

# Combined pre- and postsynaptic action of IgG antibodies in Miller Fisher syndrome

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**Article abstract**—*Background:* Miller Fisher syndrome (MFS), a variant of the Guillain-Barré syndrome, is associated with the presence of neuromuscular blocking antibodies, some of which may be directed at the ganglioside GQ1b. *Materials and Methods:* The authors investigated the in vitro effects of serum and purified immunoglobulin (Ig) G in a total of 11 patients with typical MFS during active disease, and in three of those patients after recovery. From one patient's serum, we prepared an IgG fraction enriched in anti-GQ1b antibodies by affinity chromatography. For combined pre- and postsynaptic analysis, endplate currents were recorded by a perfused macro-patch clamp electrode. Postsynaptic nicotinic acetylcholine receptor channels were investigated by an outside-out patch clamp technique in cultured mouse myotubes. *Results:* All MFS-sera depressed evoked quantal release and reduced the amplitude of postsynaptic currents. Five of the 11 sera were additionally examined by outside-out patch clamp analysis and caused a concentration-dependent and reversible decrease in acetylcholine-induced currents. The time course of activation and desensitization of nicotinic acetylcholine receptor channels was not altered by MFS-IgG. Nine patients (82 %) were positive for anti-GQ1b antibodies in ELISA and dot-blot. The enriched anti-GQ1b antibody fraction had a similar effect as whole serum. After recovery from MFS, blocking activity was lost and sera originally positive for anti-GQ1b antibodies became negative. *Conclusion:* Circulating IgG antibodies induce both pre- and postsynaptic blockade and may play a pathogenic role in acute MFS.

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Miller Fisher syndrome (MFS) is characterized by the triad of gait ataxia, external ophthalmoplegia, and areflexia.<sup>1</sup> As a variant of the Guillain-Barré syndrome (GBS), MFS is thought to be an immunopathologic disorder of the peripheral nervous system. Immunoglobulin (Ig) G autoantibodies directed at the ganglioside GQ1b are found in the acute phase sera of more than 90% of patients with MFS.<sup>2–4</sup> Gangliosides are present in high concentrations in peripheral nerve axons and myelin, and several studies indicated that different gangliosides (e.g., GM1) are present at nodes of Ranvier and at neuromuscular junctions.<sup>5</sup> The distal motor nerve terminal lacks the blood-nerve barrier, making it accessible for circulating antibodies and a suitable site to investigate the functional effects of serum-antibodies experimentally.

There is evidence that MFS sera block neuromuscular transmission in the mouse hemidiaphragm.<sup>6–9</sup> Using intracellular microelectrode techniques, a marked increase in spontaneous release (i.e., miniature endplate potential frequency) after application of MFS sera, followed by inexcitability of the muscle upon phrenic nerve stimulation has been demonstrated.<sup>6,9</sup> In that study, the effects depended entirely on the presence of complement,<sup>9</sup> and miniature

endplate potential (MEPP) amplitudes appeared unchanged.<sup>6,9</sup> With a perfused macro-patch clamp electrode, we have previously shown that the purified IgG fraction and fractions containing monovalent, antigen-binding (Fab) fragments of two patients with MFS rapidly and reversibly depressed evoked quantal release, whereas the IgM fractions had no effect.<sup>7,8</sup> Furthermore, MFS-IgG reduced the amplitude of single quanta, indicating a superimposed postsynaptic effect.<sup>8</sup>

In the current study, we addressed the postsynaptic side more directly by examining the effect of MFS-IgG on acetylcholine (ACh)-induced currents in mouse myotubes using outside-out patch clamp analysis. Using the perfused macro-patch clamp electrode we investigated whether the presence of blocking antibodies is a more general feature in patients with MFS, and whether it is disease-related.

**Materials and methods.** *Patient sera and purified IgG.* Eleven patients with MFS were defined according to standard clinical and electrophysiologic criteria.<sup>10,11</sup> Six men and five women between 23 and 71 years of age were included. One patient had a preceding gastrointestinal infection and six patients had an upper respiratory tract

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infection. Five patients (Patients 1, 3, 6, 7, 8) showed bulbar involvement in addition to ophthalmoplegia but no generalized muscle weakness. Four patients (Patients 2, 4, 9, 10) presented with ophthalmoplegia and moderate generalized weakness (Hughes grade 1 or 2).<sup>12</sup> Two patients (Patients 5 and 11) developed severe generalized muscle weakness (Hughes grade 4 to 5) after initially pure extraocular involvement.<sup>13</sup> In four patients (Patients 5, 6, 7, 8), amplitudes of compound muscle action potentials were moderately reduced around the time of serum collection. Six patients were treated with plasma exchange (Patients 1, 4, 5, 9, 10, 11), and two were treated with IV IgG (Patients 7 and 8). Except for Patient 8, who died of septicemia, all patients eventually recovered to normal or almost normal neurologic function (seven patients recovered within 4 weeks).

