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Increased Circulating T Cell Reactivity to GM1 Ganglioside in Patients with Guillain–Barré Syndrome

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Abstract

This study was performed to determine whether increased ganglioside-specific T cell reactivity can be detected in the peripheral blood of patients with Guillain–Barré syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). T cell responsiveness to the gangliosides GM1, GM3, GD1a, GD1b, GD3, GT1b, GQ1b and sulphatide was assessed in peripheral blood mononuclear cells from untreated GBS patients (57), CIDP patients (43), patients with other peripheral neuropathies (55) and healthy control subjects (74) in a standard 6-day proliferation assay. Increased T cell reactivity to GM1 occurred in GBS patients compared to healthy controls and patients with other neuropathies. There was increased reactivity to GM3 in GBS patients compared to patients with other neuropathies but not compared to healthy controls. The frequencies of increased T cell reactivity to GM1 and GM3 in CIDP patients were intermediate between those of GBS patients and controls. We suggest that T cell reactivity to gangliosides might play a contributory role in the pathogenesis of GBS and perhaps CIDP.

Keywords: Guillain–Barré syndrome; chronic inflammatory demyelinating polyradiculoneuropathy; ganglioside; T-lymphocyte

Introduction

Guillain–Barré syndrome (GBS) is an inflammatory disorder of the peripheral nervous system, characterised by the rapid onset of weakness, with disease severity ranging from mild symptoms to paralysis. Although most people recover, the duration of the illness may be prolonged.[1] Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is closely related to GBS, but is a chronic condition and is distinguished from GBS by its temporal pattern, and potential for clinical relapse.[2] Recent neurophysiological and pathological evidence suggests that GBS comprises a spectrum of diseases that could potentially have different pathological mechanisms and targets of immunological attack.[3] GBS can be subdivided into acute inflammatory demyelinating polyradiculoneuropathy (AIDP), acute motor and sensory axonal neuropathy (AMSAN) and acute motor axonal neuropathy (AMAN).[3] Miller Fisher syndrome is a related condition commonly thought to be a variant form of GBS. In Western countries, AIDP is the major form of GBS, accounting for 80–90% of cases, and involves early lymphocytic infiltration of spinal roots and peripheral nerves and macrophage-mediated segmental stripping of myelin.[3] [4] Variability also exists in the clinical features of CIDP.[5]

Autoimmune responses against peripheral nervous system antigens are believed to be important in the pathogenesis of GBS and CIDP.[3] [6] Gangliosides and other glycolipids have been increasingly studied as targets of autoimmunity in the nervous system.[7] [8] Gangliosides, which are glycolipids containing one or more sialic acid residues in their oligosaccharide chains,[9] are enriched in the plasma membranes of axons and neuronal cell bodies, and are also minor constituents of myelin.[10] Ganglioside composition varies in different parts of the nervous system.[11] [12] [13]Antibodies specific for more than 20 different glycolipids have now

been associated with a wide range of acute and chronic neuropathy syndromes, and the specificity of these shows some correlation with the clinical pattern of neuropathy.[8]

Recent studies have demonstrated that oligoclonal expansion of T cells bearing particular T cell receptor Vβ and Vδ genes frequently occurs in GBS patients, suggesting that T cells play a role in disease development.[14] Because the IgG1 and IgG3 isotypes of anti-ganglioside antibodies are frequently found in GBS,[8] [15] it is likely that T cell help is required for B cell maturation and antibody class switching. Considering the prevalence of anti-ganglioside antibodies in neuropathy and the potential role for T cell involvement in antibody production, we investigated whether ganglioside-specific T cell reactivity is increased in patients with GBS and CIDP.

