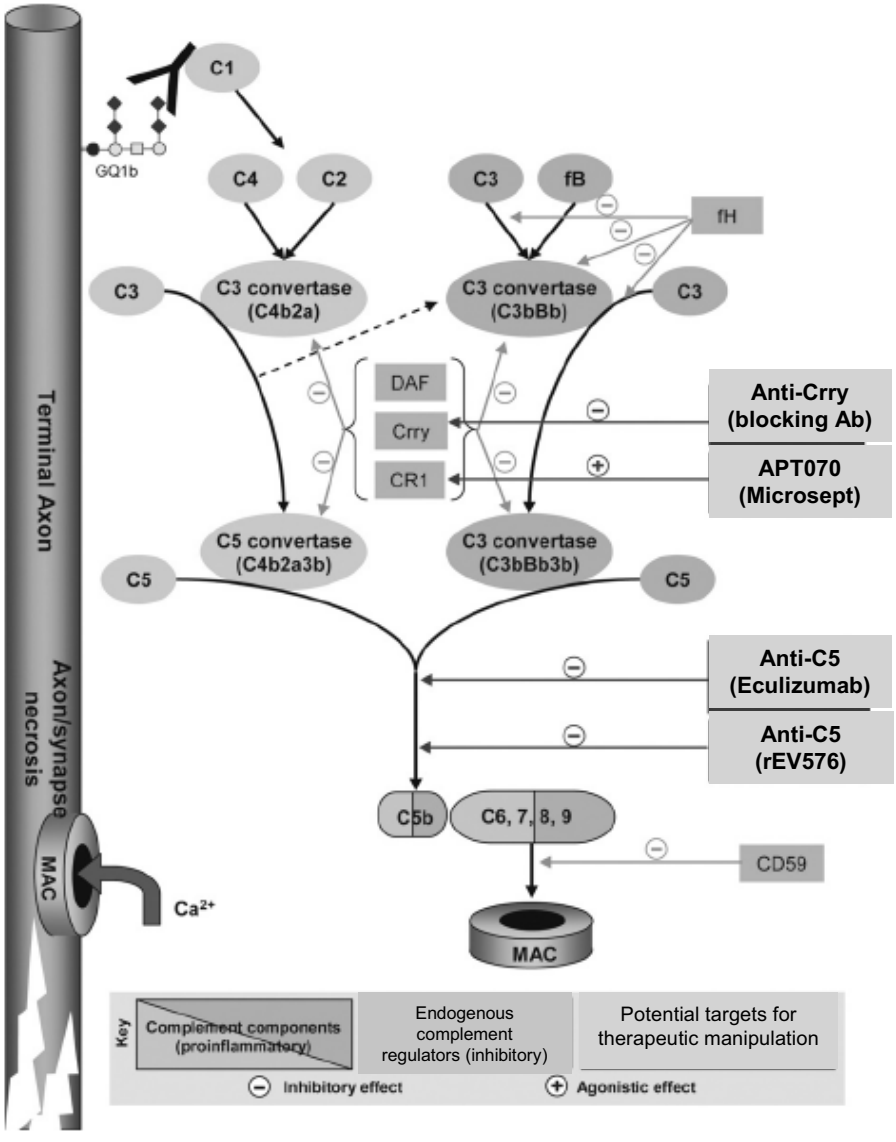

Figure 3.2.
A schematic diagram of potential therapeutic targets and strategies based on inhibition or removal of anti-ganglioside antibodies, inhibition of steps in complement activation through blocking antibodies or enhancement of complement regulation. Complement components in oval boxes: on the left, classical pathway; on the right, alternative pathway (C5b, C6, C7, C8, and C9 belong to both pathways).

Gangliosides are sialylated glycosphingolipids, concentrated in the outer leaflet of plasma membranes. They are highly enriched in the nervous system, including synaptic membranes and biosynthetically regulated by specific glycosyltransferases. A scheme is shown in Figure 3.1 (Sandhoff and Kolter, 2003). Since gangliosides are recognised as the presynaptic ectoacceptors for botulinum toxin binding, we anticipated that anti-ganglioside antibody-associated syndromes might also affect this site. Armed with this information, we established a collaboration with John Newsom-Davis and his colleagues in Oxford where we first demonstrated that the nerve terminal was indeed targeted by MFS-associated antibodies (Roberts et al., 1994). This landmark study inspired extensive subsequent work exploring the immunopathophysiology resulting from anti-ganglioside antibody and complement binding at the NMJ, as outlined in Figure 3.2. Many shared principles exist between anti-ganglioside antibody-associated syndromes and other antibody-mediated autoimmune diseases affecting the NMJ, including myasthenia gravis, Lambert-Eaton myasthenic syndrome and Isaac's syndrome (Vincent et al., 2006); in this commemorative paper we thus acknowledge the huge contribution of John Newsom-Davis and his group to our understanding of this field.