Early serum samples were obtained within 2 weeks after onset of symptoms. Plasma filtrate was obtained during therapeutic plasmapheresis; for patch clamp investigations, the filtrate of the first exchange was chosen (i.e., before any additional therapies). Convalescent serum samples were obtained in three patients after full recovery at least 6 months after onset of disease. Serum samples were stored for up to 1 week at 4 °C and then at -20 °C until used. Immunoglobulins were purified by affinity chromatography as previously described.<sup>7</sup> Lyophilized IgG was dissolved in the assay buffer just prior to use.

For control experiments (control IgG), a commercial polyclonal IgG preparation (Venimmun, Aventis-Behring, Marburg, Germany) was used. Additional control serum samples from patients with cervical disk herniation, and with *Borrelia* infection and polyradiculitis were investigated by outside-out patch clamp analysis.

Anti-GQ1b antibodies were partly purified by affinity chromatography from one serum (Patient 11). It contained high-titer anti-GQ1b (1:3,200 by ELISA and 1:32,000 by overlay high-performance thin-layer chromatography [HPTLC]), and borderline low titers of anti-GD3 (1/4,000 by overlay HPTLC) and anti-GD1b (1/500 by overlay HPTLC). In order to minimize contaminations with other immunoglobulins and complement components, the purified IgG fraction (Mab TRAp GII kit, Pharmacia, Sweden) from this patient's serum was used for further affinity chromatography. Octyl-Sepharose 4B (Pharmacia, Sweden) was used as a gel for binding the purified antigen GQ1b (Sigma Chemical Corp., St. Louis, MO) as described.<sup>14</sup> The gel was washed several times with a solution of 1:1 methanol/H<sub>2</sub>O containing 0.1 M KCl (solution 1) and equilibrated with the same solution. Partly purified GQ1b (0.4 mg) was dissolved in 1.6 mL solution 1 and warmed in a water bath at 70 °C for 30 minutes. Then, 1.5 mL gel was conjugated with the suspension of GQ1b and packed in a column for affinity chromatography. The column was washed with phosphate-buffered saline (PBS) at pH 7.4, and the IgG fraction of patient's serum was added. The column was again washed with PBS to remove unbound antibodies. For desorbing the antibodies bound on the gel, the column was eluted at room temperature with 3 M NaSCN and the eluate was collected in 1 mL fractions. Then, the mean absorbance of each sample was read at 280 nm wavelength by spectrophotometry. The fractions containing anti-GQ1b IgG were pooled, dialyzed against PBS, and concentrated. Using this procedure, we were able

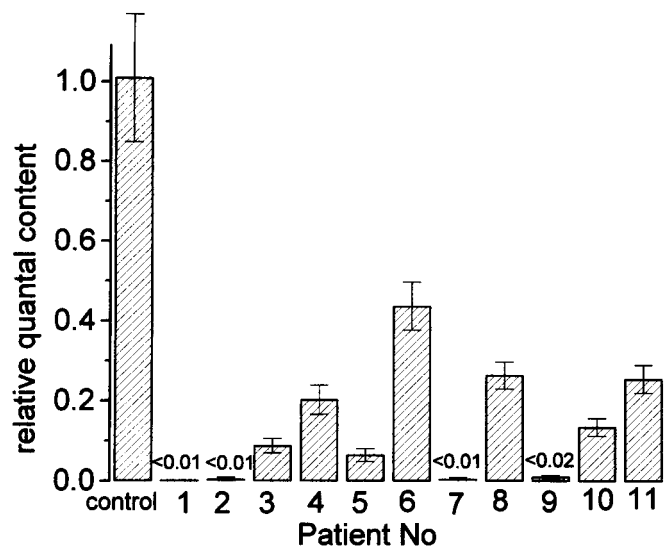
to purify only a small fraction (<10%) of the anti-GQ1b IgG present in the original patient's serum; the majority portion was eluted with the serum in the first wash. It cannot be excluded that this fraction contained antibodies reacting with other ganglioside epitopes.