Materials and methods

Patients

The numbers, sex, and age statistics of the patient groups used in this study are shown in Table 1. Patients with GBS, CIDP and other neuropathies were recruited from hospitals in South-East Queensland, Australia. GBS and CIDP patients fulfilled standard diagnostic criteria.[16] [17] GBS patients had predominantly demyelinating neuropathy and generally made a prompt recovery. Patients with Miller Fisher syndrome were not used in the study. Samples were collected from GBS patients within 10 days of the onset of neurological symptoms, either before or at the peak of disease, and before administration of any treatment. Follow-up blood samples were collected from some patients 3 months after recovery from GBS. GBS patient clinical disability was graded on the Hughes Functional Grading Scale (0 = healthy, 1 = minor symptoms or signs, 2 = independent ambulation, 3 = able to walk 5m with assistance, 4 = chair or bed bound, 5 = assisted ventilation for part of the day required, 6 = death).[18]

Table 1. Sample sizes and age data of GBS, CIDP, ON patients and healthy control subjects

Patient group	**Patient number and age**				
	Number (male:female)	**Mean age (years)**	**Age range**	**Median age**	**Mean proliferation assay background counts (cpm) ± SE**
GBS	57 (38:19)	52	18–82	54	675 ± 97
CIDP	43 (25:18)	54	18–85	57	759 ± 117
Healthy controls	74 (25:49)	34	21–72	32	745 ± 86
Other neuropathy	55 (28:27)	59	32–80	61	716 ± 115

Also included is the mean proliferation assay background counts per minute (cpm) ± SE for each of the test groups.

Of the GBS patients, 31% had a mild to medium maximum clinical severity grading of 1–3, whilst 69% had more severe clinical severity gradings of 4–5 (27% of 51 patients for whom follow-up information was available required mechanical ventilation). For GBS patients, information regarding potential precipitating factors, including recent infections or vaccinations prior to the onset of neurological symptoms, was recorded for 52 of the 57 patients studied. Of these, 29 patients (56%) had upper respiratory tract infection, 9 (17%)

gastrointestinal infections, 2 (4%) recent influenza vaccinations, 1 Epstein–Barr virus infection, 1 uveitis, and 2 fever of unknown origin. Eight remaining patients (15%) had no identifiable precipitating factors.

CIDP patients had not received any immunosuppressive medications, corticosteroids, intravenous immunoglobulin or plasma exchange for at least 2 months prior to blood collection. Patients with other neuropathies (ON) included patients with hereditary motor sensory neuropathy, toxic neuropathy and diabetic neuropathy. Healthy controls were recruited from hospital staff and at the time of blood collection had no symptoms of any infectious illnesses.

All blood samples were collected after written consent was obtained from each patient. This study was approved by the Human Research Ethics Committees of the Royal Brisbane, Princess Alexandra, Mater, Greenslopes Private, and Logan Hospitals, as well as the Medical Research Ethics Committee of The University of Queensland.

Antigens

Bovine gangliosides GM1, GD1b, GT1b and sulphatide (S) were purchased from Sigma (St. Louis, MO, USA), whilst GM3, GD1a, GD3 and GQ1b were purchased from Alexis Biochemicals (Switzerland). Structures of gangliosides used in the study are shown in Fig. 1. To determine the optimal physiological concentration of gangliosides for use in T cell proliferation assays, cells from patients and healthy controls were tested in culture against a variety of concentrations ranging from 10 μg/ml to 0.005 ng/ml. Tetanus toxoid was obtained as a gift from the Commonwealth Serum Laboratories (CSL; Melbourne, Australia). Phytohaemagglutinin (PHA) was obtained from Sigma (St. Louis, MO, USA).

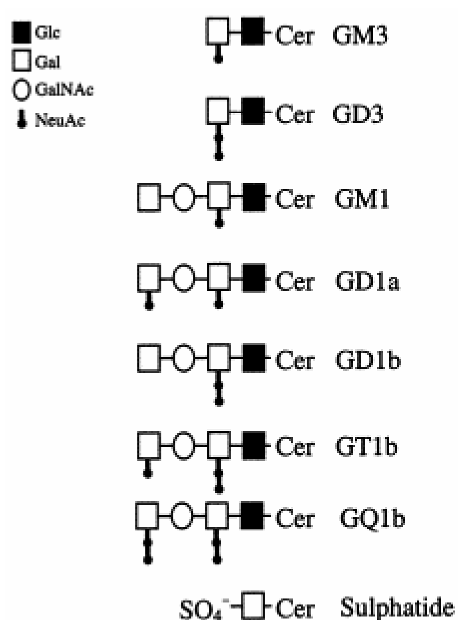


Fig. 1. Structures of gangliosides and sulphatide used in this study. Glc = glucose; Gal = galactose; GalNAc = *N*-acetylgalactosamine; NeuAc = *N*-acetylneuraminic acid; cer = ceramide.