Anti-ganglioside antibody and complement effects on neuromuscular transmission

Following on from the early studies with the Newsom-Davis group (Benatar et al., 1997; Roberts et al., 1994; Roberts et al., 1995; Willison et al., 1996), we subsequently studied the effects of MFS sera and related anti-ganglioside human and murine monoclonal antibodies (mAbs) on NMJ electrophysiology in more detail in Leiden (Plomp et al., 1999). We first tested the effects of incubation of mouse hemi-diaphragm preparations with anti-GQ1b-positive MFS sera. The sera induced a dramatic increase in the frequency (up to 300-fold at some NMJs) of spontaneously released ACh quanta, measured as miniature endplate potentials (MEPPs), without significantly altering their amplitudes. This was followed by block of evoked ACh release resulting in paralysis of the preparation, similar to the effects of the paralytic neurotoxin α-latrotoxin (αLTx) (Rohou et al., 2007). Incubation with total MFS IgG alone was without effect but subsequent treatment with freshly prepared normal human serum (NHS) readily induced high frequency MEPPs followed by transmission block, suggesting the involvement of complement in the phenomenon. Further subclass purification of IgG showed that αLTx-like activity co-eluted with anti-GQ1b activity in the same complement fixing IgG subclasses (IgG3 or IgG1), suggesting that these antibodies were the responsible factors. We demonstrated identical complement-dependent electrophysiological effects of anti-GQ1b-positive MFS/GBS sera in experiments using anti-GQ1b IgM mAbs that were derived from mice immunized with lipo-oligosaccharides which contained GD3/GT1a-like structures and originated from MFS/GBS-associated *C. jejuni* strains (Goodyear et al., 1999). In order to address whether the primary target of anti-GQ1b antibodies mediating the αLTx-like effect through complement activation were gangliosides or an unrelated (sialylated) antigen, for instance the αLTx receptor latrophilin, we exploited glycosyltransferase knockout mice that lack complex gangliosides (in collaboration with Furukawa). Through these studies we proved that the presynaptic complex ganglioside GQ1b must be the primary antigenic target of MFS-associated

antibodies and that the level of expression of gangliosides was a critical factor in influencing the extent of pathophysiology (Bullens et al., 2002). We have since demonstrated similar findings with anti-GD1a antibodies that occur in motor axonal forms of GBS (Goodfellow et al., 2005).