Anti-Ach receptor (AChR)-antibodies were determined by a radioimmunoassay as described.<sup>15</sup> Antibodies to GQ1b and GM1 were measured by ELISA and by dot-blot assays as described.<sup>16,17</sup> Purified IgG fractions were also examined by HPTLC as described.<sup>4</sup>

**Recording techniques and statistics.** Perfused macro-patch clamp technique on mouse hemidiaphragm. Experiments were performed on hemidiaphragms of male BALB/c mice that were at least 16 weeks old, at 20 ± 0.5 °C as described.<sup>8</sup> Endplate currents were recorded by means of a perfused macro-patch clamp electrode as described in detail elsewhere.<sup>18,19</sup> Statistical analysis was done with a commercial computer program (Origin, Microcal Software, Northampton, MA). Statistical *p* values were calculated using the Student's *t*-test for grouped data.

At the beginning of each experiment, the electrode was perfused with control solution, the position of the electrode was optimized, and the quantal excitatory postsynaptic currents (qEPSC) were recorded and counted. When recording conditions were stable, diluted sera (1:4) or immunoglobulins dissolved in the *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic (HEPES) buffer at a concentration of about 4 to 6 mg/mL (except for the partly purified anti-GQ1b and depleted IgG fractions) were added to the perfusate of the electrode while recording from a selected nerve terminal stimulated by a fixed depolarizing current pulse. The quantal content "*m*" is given as mean ± SD from the results of at least three independent experiments.

Outside-out patch recordings on cultured mouse myotubes. The patch clamp experiments were performed on cultured mouse myotubes prepared as previously de-



**Figure 1.** Reduction in quantal content by Miller Fisher syndrome (MFS) serum or purified immunoglobulin G (IgG). Ordinate: quantal content relative to the control normalized at 1 for each experiment; values are given as mean ± SD. Results of 11 different patients with MFS (Patients 1 to 11) and of the pooled IgG preparation from healthy donors (control) are shown.

**Table 1** Experimental data of patients

Patient	Ganglioside antibodies		Quantal content (M), mean $\pm$ SD			Time to maximal block, min
	GQ1b	GM1	Control	Application	Washout	
1						
Early	+	–	0.72 $\pm$ 0.09	0.004 $\pm$ 0.003	0.72 $\pm$ 0.08	2
Convalescent	–	–	0.72 $\pm$ 0.08	0.79 $\pm$ 0.06	ND	N/A
2	++	–	0.77 $\pm$ 0.08	0.004 $\pm$ 0.0028	0.62 $\pm$ 0.05	5
3	+++	–	0.65 $\pm$ 0.07	0.14 $\pm$ 0.03	0.20 $\pm$ 0.03	8
4	++	–	0.89 $\pm$ 0.14	0.18 $\pm$ 0.03	0.98 $\pm$ 0.07	5
5						
Early	+	–	1.77 $\pm$ 0.22	0.12 $\pm$ 0.03	1.57 $\pm$ 0.14	13
Convalescent	–	–	0.57 $\pm$ 0.09	0.68 $\pm$ 0.09	ND	N/A
6						
Early	++	–	1.77 $\pm$ 0.15	0.78 $\pm$ 0.10	1.09 $\pm$ 0.03	10
Convalescent	–	–	0.94 $\pm$ 0.07	0.98 $\pm$ 0.10	ND	N/A
7	+++	–	0.95 $\pm$ 0.13	0.006 $\pm$ 0.003	0.46 $\pm$ 0.08	20
8	+	–	0.92 $\pm$ 0.20	0.24 $\pm$ 0.03	0.65 $\pm$ 0.03	6
9	–	–	0.62 $\pm$ 0.05	0.04 $\pm$ 0.01	0.70 $\pm$ 0.14	4
10	–	–	0.51 $\pm$ 0.03	0.07 $\pm$ 0.01	0.34 $\pm$ 0.33	9
11	+++	–	0.71 $\pm$ 0.02	0.18 $\pm$ 0.03	0.53 $\pm$ 0.02	7

Patients 2, 3, 6, 7, 8, 11, whole serum; Patients 1, 4, 5, 9, 10, purified immunoglobulin G. The  $p$  value for the reduction in quantal content after application of the early MFS sera was  $p < 0.001$  when compared to the control group.