Tissue typing

Genomic DNA was prepared from heparinised whole blood either by using Nucleospin Blood XL DNA extraction kits (Macherey-Nagel, Düren, Germany) or overnight digestion with sodium dodecyl sulphate/proteinase K, followed by salting out of high molecular weight DNA. HLA DRB1, DQA1 and DQB tissue typing was performed using SSP HLA typing kits (Dynal Biotech, Oslo, Norway).

Anti-GM1 enzyme linked immunosorbent assay

Details of enzyme linked immunosorbent assay (ELISA)-based methods for detection of anti-GM1 IgG and IgM antibodies in GBS patient sera have been presented previously.[19]

Proliferation assays

Peripheral blood (~60 ml) was collected by venepuncture from each subject after informed written consent had been obtained. Peripheral blood mononuclear cells (PBMC) were separated from heparinised blood by centrifugation through Histopaque (Sigma, St Louis, MO, USA) and washed twice. One hundred thousand PBMC were cultured in 200 µl/well in quadruplicate cultures with and without test antigens in 96-well round-bottomed microtitre plates (Nunc, Denmark) in RPMI-1640 media supplemented with 10% heat-inactivated pooled human serum, 2 mM l-glutamine and 10 mM Hepes buffer. Cultures were incubated for 6 days, with 0.5 µCi [^{3}H]thymidine being added during the last 18 h of culture. Cultures were harvested and thymidine uptake was measured in counts per minute in a β-plate counter (LKB-Wallace, Turtu, Finland). Each ganglioside was tested at a range of five concentrations (1, 0.1, 0.05, 0.01 and 0.005 ng/ml). The stimulation index (SI) was determined by the formula: SI = (mean cpm of quadruplicate, ganglioside-containing wells)/(mean cpm of 24 control wells, without antigen). A positive proliferative response for each test subject's cells was scored if the patient's cells responded to the ganglioside tested at any one of the five concentrations with an SI $\geqslant$ 2.0. Individual assays with control background counts of less than 150 cpm, or greater than 5000 cpm were considered outside the normal range and rejected from the data set. Mean and standard error values for background counts for each patient group can be found in Table 1. Tetanus toxoid and the mitogen PHA were used to assess the viability and strength of cellular reactivity in each subject's cells.

Statistical analysis

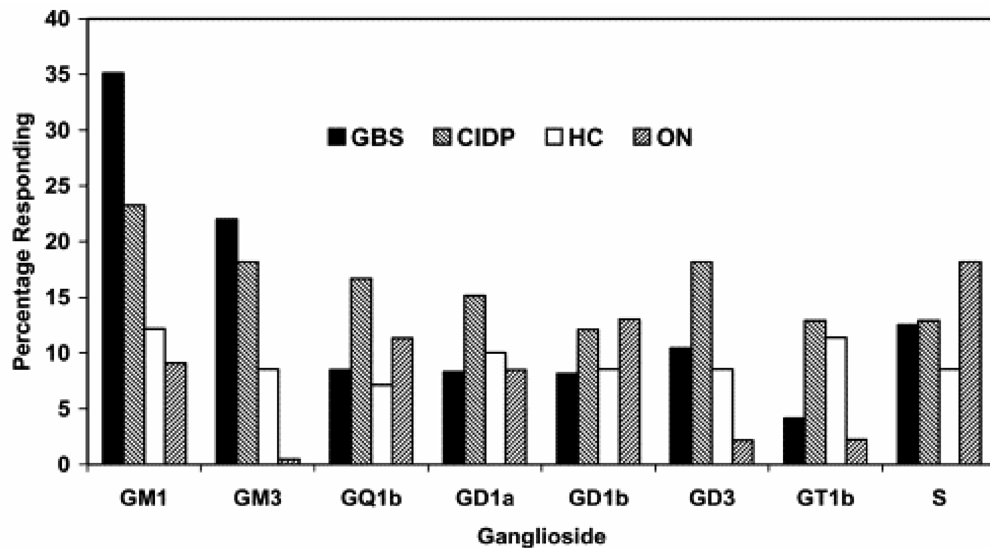
In the different patient groups, the percentages of individuals making a positive proliferative response to gangliosides were compared using the χ^2 test with Yates' correction applied as required. Odds ratios (OR) with upper and lower 95% confidence intervals were also calculated for selected comparisons. Mean SI values were compared using analysis of variance (ANOVA) to compare all the test groups simultaneously. *F*-tests were used to determine whether the variances between test group stimulation indices were significantly different, followed by the appropriate Student's *t* test to compare pairs of groups. A comparison was deemed to show statistical significance if $p \leqslant 0.05$.

