Miller Fisher syndrome is associated with serum antibodies to GQ1b ganglioside

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Abstract
A recent report described serum anti-GQ1b ganglioside antibodies in Miller Fisher syndrome (MFS), a clinical variant of Guillain-Barré syndrome (GBS). Four consecutive cases of MFS all had high titre anti-GQ1b antibodies which were absent from all control sera including those of patients with GBS.

(J Neurol Neurosurg Psychiatry 1993;56:204–206)

Antibodies directed against gangliosides are being increasingly identified in patients with a variety of acute and chronic neuropathies.1,2 In Guillain-Barré syndrome (GBS) the specificity of anti-ganglioside antibodies is very variable, although several recent reports have indicated that the presence of anti-GM1 ganglioside IgG antibodies may be particularly associated with severe predominantly motor forms of the disease.3 A recent report4 suggested that anti-GQ1b IgG antibodies may be strongly associated with Miller Fisher syndrome (MFS), a variant of GBS comprising ataxia, areflexia and ophthalmoplegia.5 We have screened the sera of recent MFS and GBS patients and appropriate controls for anti-GQ1b antibodies to substantiate and further characterise this clinical and serological association.

Patients and methods
All patients were diagnosed and treated in this institute and represent the consecutive cases of MFS and GBS admitted over the last 18 months. MFS was defined as a clinical syndrome comprising ataxia, areflexia and ophthalmoplegia with or without bulbar or facial muscle involvement and with clinically insignificant limb weakness. Serum samples were collected at the time of admission (as close to the onset of symptoms as possible) and stored in aliquots at −70°C until assayed. Sera from four patients with typical MFS and 20 patients with GBS were tested. Control sera comprised randomly selected patients with multiple sclerosis (n = 10), other neurological diseases including myasthenia gravis, myopathies and motor neuron disease (n = 14) and normal volunteers from the local community (n = 13).

Anti-ganglioside antibody assays were performed using standard enzyme linked immunosorbent assay (ELISA) and thin layer chromatography (TLC) overlay techniques as previously described.6 Briefly, ELISAs were performed by coating microtitre trays with 100 ng purified GQ1b (Novabiochem, Nottingham, UK) or 100 ng of GM1, GD1a, GD1b, GD3 and GT1b (Sigma Chemicals, Poole, UK) in 50 μl methanol per well and evaporating to dryness. Sera were serially diluted from 1/100 in fivefold dilutions in phosphate buffered saline (PBS) containing 0.1% bovine serum albumin (BSA), pH 7.4 and incubated overnight at 4°C. Plates were developed by adding substrate solution comprising 20 mg o-phenylene-diamine (Sigma) in 60 ml 0.1 M citrate buffer pH5.5 with 20 μl of 30% hydrogen peroxide. The reaction was stopped with 20 μl H2SO4 after 20 minutes and the optical densities (OD) read in an MR5000 plate-reader (Dynatech). All samples were assayed in duplicate on at least two separate occasions. Both antigen-coated wells and blank wells were incubated with test serum and the OD values and titres calculated after subtracting the blank well OD. For the TLC overlay, gangliosides were separated on aluminium backed Kieselgel 60 WF34 S HPTLC plates (Merck) in the solvent system chloroform:methanol:0.25% KCl in a volume ratio of 50:40:10. The chromatograms were air-dried, dipped in 0.1% polysobutylmethacrylate (PIMBS) beads for 20 seconds at 40°C, air dried, blocked with 1% BSA in PBS pH 7.5 for 1 hour, incubated with the test serum diluted 1/500 to 1/1000 in blocking buffer for 4 hours at 4°C, washed X5 in blocking buffer, incubated for 2–3 hours at 4°C with peroxidase-conjugated rabbit anti-human IgG diluted 1/3000 (Dako) and washed X5 in blocking buffer. Plates were developed autoradiographically for 5–30 seconds using an enhanced chemiluminescence procedure (ECL) according to manufacturer’s instructions (Amersham, UK). Gangliosides were stained with resorcinol spray using routine methods.
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Figure 1 ELISA optical density values for anti-GQ1b IgG antibodies in Miller Fisher syndrome (MFS, n = 4); Guillain Barré syndrome (GBS, n = 20), multiple sclerosis (MS, n = 10), other neurological diseases (OND, n = 14) and normal controls (NORM, n = 13). For this plot, sera are diluted 1:100.

Results
Anti-GQ1b IgG antibodies were found in high titres in all four patients with MFS (fig 1). Two patients classified as GBS had very low IgG antibody titres (less than 1/50) to GQ1b giving OD values on ELISA just above the baseline OD. One of these patients had "atypical" GBS comprising sensory ataxia, areflexia and facial weakness with little limb weakness and no ophthalmoplegia. The other weakly positive patient had very severe GBS affecting all muscle groups including facial, bulbar and respiratory muscles, extensive axonal involvement, poor recovery and very hightitre anti-GM1 IgG antibodies. None of the remaining GBS patients or the normal or disease control sera had any detectable anti-GQ1b antibody (fig 1). Of the 20 GBS patients, 2 had high titre anti-GM1 IgG antibodies, giving a frequency of anti-GM1 antibody positive GBS at 10% in this series. Both these patients had severe disease with extensive motor axonal involvement. None of the MFS patients had anti-GM1 antibodies. The titres of anti-GQ1b IgG in all four MFS cases were high, ranging from 1/960 to 1/13500 (see table). Three cases also had lower titre IgM anti-GQ1b which were again completely absent in all GBS and control cases.