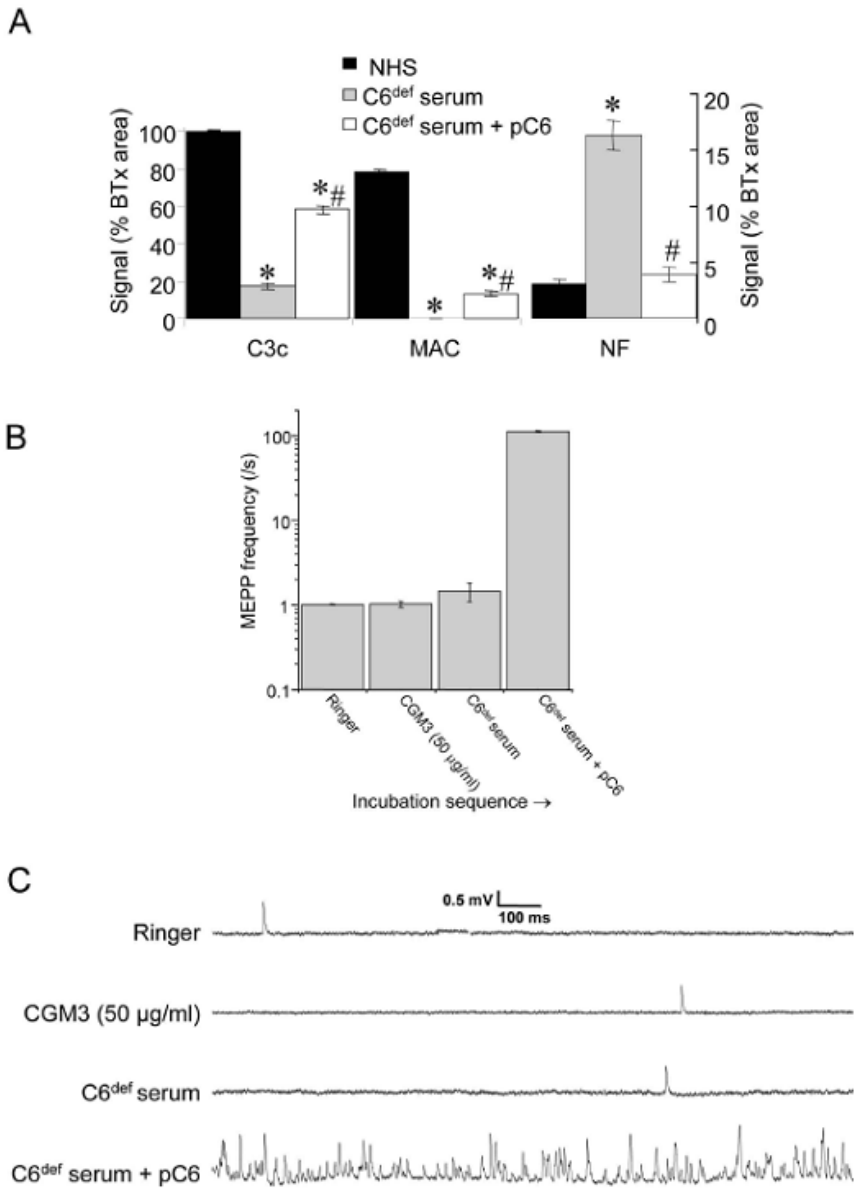

Figure 3.3.
A. Dependence of neurofilament (NF) loss on MAC deposition, assessed in total C6 deficiency (C6 deficient ($C6^{def}$) mouse with $C6^{def}$ human serum). No MAC is formed (left axis) and there is no NF loss (right axis). Reconstitution of $C6^{def}$ serum with purified C6 (pC6) leads to MAC deposits and NF loss. C3c is formed under both conditions (left axis). Student's two-tailed *t*-test: * = significantly different from NHS treated samples; # = significantly different from $C6^{def}$ serum treated samples. (BTx = α-bungarotoxin). **B and C.** Electrophysiological analysis of NMJs under $C6^{def}$ conditions: Hemi-diaphragms of $C6^{def}$ mice (*n*=2) were incubated serially in Ringer's medium, CGM3, $C6^{def}$ serum and $C6^{def}$ serum with added pC6. **B.** Spontaneous quantal ACh release at NMJs was measured as MEPPs with an intracellular microelectrode. MEPP frequency increased dramatically only when pC6 was added to the serum. Average MEPP frequency (*n*=2 muscles, 7-18 NMJs sampled per incubation). **C.** Typical examples of electrophysiological traces.

Immunopathological effects at the nerve terminal

In parallel with the electrophysiological studies described above, we assessed the immunohistological and morphological sequelae of anti-ganglioside antibody exposure to neuromuscular preparations *in vitro*, and to passively immunized mice. In topical immunostaining studies we had found that anti-GQ1b antibodies bound the NMJ (O'Hanlon et al., 2001; Willison and O'Hanlon, 1999) and thus also expected them to be deposited in passive immunization and exposure studies *in vivo* and *ex vivo* (Halstead et al., 2004). In contrast to the NMJ, we found little evidence of antibody penetration and binding to intramuscular nerve-bundles including nodes of Ranvier, despite reactive antigens being present in topical immunostaining studies. It is thus likely that antibody access to nerve fibres and nodes of Ranvier beyond the NMJ is limited by the blood nerve barrier, as we have previously observed for sheathed sciatic nerve preparations (Paparounas et al., 1999) and this finding reinforces our preference for understanding pathophysiological pathways using the NMJ as a model, rather than more proximal nerve sites.

Using confocal and immuno-electron microscopy (EM), we were able to distinguish the presynaptic neuronal elements, the perisynaptic Schwann cell (pSC) and the post-synaptic structures which comprise the NMJ, and the detailed localization of anti-ganglioside antibody and complement deposits at the NMJ using these methods has been examined. Depending upon the gangliosides examined and the strain of mouse used for the studies, extensive immunoglobulin deposits can be detected on presynaptic axonal membranes or pSC membranes, or both. At the fluorescence microscopy level, the former can be identified by proximity to α-bungarotoxin staining (marking ACh receptors and therewith the postsynaptic membrane), and the latter by observing that the labelling pattern extends around the nuclei of pSCs and co-locates with the Schwann cell specific marker, S100 in double staining studies. For anti-GQ1b antibodies, this has also been resolved by immuno-gold EM (Halstead et al., 2004).

At NMJs in mouse hemi-diaphragm preparation exposed to anti-GQ1b antibody in presence of NHS, complement products are deposited in a very similar distribution to anti-GQ1b immunoglobulins. Complement fragment C1q, C4, C3c, C5 and the pore-forming membrane attack complex (MAC, also termed C5b-9) can all be readily detected. In an extensive series of *ex vivo* and *in vivo* experiments using C6 complement component deficient sera and mice, we have demonstrated that the αLTx-like effects do not occur in the absence of MAC. Key features of these published data are shown in Figure 3.3. Whilst this does not preclude important pro-inflammatory roles for earlier complement components including C3a and C5a, it indicates that the major acute injury in this model is mediated by MAC. Extensive MAC-dependent cytoskeletal and mitochondrial changes occur concomitantly with the αLTx-like effect. The axonal cytoskeleton is severely affected, as assessed by loss of neurofilament (NF) and beta-tubulin immunoreactivity and abnormal EM appearance, when compared to controls. At the EM level, the intra-terminal mitochondrial also show severe structural damage. Using this information, we have formulated a model of injury in which the dominant event is uncontrolled Ca^{2+} influx through MAC pores into the nerve terminal, resulting in uncontrolled ACh exocytosis, calpain activation with NF degradation and mitochondrial injury (O'Hanlon et al., 2003). This model is summarized in Figure 3.4.