T cell reactivity to different antigens in the patient groups (GBS or CIDP) was deemed to be of significance only if both the comparison of the percentages of patients showing increased T cell reactivity, and the comparison of the T cell reactivity mean SI values were significantly increased when compared to both healthy controls and ON patients.

Results

T cell reactivity to gangliosides: frequencies of responses with an SI $\geqslant$ 2.0

The percentages of individuals in each subject group with a T cell reactivity SI $\geqslant$ 2.0 were determined for each ganglioside and compared by χ^2 analysis (Fig. 2). The percentage of GBS patients reacting with an SI $\geqslant$ 2.0 to GM1 was 35%, which was significantly higher than the percentage of healthy subjects (12%) and of ON patients (9%). Although the percentage of CIDP patients with an SI $\geqslant$ 2.0 for GM1 reactivity (23%) was higher than that of healthy controls and ON patients, this increase was not statistically significant.



	GM1	GM3	GQ1b	GD1a	GD1b	GD3	GT1b	S
4 x 2	0.002	0.004	0.5	0.5	0.5	0.108	0.152	0.5
GBS vs HC	0.002	0.035	0.463	0.662	0.701	0.535	0.236	0.354
GBS vs ON	0.002	0.002	0.859	0.759	0.646	0.225	0.943	0.426
CIDP vs HC	0.117	0.090	0.081	0.282	0.311	0.090	0.252	0.194
CIDP vs ON	0.099	0.009	0.513	0.463	0.840	0.037	0.143	0.655

Fig. 2. Percentages of patients with GBS, CIDP, healthy control subjects (HC) and other neuropathy patients (ON) responding with an SI ⩾ 2.0 to specific gangliosides. The *p* values for the comparisons of the responses of the four groups together (4 × 2) and the *p* values for the comparisons of the pairs of groups (χ^2 analysis) are shown directly below the ganglioside to which they refer. S = sulphatide.

The proportions of patients showing an SI ⩾ 2.0 for GM3 T cell reactivity in GBS patients (22%) were significantly higher than those obtained for both healthy controls (9%) and ON patients (0%). CIDP patient anti-GM3 reactivity (18%) was significantly greater than that of ON patients (0%), but not significantly greater than healthy controls (9%). The percentage of CIDP patients with increased T cell reactivity to GD3 (18%) was significantly higher than that of ON patients (2%), but not that of healthy controls (8%). Seven of the 20 GBS patients (35%) with increased T cell reactivity to GM1 also had increased reactivity to GM3, and of the 11 GBS patients with increased reactivity to GM3, 7 (63%) displayed increased anti-GM1 reactivity. Table 2 shows the detailed statistical comparison of reactivity to GM1 and GM3 between pairs of test groups. Ten GBS patients who initially responded to GM1 were re-tested for reactivity 3 months after disease recovery. Only one of these patients retained increased T cell reactivity to GM1.