+ = titer 1:100; ++ = titer 1:200; +++ = titer > 1:400; N/A = not applicable; ND = not done.

scribed.<sup>20</sup> The embryonic-type of nicotinic AchR (nAChR)-channel, made up of  $\alpha 2\beta\gamma\delta$  subunits, is found exclusively.<sup>20</sup> Patch clamp experiments were performed in the outside-out configuration using standard protocols<sup>21</sup> as described in detail elsewhere.<sup>22</sup>

For application of Ach to outside-out patches, we used a piezo-driven tool for ultrafast solution exchange.<sup>23</sup> In our set-up, the solution exchange typically took 90  $\mu$ s.<sup>24</sup> In the present study we applied short Ach-pulses of 20 ms duration with a frequency of 0.25 Hz. For experiments with MFS-IgG, the respective IgG concentration was applied via the background flow of the application system as previously described.<sup>22</sup> The solution exchange of the background system took around 100 ms.<sup>25</sup>

**Results.** *Effects of MFS sera or IgG on presynaptic transmitter release.* Application of serum or IgG from all 11 MFS patients significantly reduced quantal content, whereas control IgG were without blocking effect (figure 1). The blockade by the MFS-IgG or MFS-sera could be fully reversed by washing with control solution in four of 11 patients, and partly in six of 11 patients. In only one serum (Patient 3), the blockade was not reversible. The final effects of each serum or IgG studied are shown in table 1.

In every patient, the blockade could be overcome by stronger depolarization of the nerve terminal<sup>8</sup> (data not shown), confirming the presynaptic nature of the blockade and excluding technical malfunction as a reason for the observed decrease in quantal content.

In addition, we studied serum, purified IgG, and the

IgG fraction enriched for anti-GQ1b antibodies from the same patient (Patient 11) with a high titer of anti-GQ1b antibodies (1:3,200 in serum) separately. All fractions revealed blocking properties with a significant reduction in evoked quantal release. The mean reduction in quantal content  $m$  relative to the control for whole serum (1:4 diluted) was  $0.26 \pm 0.04$ ; and for purified MFS-IgG at a concentration of 2.6 mg/mL, it was  $0.22 \pm 0.03$ . The IgG fraction enriched for anti-GQ1b antibodies had a much lower IgG concentration of 0.036 mg/mL, and induced a tenfold stronger reduction in quantal content ( $m = 0.029 \pm 0.01$ ). In contrast, the IgG fraction partially depleted from anti-GQ1b antibodies (residual anti-GQ1b titer 1:400) was less effective in blocking evoked quantal release ( $m = 0.43 \pm 0.15$ ) at an equivalent IgG concentration.

*Effect of MFS sera or IgG on postsynaptic currents. Macro-patch clamp analysis.* To investigate the postsynaptic action of the MFS sera amplitudes of (qEPSC) were analysed. Figure 2 depicts amplitude distributions before, during, and after application of the MFS-IgG of Patient 11. Under control conditions (see figure 2A), the histogram revealed two peaks indicating release of single and double quanta. For single quanta, the mean amplitude was  $-1648$  pA. Even the smallest amplitudes were well above the noise level of the recording system. In the presence of the MFS-IgG (see figure 2B), the mean amplitude was markedly reduced when compared to control. Due to the presynaptic blockade, only single quanta were released and the histogram showed only one peak with a mean amplitude of  $-1200$  pA. After wash-out, mean amplitude and distribution were almost identical to control conditions (see figure

**Table 2** Postsynaptic blockade, macro-patch-clamp analysis

Patient	Mean amplitude (-pA)		
	Control	Application	Washout
1			
Early	1513 ± 340	620 ± 149	1481 ± 327
Convalescent	1323 ± 194	1513 ± 340	1481 ± 327
2	1133 ± 186	630 ± 407	1362 ± 233
3	1763 ± 782	235 ± 204.5	ND
4	1004 ± 173	389 ± 165	1234 ± 301
5			
Early	1619 ± 401	1347 ± 250	1649 ± 561
Convalescent	821 ± 150	848 ± 200	ND
6			
Early	1064 ± 170	1055 ± 168	1023 ± 198
Convalescent	1324 ± 255	1355 ± 295	1404 ± 283
7	913 ± 167	530 ± 125	1235 ± 242
8	1682 ± 714	760 ± 184	703 ± 167
9	1060 ± 276	522 ± 349	1294 ± 201
10	1406 ± 274	464 ± 80	440.6 ± 133
11	1642 ± 257	1204 ± 251	1614 ± 221

Amplitude of single quanta (mean ± SD; see figure 2) before, during, and after application of the MFS serum; the *p* value for the reduction in amplitude after application of early MFS immunoglobulin G was *p* < 0.001 when compared to the control group.

ND = not done.