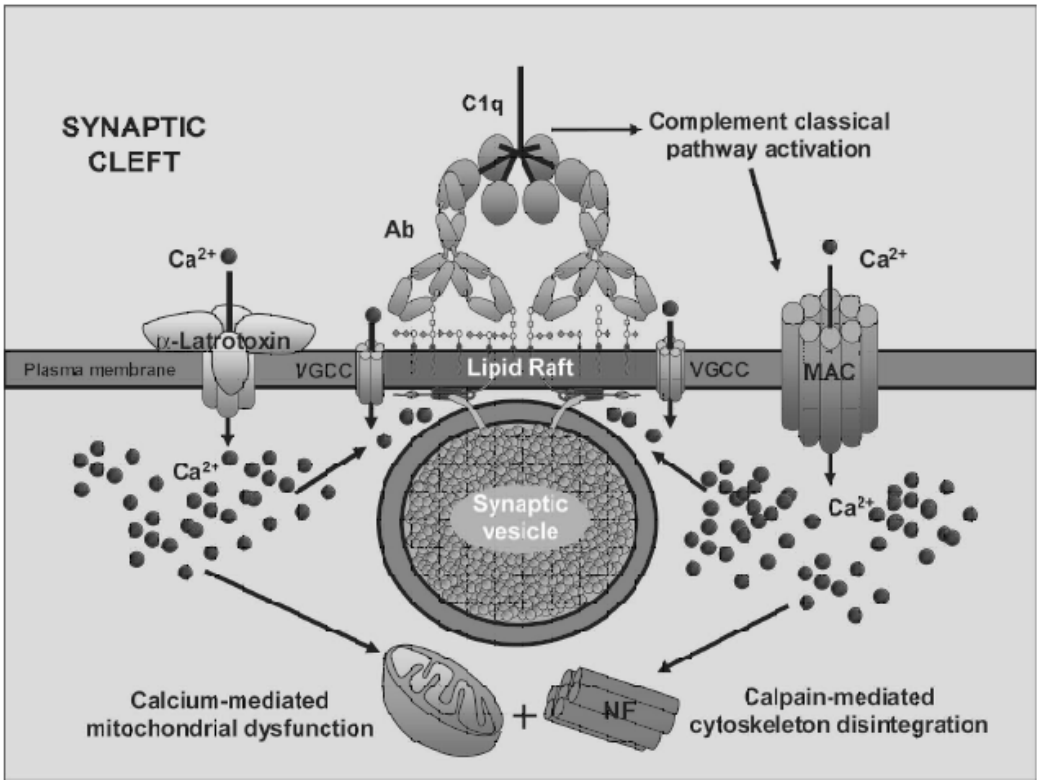

Figure 3.4. A schematic diagram of the events occurring at nerve terminals in experimental nerve-muscle preparations exposed to anti-ganglioside antibodies in the presence of complement. Exocytosis is normally initiated by highly regulated Ca^{2+} entry through voltage-gated Ca^{2+} channels (VGCC) that activates the adjacent exocytotic protein complex and initiates synaptic vesicle fusion with the pre-synaptic membrane. When MAC pores (on the right) are deposited in the pre-synaptic membrane, unregulated Ca^{2+} entry triggers massive, uncontrolled exocytosis and Ca^{2+}/calpain mediated intra-terminal injury, including cytoskeletal degradation, assessed by measuring neurofilamant (NF) levels, and mitochondrial injury. The pore forming toxin, α-latrotoxin (on the left), is believed to act in part through a similar mechanism, allowing unregulated Ca^{2+} influx with similar electrophysiological and morphological sequelae to those resulting from MAC pores.

The experimental data described above is derived from mouse modelling and what remains unclear is the extent to which the distal motor axon is a site of injury relevant to human disease. The availability of (motor point) muscle biopsy tissue from human cases at the appropriate time point in the clinical course is practically very limited, either in MFS or in GBS, and even if it were available the interpretation is likely to be confounded by the co-occurence of Wallerian degeneration arising from more proximal axonal injury. Direct electrophysiological assessment of human distal motor axonal function is relatively crude and imaging this site is not feasible. These factors make is difficult to assess the validity of these mouse data as a direct model of the human disease. However, indirect evidence indicates that the distal motor axon is an affected site in axonal forms of GBS (Ho et al., 1997; Kuwabara et al., 2003), along with other sites including nerve roots and trunks. Furthermore, several case studies have supplied clinical electrophysiological indications for NMJ defects in MFS (Lange et al., 2006; Lo et al., 2006; Sartucci et al., 2005; Uncini and Lugaresi, 1999; Wirguin et al., 2002). Irrespective of the human clinical relevance of the distal motor axon, the principles governing the effects of antibody binding with complement fixation at ganglioside-rich membrane sites are likely to be applicable to axonal and glial membranes in general.

The influence of endogenous complement regulators on motor nerve terminal injury

One perplexing issues surrounding our model of anti-GQ1b and MAC mediated injury is its dependence upon an exogenous, heterologous source of complement to induce the neuropathic lesions in mouse tissues; in our case we achieve this with NHS. Previously, we have attempted to generate a model by intraperitoneal injection of anti-ganglioside mAb, relying on mouse serum as a source of complement; whilst we could demonstrate clear antibody deposits overlying presynaptic nerve terminals accompanied by subtle electrophysiological abnormalities, no behavioural defects were observed (Willison et al., 1996). In subsequent studies we have sought mouse complement deposits and found them to be very sparse. We considered factors that might influence this failure of anti-ganglioside antibodies to activate mouse complement on the presynaptic membrane. To this end, we have conducted a range of studies in parallel with a positive control study in the form of a complement fixing anti-acetylcholine receptor (AChR) rat mAb (McAb3, kindly provided by Vanda Lennon, Mayo Clinic), that is known to induce complement-dependent experimental myasthenia gravis on passive immunization in mice in the absence of a heterologous complement source. As this antibody binds on the postsynaptic membrane, only ~50 nM distant from the anti-ganglioside mAb binding region on the presynaptic membrane, it would appear to be a good control in terms of the diffusion dynamics of antibody and complement components from the circulation.