Table 2. χ^2 ***p*** **values, odds ratios and upper and lower 95% odds ratio confidence intervals for multiple two-way comparisons for increased T cell reactivity to GM1 and GM3**

Comparison	GM1			GM3		
	p value	Odds ratio	Upper and lower 95% CI	*p* value	Odds ratio	Upper and lower 95% CI
GBS vs. HC	0.002	3.90	1.61–9.45	0.035	3.00	1.03–8.78
GBS vs. ON	0.002	5.40	1.85–15.73	0.002	13.25	1.60–107.2
CIDP vs. HC	0.117	2.00	0.74–5.39	0.090	2.37	0.70–8.01
CIDP vs. ON	0.099	2.77	0.87–8.82	0.009	10.44	1.19–91.4

The percentages of GBS and CIDP patients with increased T cell reactivity to the gangliosides GD1a, GD1b, GT1b, GQ1b and sulphatide were not significantly greater than those of healthy controls or ON patients. There were no significant differences in anti-ganglioside reactivity between GBS patients and CIDP patients.

T cell reactivity to gangliosides: mean stimulation indices

Fig. 3 shows a scatter plot of individual patients' proliferative responses to GM1 and GM3. For GM1, the mean maximum SI for GBS patients (2.43 ± 0.53) was significantly higher than for healthy controls (1.43 ± 0.07; $p = 0.036$) and ON patients (1.28 ± 0.09; $p = 0.037$). For GM3 reactivity, the mean maximum SI for GBS patients (1.52 ± 0.13) was significantly greater than that of ON patients (1.20 ± 0.04; $p = 0.038$), but not than that of healthy controls (1.37 ± 0.07). Of the GBS patients reacting to GM1 with an SI $\geqslant$ 2.0, the mean SI was 4.69 ± 1.42 (range 2.06–28.67). Of the positive GBS responders to GM3, the mean SI was 2.93 ± 0.92 (range 2.02–5.17). For the gangliosides GD1a, GD1b, GD3, GT1b, GQ1b and sulphatide, there were no significant differences in means of stimulation indices between GBS or CIDP and healthy controls and ON patients.

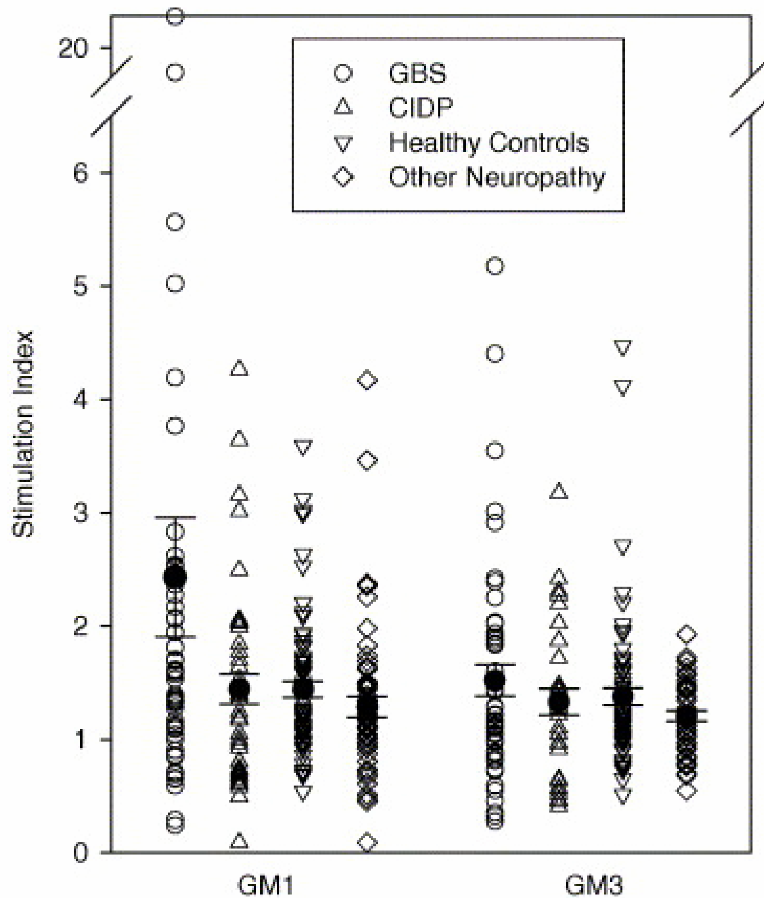


Fig. 3. Scatter plot of T cell reactivity to GM1 and GM3 expressed as stimulation indices for each patient in each of the test groups. Filled black circles indicate the mean stimulation index for each group, whilst error bars indicate standard error.