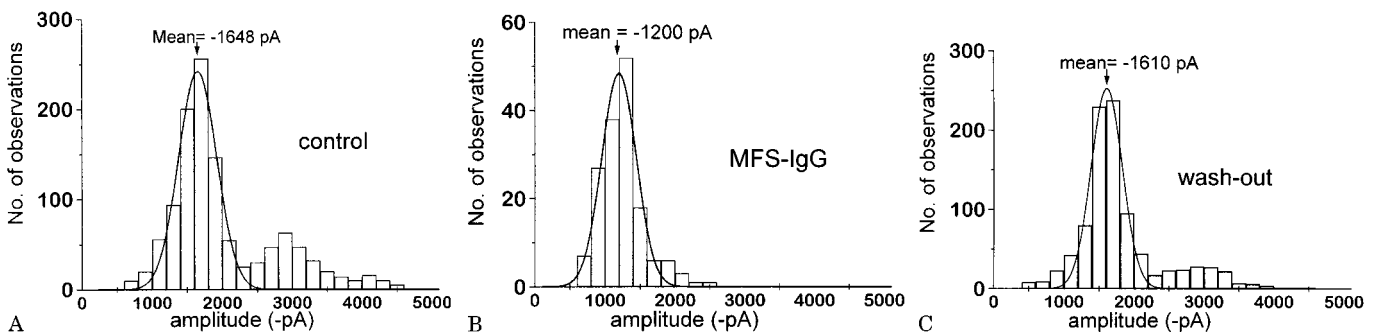
2C). In the presence of the MFS-serum, amplitudes of single quanta were reduced in 10 out of 11 patients; in seven of these 10 patients, the amplitudes recovered completely after wash-out (table 2).

**Outside-out patch clamp analysis.** To study the postsynaptic effects of the MFS-IgG avoiding interference with the presynaptic depression of release, we investigated the MFS-IgG of five patients by patch clamp technique in the outside-out configuration on mouse myotubes. The peak current amplitude elicited by a saturating concentration of 1.0 mM Ach was evaluated before, during, and after

incubation of outside-out patches with the respective MFS-IgG concentration. A representative experiment showing the time course and the reversibility of the blockade of the peak current amplitude by MFS-IgG is depicted in figure 3. In the presence of 70 mg/L MFS-IgG of Patient 5, the amplitude of Ach-induced current was reduced by about 97% within 20 seconds. Amplitudes recovered fully after wash-out within 60 seconds. The extent of the reduction in amplitude of nAChR channel currents and its time course depended on the MFS-IgG concentration applied. The dose-response relationship was steepest when between 0.7 and 7.0 mg/L MFS-IgG was added to the background solution. Whereas the time course of block depended on the MFS-IgG concentration applied, the time for wash-out was independent (data not shown). The time course of activation and desensitization of nAChR channel currents was not altered by the MFS-IgG (data not shown). The final effects of each MFS-IgG preparation investigated by outside-out patch analysis are summarized in table 3. Application of control IgG from healthy donors and two patients with other neurological diseases were without blocking effect.

**Effect of convalescent serum on quantal content and qEPSC.** To further examine the pathogenic relevance of the observed blocking effects, sera from the acute phase of disease and sera obtained after full recovery were investigated at the same nerve terminal (figure 4). After application of the MFS-IgG from Patient 1 during the height of the illness, quantal release was almost completely blocked within 5 minutes. Returning to control solution, quantal content recovered fully within 7 minutes. During superfusion with the convalescent serum, quantal content remained stable at the same level for more than 30 minutes. Neither the amplitude of single quanta nor the decay time constant was affected by the convalescent serum, indicating that the original postsynaptic effects had also subsided. The experiment shown in figure 4 is representative for nine experiments of this type with the sera of three different patients (see tables 1 and 2).

**Serum antibodies.** Specific reactivity to GQ1b ganglioside was found at high titer in ELISA and dot-blot assays in nine out of 11 patients in the IgG, but not in the IgM, fraction. No IgM or IgG binding to GM1 was detected. In two patients (Patients 9 and 10), no anti-GQ1b or anti-



**Figure 2.** Amplitude distribution of quantal excitatory postsynaptic currents (qEPSC) before, during, and after application of the Miller Fisher syndrome (MFS) immunoglobulin G (IgG). Ordinates: numbers of observations per bin. Abscissae: amplitude of qEPSC at a constant depolarizing current ( $-0.4\mu\text{A}$ ), bin width 200 pA. (A) Control solution; quantal content  $m = 0.7$ . (B) MFS-IgG of Patient 11; quantal content  $m = 0.2$ . (C) Wash-out (control solution); quantal content  $m = 0.5$ . All data were recorded from the same nerve terminal. The number of records evaluated for each distribution were 1,500 to 3,000, depending on quantal content. The solid line marks the gaussian distribution of single quanta.