In this comparative study we first confirmed that all the mouse mAbs we were using in passive immunization studies were able to fix mouse complement in an equivalent manner to the rat anti-AChR mAb, McAb3 (primary data, Figure 3.5). There are variations both the complement fixing abilities of different antibodies and in complement activity in laboratory mice strains that confound these issues (Rice, 1950). For example, the low haemolytic capacity of mouse serum may be in part due to the inability of the C4b subunit of the classical pathway C5 convertase to bind C5 (Ebanks and Isenman, 1996). Nevertheless, these data show that the mouse anti-glycolipid mAbs used in our studies are as able to effectively fix mouse complement as a known myasthenia gravis-inducing pathogenic antibody reactive to AChR, using C57bl/6 serum in solid-phase ELISA studies, conducted according to Zwirner with minor modifications (Zwirner et al., 1998). From this starting point it seems very likely that unknown inhibitory factors must present in the presynaptic membrane that differ from those in the postsynaptic membrane. Candidate molecules include the murine complement regulators complement receptor-1 related gene/protein Y (Crry), CD59a, decay accelerating factor 1 (DAF1, CD55) and Factor H that are widely distributed and limit the formation of activated complement products (Figure 3.2). We can clearly observe CD59a by immunofluoresence microscopy in the presynaptic axonal and pSC membranes using mouse anti-CD59 mAb 7A6 (Donev et al., 2008). This is illustrated in Figure 3.6 (primary data), using transgenic mice provided in collaboration with the Thompson laboratory that also express the endogenously fluorescent proteins cyan fluorescent protein (CFP) and green fluorescent protein (GFP) respectively in the cytoplasm of these NMJ components (Feng et al., 2000; Zuo et al., 2004). We have also previously shown that CD59a deficiency plays an aggravating role in our model of injury, although in these studies the complement source was provided by NHS, a heterologous source of complement whose degree of regulation by murine CD59 is not clearly delineated (Halstead et al., 2004). The

expression of membrane-associated DAF1 and Crry has been more difficult to detect at this site by immunohistology.
Using CD59a and DAF1 deficient mice in the McAb3 passive immunization model of myasthenia gravis, exacerbation of pathology has clearly been shown in comparison to wildtype, the presumption therefore being that both CD59a and DAF1 are present in the postsynaptic membrane (Lin et al., 2002; Morgan et al., 2006). In collaboration with the Medof laboratory, our passive immunization experiments conducted to date in CD59a/DAF1 double knockout mice with anti-ganglioside antibody (in the *absence* of NHS as a source of complement) have not clearly demonstrated any major clinical or morphological differences when compared with wildtype controls. This is in clear contrast to the situation with the myasthenia gravis model, both from the published data and from experiments we have also conducted in Glasgow in parallel by way of control. We are currently assessing these double knockout mice for subtle electrophysiological abnormalities (Zitman and Halstead, unpublished observations). Although we do observe an increase in C3c deposits in these double knockout mice, one of the limitations in detailing subtle quantitative changes in MAC levels in these studies has been the absence of suitable mouse MAC probes producing the very low backgrounds needed for our quantitative analytical methods, in comparison with the widely available reagents for human MAC. We therefore cannot conclude that no MAC is formed, but nevertheless cannot observe it at significant levels compared with control tissue.