T cell reactivity to tetanus toxoid

For all assays, tetanus toxoid was included as a known, strong inducer of recall T cell reactivity, and serves as a useful indicator for comparison with anti-ganglioside specific responses. Table 3 shows the percentages of positive T cell reactivity as well as the means ± SE of tetanus toxoid-specific responses in each of the test groups. The *p* values for comparison of frequencies of increased reactivity and for comparison of means between pairs of test groups are also shown. No significant differences in proportions of tetanus toxoid-specific T cell reactivity were seen in GBS patients when compared to healthy controls or ON patients. CIDP patients had significantly lower reactivity than healthy controls, but not compared with ON patients.

Table 3. Frequencies (%) and mean maximum stimulation indices (SI ± SE) of T cell reactivity to tetanus toxoid for GBS, CIDP, ON patients and healthy controls

Patient group	**Frequency SI ⩾ 2 tetanus toxoid**	**Mean SI ± SE tetanus toxoid**
GBS	73.8%	27.9 ± 5.4
CIDP	66.7%	20.5 ± 4.1
Healthy controls	83.5%	35.3 ± 4.9
Other neuropathy	72.1%	34.5 ± 6.2
Groups compared	**χ^2 *p* values**	**Student's *t* test *p* values**
GBS vs. HC	0.150	0.317
GBS vs. ON	0.838	0.426
CIDP vs. HC	0.021	0.034
CIDP vs. ON	0.525	0.069

Student's *t* test was used to compare the means of pairs of test groups, whilst the χ^2 test was used to compare frequencies of reactivity between test groups.

Correlation of T cell reactivity with clinical features and HLA typing

Table 4 compares GBS patient anti-GM1 T cell reactivity with positive anti-GM1 antibody titre, disease severity as denoted by requirement for patient ventilation, and antecedent illness. The proportion of patients with increased IgG or IgM anti-GM1 antibody levels was higher in patients with increased GM1-specific T cell reactivity (67%) than in patients without elevated T cell reactivity to GM1 (41%), although this was not significant.

Table 4. Comparison of GBS patient T cell reactivity to GM1 ganglioside with positive anti-GM1 antibody titre, disease severity and antecedent illness

Total GBS patients	GM1 T cell reactivity	IgG or IgM GM1 antibody	Disease severity (ventilation required)	Antecedent illness
		8 +ve (67%)	1 ventilated (6%)	8 URTI (42%)
n = 57	20 +ve (35%)	4 −ve (33%)	17 not ventilated (94%)	5 gastro (26%)
		8 not tested	2 not known	2 other (11%)
				4 none (21%)
				1 not known
		9 +ve (41%)	13 ventilated (39%)	21 URTI (64%)
	37 −ve (64.9%)	13 −ve (59%)	20 not ventilated (61%)	4 gastro (12%)
		15 not tested	4 not known	4 other (12%)
				4 none (12%)
				4 not known

Interestingly, only one (6%) of 18 GBS patients who had increased T cell reactivity to GM1 required ventilation (disease severity score of 5), whereas ventilation was required in 13 (39%) of 33 patients who did not have increased T cell reactivity. This negative association between disease severity and anti-GM1 T cell reactivity was statistically significant ($p = 0.023$, OR = 0.09, 95% CI of OR = 0.011–0.76).

When GBS patients were divided into two groups based on the type of preceding infection prior to onset of neurological symptoms, 56% (5 of 9 patients) with gastrointestinal infections as a preceding illness had increased T cell reactivity to GM1, whereas only 26% (8 of 29 patients) with non-specific upper respiratory tract infections as a preceding illness displayed increased reactivity to GM1, although this was not statistically significant.

HLA-DR and HLA-DQ typing of GBS patients revealed no associations between T cell reactivity to GM1 or any of the other gangliosides tested and HLA-DR or HLA-DQ type (either at the molecular level, or when results were grouped into conventional serological typing groups).