**Table 3** Outside-out patch-clamp analysis

Patient	IgG, mg/L	Normalized peak current amplitude*	Time course of block, s	Time course of washout, s
1	180	0.28 ± 0.11 (5)	55.2 ± 28.8 (6)	51.2 ± 17.3 (5)
4	70	0.42 ± 0.24 (5)	39.2 ± 13.2 (5)	24.0 ± 6.3 (9)
5	70	0.14 ± 0.03 (9)	20.0 ± 12.1 (8)	36.0 ± 11.0 (14)
9	60	0.42 ± 0.21 (6)	60.0 ± 28.9 (6)	32.0 ± 11.3 (6)
10	70	0.36 ± 0.23 (4)	47.0 ± 29.1 (4)	26.0 ± 5.2 (4)
Cervical disc	70	0.99 ± 0.01 (32)		
	700	0.98 ± 0.02 (5)		
Borreliosis with polyradiculoneuritis	120	1.02 ± 0.02 (12)		
IgG (healthy donors)	3000	0.96 ± 0.05 (3)		

Values are mean ± SD (number of patches studied).

\*Peak current amplitudes were normalized to the current elicited by 1.0 mM ACh when no serum or IgG was present.

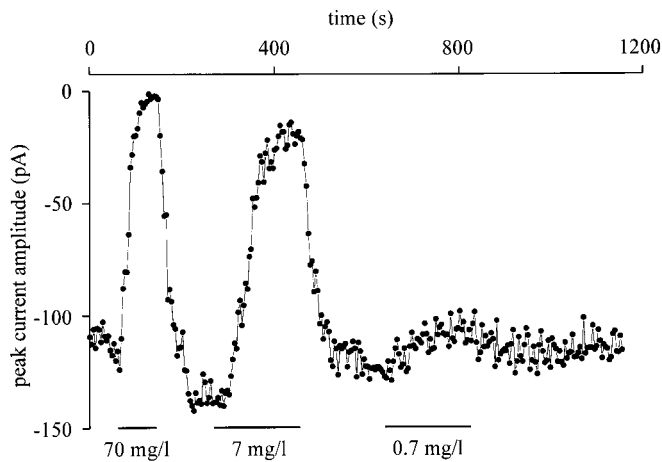
IgG = immunoglobulin G.

GM1 antibodies could be detected by standard ELISA. In patients with follow-up serology 2 to 6 months later, specific IgG binding to these gangliosides had disappeared. None of the patients' sera showed elevated titers (>0.4 nmol/L) of antibodies to nAChR in the standard immunoprecipitation assay.

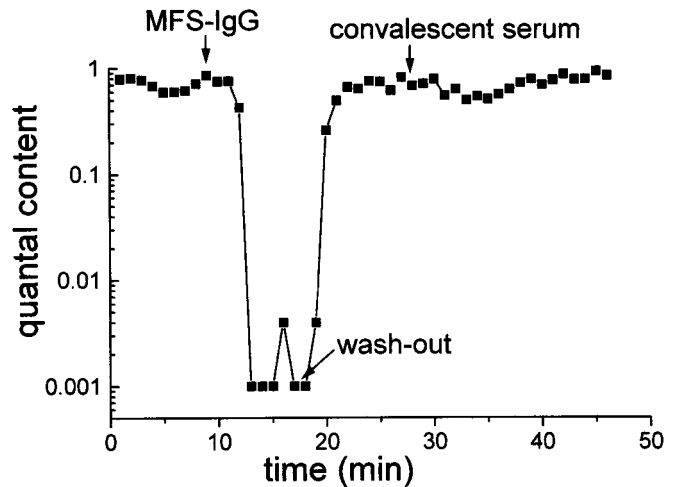
**Discussion.** Our study shows that neuromuscular blocking antibodies are common in MFS. Postsynaptically, the antibodies directly interfere with ACh receptor channels. All sera lost pre- and postsynaptic blocking properties after complete clinical recovery.

*Presynaptic blockade by MFS-IgG.* The presynaptic depression of evoked quantal release may be explained by an interference of the MFS-IgG with

voltage-gated calcium channels<sup>8</sup> (not unlike the action of Lambert-Eaton myasthenic syndrome [LEMS]-IgG<sup>26,27</sup>), or may indicate changes within the release machinery similar to the action of botulinum toxin.<sup>28</sup> We found a slight increase in late releases which may reflect increase in spontaneous release after application of MFS-IgG.<sup>8</sup> We did not observe a profound increase in spontaneous release followed by complete inexcitability of the nerve terminal as described after bath incubation of the whole nerve muscle preparation with MFS serum together with active complement.<sup>6,9</sup> The fact that we stimulate and treat only a small patch of the synaptic region,<sup>19</sup> and that the



**Figure 3.** Concentration-dependent reduction in peak current amplitude by Miller Fisher syndrome (MFS) immunoglobulin G (IgG) from Patient 5. Amplitudes of single pulses of 1.0 mM acetylcholine to an outside-out patch were plotted versus time. Pulses were applied with a frequency of 0.25 Hz. The horizontal bars indicate duration of IgG application at the respective IgG concentration. Note that the reduction in peak current amplitude comes on faster and is more pronounced with high antibody concentrations.