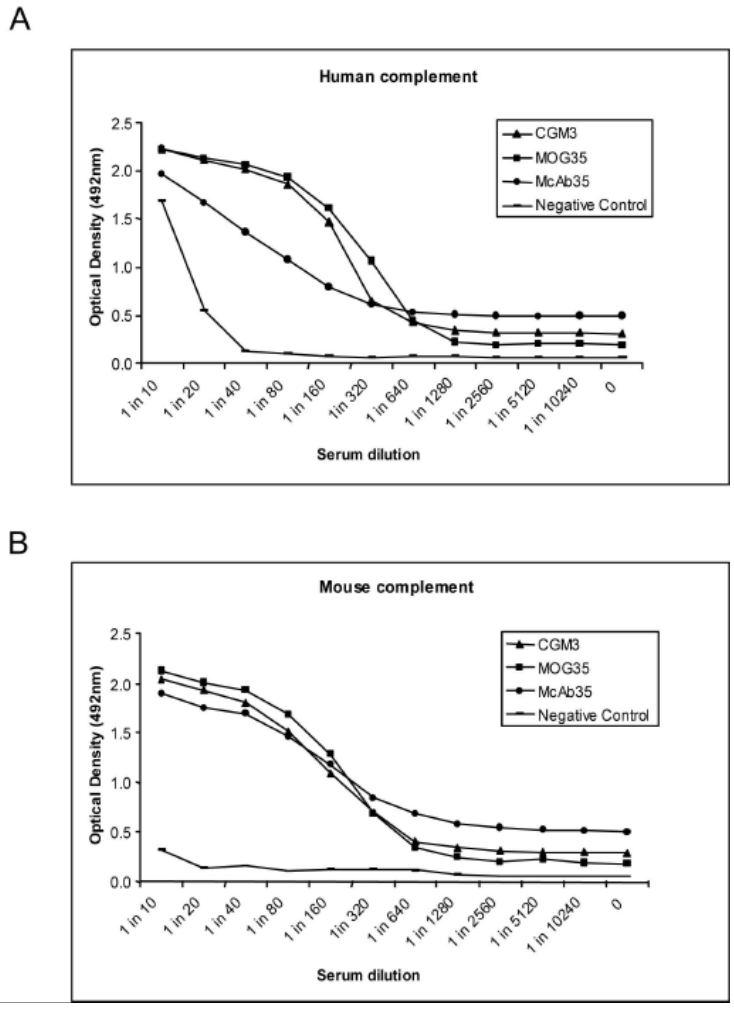

Figure 3.5. Complement activation ELISA
Activation of complement in human and mouse serum was compared between 3 antibodies of different specificities, species and subclasses. MAbs CGM3 (mouse IgM), MOG35 (mouse IgG2b) and McAb3 (rat IgG) were captured on micro-titre plates and serial dilutions of human (**A**) or mouse serum (**B**) was applied. The complement activation capacity of the antibodies was assessed by detecting the presence of activated C3 with an anti-C3c antibody (Dako). Mouse mAbs CGM3 and MOG35 had comparable levels of human C3, while rat mAb McAb3 fixed human C3 to a slighter lesser degree. All 3 mAbs activated mouse C3 to similar levels. Method based on Zwirner (Zwirner et al., 1998).

Many other complement regulators exist, and cross-regulation across species is complex and controversial (Morgan et al., 2005). Our previous studies using NHS in passively immunized mice have demonstrated activation of the complement cascade via the classical pathway (Halstead et al., 2004); however classical pathway deposited C3b can also serve as a nidus for the alternative pathway positive feedback loop, thereby resulting in the further amplification of the cascade (Fearon and Austen, 1980). A key regulator of the alternative pathway is the soluble glycoprotein Factor H (fH), which has both decay accelerating activity against the alternative pathway C3 convertase (Pangburn and Muller-Eberhard, 1978; Weiler et al., 1976) and cofactor activity for Factor I mediated C3b cleavage (Pangburn et al., 1977). Sialic acid enriched membrane (in our case the presynaptic membrane) has also been shown to prevent alternative pathway activation, by increasing the affinity of fH for C3b (Fearon, 1978; Kazatchkine et al., 1979). Indeed, we see heavy deposits of fH at NMJs as detected with polyclonal sheep anti-fH antibody (Abcam; primary data, Figure 3.7), but only in the presence of activated complement in our anti-ganglioside antibody and NHS passive immunization model. The amount of fH deposited in other models has not been reported in detail.

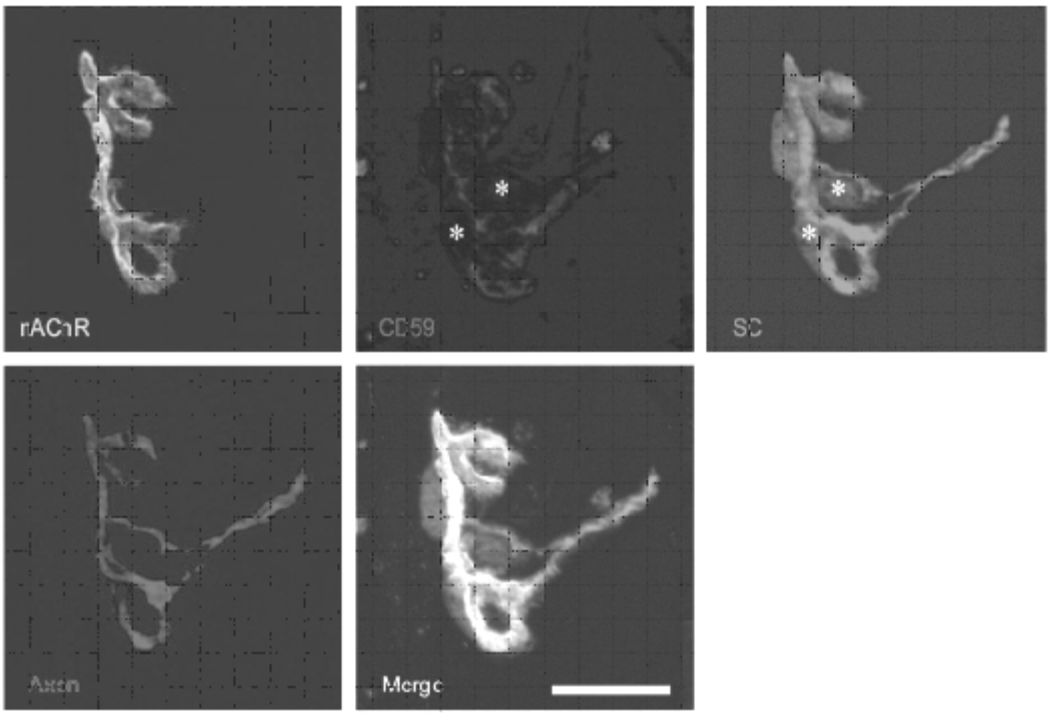

Figure 3.6. Illustrative immunofluorescence image of whole-mount triangularis sternae nerve-muscle preparation demonstrating the presence of CD59a at the NMJ
Transgenic mice which express cytoplasmic green fluorescent protein in their Schwann cells (SC; upper right panel) and cyan fluorescent protein in their axons (Axon; lower left panel) were stained with α-bungarotoxin to delineate their postsynaptic nicotinic acetylcholine receptors (nAChR; upper left panel) and anti-mouse CD59 (upper middle panel). CD59 is present on both the motor axon and perisynaptic Schwann cells (asterisk).
Scale bar: 20μm.