Discussion

The present study has shown that patients with GBS have increased circulating T cell reactivity to GM1 compared to healthy controls and ON patients. The finding of elevated reactivity in GBS patients, but not in patients with other peripheral neuropathies, shows that the immunoreactivity is not a consequence of peripheral nerve damage. Indeed, the T cell reactivity against GM1 tended to be lower in patients with other neuropathies than in healthy controls, as we have previously reported for T cell reactivity to gangliosides in patients with central nervous system diseases other than multiple sclerosis.[20] Elevated T cell reactivity to GM1 in GBS patients is congruent with previous reports of elevated anti-GM1 antibodies in patients with either demyelinating or axonal forms of GBS.[21] [22] [23] [24] [25] [26] [27] In the present study, there was an increased frequency of elevated anti-GM1 antibody levels in those

GBS patients demonstrating increased T cell reactivity to GM1, although this failed to reach statistical significance. GM1 is relatively enriched in human ventral roots compared with dorsal roots,[28] and antibodies specific to GM1 are commonly associated with motor neuropathy, especially axonal forms of GBS,[27] CIDP and multifocal motor neuropathy. As the majority of our patients had demyelinating forms of GBS, we were unable to examine the frequency of GM1 reactive T cells in axonal forms of GBS.

Recently, the role of the MHC-like CD1 family of molecules in presentation of lipid and glycolipid antigens to T cells and natural killer T (NKT) cells has been elucidated. T cells and NKT cells of both pro-inflammatory and immunoregulatory phenotypes can be stimulated by presentation of lipid and glycolipid antigens through CD1, suggesting that CD1-restricted T cells and NKT cells might play a role in determining the balance between tolerance and autoimmunity to these antigens.[29] GM1-specific T cell clones have been produced from the peripheral blood of MS patients and shown to be restricted by CD1b,[30] [31] which is expressed by dendritic cells, but not by monocytes and macrophages in the blood.

Generally, the stimulation indices of ganglioside-specific T cell reactivity was of a lower level than responses to MHC class II-restricted control antigens like tetanus toxoid. Our assay system could have been limited by a lack of cells capable of presenting gangliosides to CD1b-restricted ganglioside-specific T cells, and could have under-estimated the level of reactive cells as dendritic cell numbers in the blood are generally much lower than the levels of monocytes and macrophages. Indeed, as we found no correlation of HLA-DR or HLA-DQ typing with anti-GM1 T cell reactivity in our GBS patients, it is possible that these T cells are CD1 restricted. Gangliosides are relatively insoluble in aqueous solutions, and this could also influence the efficiency of their uptake and presentation by antigen-presenting cells in our assay cultures.

There was no significant difference in reactivity to the positive control antigen tetanus toxoid between GBS patients and healthy controls or ON patients.
Ninety percent of GBS patients showing increased anti-GM1 T cell reactivity did not have increased reactivity 3 months after recovery from the disease, perhaps indicating a lack of induction of conventional T cell memory mechanisms, or that these cells are deleted from the T cell repertoire during recovery from GBS.

Despite many reports characterising specificity of anti-ganglioside antibodies in inflammatory neuropathy, this is the first report of ganglioside-specific T cells in GBS and CIDP. Recently, there has been a report that GBS patients might show T cell proliferation in response to non-protein antigens of *Campylobacter jejuni*, which could represent a response to gangliosides found in *C. jejuni*[32] Chemical studies of the core oligosaccharide of *C. jejuni* lipo-oligosaccharides have revealed structures that mimic human gangliosides including GM1, GD1a, GD2, GD3, and GM2,[33] strengthening the view that molecular mimicry between pathogen glycolipids and self-gangliosides, particularly GM1, could be an important factor in the pathogenesis of GBS.[34] It has been generally found that GBS follows gastrointestinal or respiratory infections in two-thirds of cases.[4] Seventy-three percent of the GBS patients in this study had preceding non-specific upper respiratory tract or gastrointestinal infections prior to the onset of neurological symptoms. Although not statistically significant, we found that a greater proportion of patients with preceding gastrointestinal infections had increased anti-GM1 T cell reactivity than did those patients with preceding non-specific upper respiratory tract infections. In GBS, anti-GM1 antibodies have been found to be associated with severe cases and a poor prognosis, and also with GBS following infection with *C. jejuni*[23] [24] [35] In contrast to the finding that anti-GM1 antibodies are associated with more severe forms of GBS, we found that patients with increased anti-GM1 T cell reactivity were significantly less likely to need mechanical ventilation than those without such reactivity. This raises the possibility that increased GM1-specific T cell reactivity might have an immunoregulatory or neuroprotective role.