**Figure 4.** Time course of the depression of evoked quantal release by the Miller Fisher syndrome (MFS) immunoglobulin G (IgG) (Patient 1) obtained during the active stage of the disease and of the same patient after recovery from disease. Quantal content (logarithmic ordinate scale) versus time in minutes (abscissa). Each point of the curve was determined from the results of at least 256 stimuli; quantal content *m* was calculated using the Poisson formula. The arrows denote the instant of solution change in the electrode. Note that recovery serum did not block transmission.

combined pre- and postsynaptic blockade is seen without addition of active complement, may account for some basic differences in comparison to the results published on bath application with long incubation times and intracellular recordings.<sup>9,29</sup> Because of the complement independence and the reversibility of the blockade, our data are best explained by an immunopharmacologic effect of IgG antibodies. One hypothesis to reconcile both these observations would be that these are different yet complementary functions of antibodies, not unlike the situation in myasthenia gravis where both complement-dependent alterations and direct immunopharmacologic blockade of the AchR are operative.<sup>30,31</sup>

**Postsynaptic blockade.** The additional action of the MFS-IgG on postsynaptic nAChR channels first observed with the macro-patch clamp technique was corroborated and now shown to be an independent effect by single channel measurements. Our data indicate binding of the MFS-IgG to the nonliganded state of the AchR-channel, as described for myasthenic antibodies.<sup>22,31</sup> Because the exact amount of specific activity in the IgG-fraction is unknown, the rate constants of binding and unbinding were not calculated. The time course of the blockade by MFS-IgG seems to be slightly faster than for purified IgG from patients with myasthenia gravis.<sup>22</sup> Despite the functional evidence of MFS antibodies acting at the nAChR, binding of the MFS-IgG was not found by the diagnostic immunoprecipitation assay. This is not unexpected, since antibodies binding at or near the binding site of nAChR channels are usually not detected by the diagnostic immunoprecipitation assay.<sup>15,32</sup> When comparing the observations obtained by the macro-patch clamp technique with those obtained by the outside-out patch clamp method, one has to take into account that the AchR may not be as easily accessible to the antibody in an intact nerve muscle preparation as it is in myotubes expressing nAChR channels. This may account for quantitative differences in the dose-response relationship. Altogether, the data obtained by the two independent methods are in good agreement, clearly indicating a postsynaptic effect of MFS-IgG. The results for MFS-IgG resemble our previous findings with IgG fractions from patients with GBS, yet with some remarkable differences.<sup>19</sup> In our experiments with GBS sera, the postsynaptic currents were affected in only four out of 10 patients. Furthermore, the time course of blockade with GBS-IgG tended to be slower, and the blockade induced by GBS-filtrate could not be reversed after prolonged incubation times. It is conceivable that different antiganglioside antibodies show different modes of binding and neuromuscular blockade.

**The possible role of anti-ganglioside antibodies.** From the present data, there is no hint as to whether the blocking antibodies are directed against one homologous epitope expressed both pre- and postsynaptically, or whether this combined blocking action is due to a polyclonal immune response with multiple

antibodies directed at different epitopes. A number of different studies have suggested that GQ1b is the candidate antigen in MFS,<sup>9,29,33-35</sup> but the putative pathogenic role of anti-GQ1b antibodies still remains speculative because direct proof by experimental data is not yet available. Our investigations using an immunoglobulin fraction enriched for anti-GQ1b antibodies also does not unequivocally prove the pathogenic relevance of GQ1b gangliosides, since cross-reactivity with other ganglioside epitopes cannot be ruled out. The fact that anti-GQ1b antibodies were no longer detectable after recovery, along with the loss of blocking activity, is suggestive but not conclusive evidence. Future studies should make use of monoclonal IgG antibodies binding exclusively to GQ1b.

In two out of 11 patients, we did not find detectable anti-GQ1b antibodies by ELISA, although their sera reduced evoked quantal release to the same extent as anti-GQ1b positive sera. We assume that our detection system (ELISA and dot-blot) may have failed to detect low titer activity, as has been described earlier.<sup>36,37</sup> Specifically, our assay uses bovine brain gangliosides, and therefore antibodies to specific peripheral nerve gangliosides may not be detected. Indeed, for serum anti-GM1 antibodies, different binding affinities to gangliosides derived from peripheral and central nervous systems have been described in a patient with GBS.<sup>38,39</sup> Alternatively, the antibodies may not have been detected by ELISA due to conformational differences, although they may be functionally active. This would resemble findings in some patients with seronegative myasthenia gravis.<sup>22</sup> Finally, it cannot be excluded that functionally relevant antibodies react with glycoconjugates other than GQ1b.