The GQ1b positive MFS cases showed interesting reactivity with other gangliosides which may be cross-reactivity or may represent additional polyclonal antibodies (table). Case 4 reacted with GQ1b alone and showed no other anti-ganglioside reactivity. Cases 1–3 also reacted at lower titres with GD3, GD1a and GD1b, mainly in the IgG fraction. Reactivity with GT1b remains uncertain since TLC analysis of our GT1b (obtained from Sigma) showed significant contamination (estimated at 5%) with GQ1b thus making it impossible to determine anti-GT1b titres by ELISA. TLC-overlay confirmed the reactivity with GQ1b in all cases (see figure 2) and also showed clear reactivity with GD3 in case 2 but no binding to GT1b. The lower titres of antibody to GD1a and GD1b were not visible on TLC-overlay with antigen concentrations, serum dilutions and developing conditions designed to demonstrate the reactivity with GQ1b, as for example seen in fig 2.

The CSF in 2 of the 4 MFS cases contained low but detectable anti-GQ1b IgG (titres of 1/17 and 1/25), but the other two were negative. No anti-QQ1b IgM was detected in CSF and no control CSF samples (n = 10) contained any anti-GM1 antibody.

Discussion
The association between MFS and anti-GQ1b antibodies that we and Chiba et al have observed is remarkable as there appears to be a complete and exclusive correlation between the two. Equally striking is the absence of anti-GQ1b IgG or IgM antibodies in any of our control sera. This is particularly unusual since many other anti-ganglioside antibodies, such as anti-GM1 antibodies, are frequently found...

Table Anti-ganglioside antibody titres in patients with Miller Fisher syndrome

<table>
<thead>
<tr>
<th></th>
<th>GQ1b</th>
<th>GD1b</th>
<th>GD1a</th>
<th>GD3</th>
<th>GM1</th>
</tr>
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<tr>
<td>IgG IgM</td>
<td>IgG</td>
<td>IgG</td>
<td>IgG</td>
<td>IgM</td>
<td>IgM</td>
</tr>
<tr>
<td>Case 1</td>
<td>960</td>
<td>110</td>
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<td>145</td>
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<tr>
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<td>13500</td>
<td>300</td>
<td>470</td>
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<td>550</td>
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<td>700</td>
<td>50</td>
<td>0</td>
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<tr>
<td>Case 4</td>
<td>7800</td>
<td>0</td>
<td>0</td>
<td>15</td>
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</table>

Figure 2 TLC-overlay showing reactivity of serum from Case 2 (see table) with GQ1b. Lane 1 is the TLC plate loaded with 7mg of each of the migrated gangliosides as labelled and stained with resorcinol reagent. Lane 2 is an autoradiogram following immuno-overlay of a 1/1000 serum dilution onto 1mg of each of the gangliosides as shown in lane 1. The faint immune reactive band seen in lane 2 migrating between GD1a and GD1b is an unidentified contaminant or degradation product from one of the purified ganglioside stocks. Strong staining can be seen over GQ1b, with no staining of other gangliosides.
in low titre in normal controls. The patients reported here constitute a consecutive series of cases referred to us from our catchment area (population: 1.5 million) and include all patients with MFS known to have occurred in this area during the last year. Thus they do not represent a pre-selected group of MFS patients and we have not yet seen any MFS patients without anti-GQ1b antibodies.

Although the numbers of patients so far reported is small, it is clear that these anti-GQ1b antibodies are closely related to the disease and may play a role in pathogenesis. Equally, this antibody specificity may be a bystander phenomenon resulting from a unique preceding infection that through some other mechanism induces MFS. Although GQ1b is known to be widely distributed in neural tissue, its precise localisation and function remain unknown. The impetus is now present to expand these observations by determining the fine specificity and cross-reactivity of anti-GQ1b antibodies, the regional localisation in peripheral nerve and brain of anti-GQ1b antibody reactive antigens (which may include polysialylated glycoproteins), the uniqueness or otherwise of the preceding infection and to perform pathogenetic studies in appropriate model systems.

MFS can occasionally be difficult to distinguish from intrinsic brainstem disease, especially encephalitis. Indeed, a long-standing conflict exists about the relative contribution of central and peripheral nervous system involvement in this syndrome. It will be particularly interesting to analyse the relationship between these conditions through anti-GQ1b antibody analysis to address this issue and to determine whether anti-GQ1b antibodies may also be a useful diagnostic marker for MFS in this context.