Antibody reactivity to GD1a has been found in patients with acute motor axonal neuropathy and predominantly motor forms of GBS,[36] [37] and antibodies to GD1a preferentially label motor fibres whereas antibodies to GD1b preferentially label large dorsal root ganglion neurons.[13] We did not find significantly increased T cell reactivity to GD1a in

our patients with GBS or CIDP, possibly because the majority of our GBS patients had predominantly demyelinating, rather than axonal forms of disease. Antibody to GD1b and GT1b has been found in patients with predominantly sensory neuropathy,[38][39][40] but we did not find increased T cell reactivity to these antigens in the present study, possibly because our patients had predominantly motor neuropathies. Similarly, we found no elevated reactivity to sulphatide, although this has also been a target of antibody in neuropathy.[41]
CIDP patients had increased T cell reactivity to the gangliosides GM1, GM3, GD3 and possibly GQ1b more frequently than healthy controls and ON patients but the differences were not statistically significant. The proportions of CIDP patients with increased T cell reactivity to gangliosides may be an underestimate, as it is possible that the level of this reactivity may fluctuate with the disease course of CIDP, as we have demonstrated for T cell reactivity to myelin antigens in multiple sclerosis.[42] Although CIDP patients had not received any immunomodulatory therapy for at least 2 months prior to blood collection, previous treatments in some patients might have reduced the levels of ganglioside-specific T cells. Gangliosides could still represent targets of autoimmune T cell attack in CIDP. Studies of nerve biopsies from CIDP patients have revealed evidence of NKT and T cells of a γδ TCR phenotype, consistent with a possible role for cellular immune responses against non-protein antigens.[43] [44]

The present study does not directly determine whether these ganglioside-specific T cells contribute to the pathogenesis of inflammatory neuropathy. Previous experimental evidence in animal models indicates that autoreactivity to gangliosides can cause neuropathy,[45] [46] [47] and a positive association between injection of patients with bovine gangliosides and increased incidence of GBS provides further evidence.[8] [48] One possible role for ganglioside-reactive T cells might be to facilitate production of antibody that may itself have a role in pathogenesis. Indeed, it has been shown that the B cells producing GM1 antibody in GBS and multifocal motor neuropathy are T cell dependent,[49] whilst T cells of the γδ type have been found in the blood of patients with GBS and were thought to provide help for glycolipid antibody production.[50] Ganglioside-reactive T cells may also have a more direct role in pathogenesis. In GBS and CIDP, there is infiltration of the nerves with T cells and macrophages.[3] and [6] In experimental allergic neuritis, an animal model of GBS, both NK cells and γδ T cells have been found in the nerves, where they could play a direct role in pathogenesis,[51] or in providing B cell help.[52] In GBS, γδ T cells have been cultured from nerve biopsies, where they have been thought to have a role in responding to non-protein antigens,[44] [53] and oligoclonal expansion of T cells bearing particular T cell receptor Vβ and Vδ genes frequently occurs in GBS, suggesting that T cells play a role in disease development.[14]

In GBS, ganglioside-specific T cells could cause damage to nerves through a number of mechanisms including direct axonal or Schwann cell cytotoxicity, or by secretion of proinflammatory cytokines to induce damage directly or act to recruit and activate macrophages. Early infiltration of nerves with activated, pro-inflammatory T cells could help to open the blood–nerve barrier to pathogenic antibody.[54] Alternatively, specific sub-populations of ganglioside-specific T cells could act by the secretion of downregulatory cytokines to terminate the acute inflammatory process.

In summary, we report the finding of increased T cell reactivity to GM1 in GBS patients, and suggest that further studies are required to characterise these cells phenotypically, to determine whether they contribute to the pathogenesis of disease, or serve to act in an immunoregulatory or neuroprotective capacity.

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