We therefore hypothesize that the failure of endogenous mouse complement to generate a pathological lesion in our anti-ganglioside antibody passive immunization model results from the relative inability of the classical pathway C5 convertase to cleave C5 and progress to MAC formation in the presynaptic membrane. In other sites, for example the postsynaptic membrane that is targeted in myasthenia gravis, this might be overcome by amplification of the alternative pathway loop which is activated through the deposition of classical pathway C3b. On the presynaptic membrane, where anti-ganglioside antibodies bind their sialic-acid containing ganglioside targets in glycolipid-enriched microdomains (functional rafts), it is possible that the alternative pathway loop might be especially highly regulated by fH because of the rich sialic acid environment at this site. fH could thereby more tightly regulate the progression of the cascade to MAC in this site compared with other sites where autoantibodies bind protein targets such as AChR, i.e. the postsynaptic membrane. This hypothetical model remains consistent with our unpublished observation of a rise in C3c levels in the DAF1/CD59a double knockout as the relative deficiency in the classical pathway C5 convertase (component C4b), along with the fH regulation discussed above, follows after the step at which DAF1 exerts its C3 convertase regulation. The implication of this is that the deficiency of CD59a, which regulates at the C5b-8 level, does not overcome the inhibitory events earlier in the pathway as there are no or very low levels of activation products for it to regulate. Further studies are needed to address these complex issues surrounding the activation and regulation of complement in different sites and species in models of antibody mediated autoimmune disease.

Therapeutic strategies for the attenuation of complement activation

As a result of the apparent inability of anti-glycolipid antibodies to fix mouse complement in the presynaptic membrane, our studies have been pursued with NHS as a source of heterologous complement. The mechanism(s) by which activation of human complement is able to proceed, where mouse complement fails is unknown, but presumably relates to a relative inefficiency of heterologous regulation. In our situation this species-specific effect has the fortuitous benefit of allowing us to test any complement therapeutics in a model system in which the complement component proceeding to MAC has essentially been “humanized”, and thus has added relevance to preclinical testing of agents that might be suitable for human clinical trials. Although anti-ganglioside antibodies may exert some of their paralytic effects directly (independent of complement) as shown in an extensive series of studies from the Toyka group (Buchwald et al., 2001), tissue bound antibody of the appropriate class as found in GBS (human IgG1 and IgG3) inevitably fixes complement to exacerbate injury unless complement regulation is highly active. Thus, one key approach that might be expected to suppress disease activity in anti-ganglioside antibody-mediated neuropathy is the suppression of complement activation. Since it is very clear that complement activation with MAC formation drives neural membrane injury in anti-ganglioside antibody treated mouse tissue and in rabbit models of acute motor axonal neuropathy (Susuki et al., 2007a), it would appear likely that blocking MAC formation locally should prevent MAC-dependent tissue injury, even if anti-ganglioside antibodies are deposited in neuronal or myelin membranes. We recently

investigated this using the complement inhibitor APT070 that contains the C3/C5 convertase inhibiting region of complement receptor 1 and a membrane-localizing peptide that allows it to inhibit complement accumulation. APT070 pretreatment completely abrogated MAC formation and acute tissue injury of anti-ganglioside antibody immunized mice both *ex vivo* and *in vivo* (Halstead et al., 2005a).