**Clinical implications.** A combined pre- and postsynaptic defect may add up to compromise the safety margin of neuromuscular transmission. Muscles with the lowest safety margin, such as the extraocular muscles, could be preferentially involved.<sup>40</sup> Indeed, a clinical electrophysiologic case study suggests that motor nerve terminal block may induce muscle weakness in MFS.<sup>41</sup> In MFS patients, however, we did not observe abnormal fatigability and fluctuation of muscle weakness as one might expect in assuming a myasthenia-like action of blocking antibodies. This may be due to the combination of a pre- and postsynaptic blockade<sup>42,43</sup> whereby the postsynaptic transmission defect cannot be partially compensated for by presynaptic increase in quantal content.<sup>44,45</sup> Interestingly, the clinical pattern of primarily affecting extraocular muscle and cranial nerves, and the mode of paralysis in MFS are very similar to those found with botulism, a toxin-induced disorder of impaired presynaptic transmitter release.<sup>46</sup>

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# Sural nerve T-cell receptor V $\beta$ gene utilization in chronic inflammatory demyelinating polyneuropathy and vasculitic neuropathy

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**Article abstract**—*Objective:* To investigate the utilization of T-cell receptor (TCR) variable (V) regions in infiltrates of sural nerve biopsies of patients with chronic inflammatory demyelinating polyneuropathy (CIDP) and vasculitic neuropathy. *Background:* The presence of infiltrating T lymphocytes in sural nerve biopsies may suggest a T cell-mediated immune mechanism in the pathogenesis of CIDP and vasculitic neuropathy. *Patients and methods:* The utilization of TCR V $\beta$  regions in sural nerves of 13 patients with CIDP and five patients with vasculitic neuropathy was determined by immunohistochemistry, reverse-transcription PCR, and nucleotide sequence analysis. These techniques were also applied in four patients with chronic idiopathic axonal polyneuropathy (CIAP) who acted as noninflammatory controls, and in five autopsy controls. *Results:* The TCR V $\beta$  utilization of infiltrating T cells in sural nerves of patients with CIDP, vasculitic neuropathy, and noninflammatory controls is heterogeneous. A dominant TCR V $\beta$  utilization was not found in any of the patients or controls. *Conclusion:* There is no evidence for the presence of clonally expanded T cells in sural nerves of patients with CIDP and vasculitic neuropathy.

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Evidence for a pathogenic role of T cells in chronic inflammatory demyelinating polyneuropathy (CIDP) includes an increased frequency of circulating activated peripheral T cells,<sup>1,2</sup> and elevated serum levels of interleukine-2 (IL-2) and soluble IL-2 receptors.<sup>3</sup> In both CIDP and vasculitic neuropathy, increased numbers of T cells in the sural nerve biopsy specimens may indicate a role for T cells in the disease mechanism.<sup>4–6</sup> It is currently not clear whether sural nerve T cells, their numbers increased or not, are disease-specific autoreactive T cells, or whether these are attracted nonspecifically to the peripheral nervous tissue.

Analysis of T-cell receptors (TCR) in affected tissue may show 1) a broad TCR repertoire without V $\beta$

family overrepresentation (similar to that found in peripheral blood or normal lymph nodes), indicating that the T cells have been attracted nonspecifically by a proinflammatory environment; 2) a restricted TCR repertoire with V $\beta$  family overrepresentation but without evidence of clonal expansion, indicating that there may be a superantigen-driven T cell expansion; or 3) a restricted TCR repertoire with V $\beta$  family overrepresentation and clonal expansion, indicating that the T cells have been stimulated by specific antigens.<sup>7</sup> Patterns of restricted TCR V $\beta$  gene expression have been described in studies of multiple sclerosis,<sup>8</sup> inflammatory myopathies,<sup>9–16</sup> psoriasis,<sup>17</sup> and Sjögren's syndrome<sup>18</sup>; whereas other studies of MS,<sup>19</sup> rheumatoid arthritis,<sup>20</sup> and anti-Hu paraneoplastic encephalomyelitis/sensory neuronopathy<sup>7</sup> have indicated a more heterogeneous TCR V $\beta$  utilization of T cells in situ.

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## Combined pre- and postsynaptic action of IgG antibodies in Miller Fisher syndrome

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