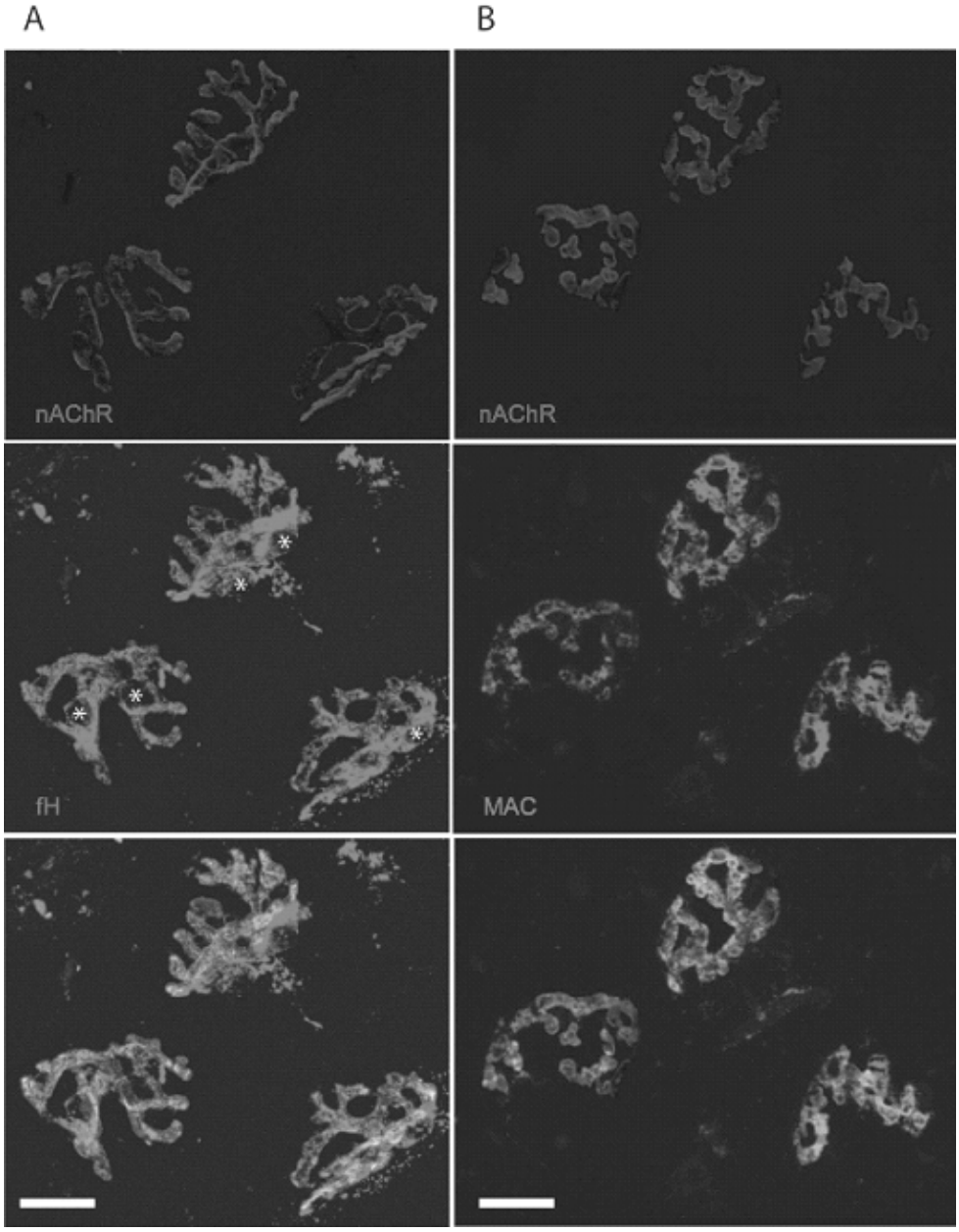

Figure 3.7. Illustrative immunofluorescence images of mouse triangularis sternae nerve-muscle organ bath preparations *in vitro*, demonstrating complement deposition at the NMJ
Live mouse tissue was incubated with the anti-ganglioside IgM mAb, CGM3, followed by 40% normal human serum (NHS) as a source of complement, according to Halstead (Halstead et al., 2005b). **A.** Postsynaptic nicotinic acetylcholine receptors (nAChR) delineate the NMJ (top). Factor H (fH, middle) is extensively deposited present on the terminal motor axon and perisynaptic Schwann cells (asterisk). **B.** In this model, the complement cascade in human serum is activated, culminating in human membrane attack complex (MAC; middle) deposits over presynaptic neuronal and Schwann cell membranes.
Lower panels show merged views. Scale bar: 20µm

We have also assessed the efficacy of the humanized anti-C5 mAb eculizumab, which neutralizes C5 and thereby blocks the formation of human C5a and C5b-9, in preventing the anti-ganglioside antibody and complement-mediated motor nerve terminal injury (Halstead et al., 2008a). In these studies, eculizumab completely prevented electrophysiological and structural lesions at anti-GQ1b antibody pre-incubated NMJs *in vitro* when using NHS as a complement source. In our *in vivo* mouse model of MFS generated through intraperitoneal injection of anti-GQ1b antibody and NHS, mice developed respiratory paralysis due to transmission block at diaphragm NMJs, resulting from anti-GQ1b antibody binding and complement activation. Intravenous injection of eculizumab effectively prevented respiratory paralysis and associated functional and morphological hallmarks of terminal motor neuropathy. Very recently we have also assessed whether the complement C5 inhibiting recombinant protein, rEV576, derived from tick saliva (Hepburn et al., 2007), is able to prevent neural injury in the mouse model for MFS (Halstead et al., 2008b). Again, mouse hemi-diaphragm preparations were treated with anti-GQ1b antibody and NHS as a source of complement with added rEV576 or control protein. In control tissue C3c and MAC were deposited at NMJs, along with terminal motor axonal NF degradation, pSC injury and electrophysiological and functional impairment. In tissue treated with rEV576, all these indicators of motor neuronal damage were absent, except for the presence of C3c, indicating effective inhibition of C5. These results demonstrate that rEV576 is a further agent that effectively prevents development of neuronal and pSC damage in experimental murine neuropathy.

Conclusions

It is well established that MAC is present in human GBS nerve biopsy and autopsy material and that this is likely to be an important step in generating pathology and functional loss. It is also very clear that complement activation with MAC formation drives neural membrane injury in our models of anti-ganglioside antibody treated mouse tissue. Since MAC is a convergence pathway in many antibody and complement-mediated diseases it would seem an excellent target for human therapeutic trials. Whilst our studies focus on ganglioside targets in the presynaptic membrane, other presynaptic targets and the postsynaptic membrane that is targeted by antibody in myasthenia gravis are also important candidates for therapeutic complement inhibition. The use of complement inhibition trials in clinical practice is thus eagerly awaited across several disease areas of antibody-mediated clinical neuroimmunology at the NMJ, to which the pioneering contribution of John Newsom-Davis was substantial.