Anti-GD1a Antibody Is Associated with Axonal But Not Demyelinating Forms of Guillain-Barré Syndrome

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Immunopathological studies suggest that the target of immune attack is different in the subtypes of Guillain-Barré syndrome (GBS). In acute motor axonal neuropathy (AMAN), the attack appears directed against the axolemma and nodes of Ranvier. In acute inflammatory demyelinating polyneuropathy (AIDP), the attack appears directed against a component of the Schwann cell. However, the nature of the antigenic targets is still not clear. We prospectively studied 138 Chinese GBS patients and found that IgG anti-GD1a antibodies were closely associated with AMAN but not AIDP. With a cutoff titer of greater than 1:100, 60% of AMAN versus 4% of AIDP patients had IgG anti-GD1a antibodies; with a cutoff titer of greater than 1:1,000, 24% of AMAN patients and none of the AIDP patients had IgG anti-GD1a antibodies. In contrast, low levels of IgG anti-GM1 antibodies (>1:100) were detected in both the AMAN and the AIDP forms (57% vs 35%, NS). High titers of IgG anti-GM1 (>1:1,000) were more common in the AMAN form (24% vs 8%, NS). Serological evidence of recent Campylobacter infection was detected in 81% of AMAN and 50% of AIDP patients, and anti-ganglioside antibodies were common in both Campylobacter-infected and noninfected patients. Our results suggest that IgG anti-GD1a antibodies may be involved in the pathogenesis of AMAN.

Guillain-Barré syndrome (GBS) is a heterogeneous disorder with different clinical, electrophysiological, and pathological subtypes. In North America, Western Europe, and Australia, the major subtype is acute inflammatory demyelinating polyneuropathy (AIDP). AIDP is characterized electrophysiologically by demyelination of both motor and sensory nerves and pathologically by various degrees of lymphocytic infiltration and demyelination.1–4 In northern China, Japan, and Mexico, acute motor axonal neuropathy (AMAN) is frequently encountered.5–9 AMAN is characterized electrophysiologically by low motor-evoked amplitudes without features of demyelination and pathologically by macrophage-mediated attack on motor axons especially at the nodes of Ranvier, with variable amounts of Wallerian-like degeneration, but little inflammation or demyelination.5–7,10

Several studies have shown that infection with Campylobacter jejuni has been associated with AIDP and AMAN.5,11–14 Because anti-ganglioside antibodies are found in many GBS patients5,11–13,15,16 and the lipopolysaccharide of some C. jejuni strains isolated from GBS patients contains ganglioside-like epitopes17–19 molecular mimicry between epitopes on the surface of C. jejuni and the neural targets has been proposed as a possible mechanism for C. jejuni-associated GBS.20 The difference in the pattern of nerve damage and differential localization of antibody and complement in AIDP and AMAN suggest different targets of immune attack.21,22 The targets of immune attack have been proposed to be gangliosides and/or structurally related glycoconjugates that are localized either at the abaxonal surface of the Schwann cell in the case of AIDP or at the motor nodes of Ranvier and adjacent axolemma in the case of AMAN.21,22

Many anti-glycoconjugate antibodies have been

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described in GBS patients. The strongest association is between the Fisher syndrome and IgG anti-GQ1b. However, the association of anti-glycoconjugate antibodies with AIDP and AMAN is variable. We examined the anti-glycoconjugate profile in a group of well-characterized, prospectively studied GBS patients with a balanced proportion of AMAN and AIDP subtypes. We then compared our findings with those of GBS patients from the United States.

Patients and Methods

Patients
The study population included all patients with clinically defined GBS admitted to the Second Teaching Hospital of Hebei Medical University (Shijiazhuang, People’s Republic of China) between January 1, 1992, and December 31, 1997. To be included, patients had to have their first neurological symptoms within 30 days before admission. One hundred seventy-eight patients with clinically defined GBS were admitted; of these, 138 patients who fulfilled the inclusion criteria were enrolled in the study. After informed consent, each patient underwent serial clinical examinations, serological and electrodiagnostic studies, and stool cultures as previously described. All studies were approved by the appropriate institutional review boards.

Control Subjects
During the study, control sera were collected from the following groups: (1) 39 age-matched and sex-matched controls from two Chinese villages during the summer, the peak season for GBS in China; (2) 10 hospitalized Chinese patients with other neurological diseases; (3) 18 patients from the United States with culture-positive uncomplicated Campylobacter infection; and (4) 13 family members of patients.

Electrophysiological Studies
Motor and sensory conduction studies were performed with a Dantec Cantata (Skoulunde, Denmark) machine by standard methods. The diagnostic definitions for AIDP and AMAN were based on our previous criteria, except that nerves with very low compound motor-evoked amplitude (<10% of lower limit of normal) were classified as equivocal. This change was made because of the recognition that the latency and conduction velocity may be abnormal and in the demyelinating range as a result of loss of fast conducting fibers alone.

Anti-Glycolipid Serology
Enzyme-linked immunosorbent assays were performed on the initial acute sera from GBS patients, and control groups according to Willison and colleagues. GM1, asialo-GM1 (GA1), GD1a, and GD1b (Sigma, St Louis, MO) were tested and a positive serological response analyzed by using two cutoff values for titers—greater than 1:100 and greater than 1:1,000.

Anti-C. jejuni Antibodies
Antibodies against C. jejuni were measured as previously described. Sera from GBS patients and controls obtained in 1992 were reported previously. Positivity was defined as an optical density ratio (ODR) of either 1 or more, or 2 or more, in two or more immunoglobulin classes. Previous studies of patients with culture-confirmed infections and of healthy controls showed that using an ODR definition of 1 or more was 100% sensitive and 90% specific, and 2 or more was 65% sensitive but 97% specific.

Statistical Analysis
For statistical analysis, only patients with AIDP or AMAN were included; patients with inexcitable nerves or equivocal physiological studies were not included. Comparative analyses were done in a univariate fashion by using the Mantel–Haenszel $\chi^2$ and two-tailed Fisher exact tests, when indicated (Stata 5.0, College Station, TX). Because of multiple

<table>
<thead>
<tr>
<th>Titters &gt;1:100</th>
<th>AMAN (n = 68)</th>
<th>AIDP (n = 26)</th>
<th>Odds Ratio (95% Confidence)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG anti-GD1a</td>
<td>60%</td>
<td>4%</td>
<td>38 (4.9–297)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>IgG anti-GM1</td>
<td>57%</td>
<td>35%</td>
<td>2.5 (1–6.5)</td>
<td>0.06</td>
</tr>
<tr>
<td>Titters &gt;1:1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG anti-GD1a</td>
<td>24%</td>
<td>0%</td>
<td>7.7 (1–61)*</td>
<td>0.005*</td>
</tr>
<tr>
<td>IgG anti-GM1</td>
<td>24%</td>
<td>8%</td>
<td>3.7 (0.8–17)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*Significance after Bonferroni corrections.
*Estimated odds ratio and 95% confidence interval, assuming the 0% cell is 1.

AMAN = acute motor axonal neuropathy; AIDP = acute inflammatory demyelinating polyneuropathy; GBS = Guillain-Barré syndrome.
comparisons, the significant levels were adjusted to $p = 0.00625$ for Table 1 (eight comparisons) and $p = 0.005$ for Table 2 (10 comparisons), using the Bonferroni correction.

### Results

Of the 138 Chinese patients enrolled in the study, 72 were classified as AMAN (52%), 26 as AIDP (19%), 33 as equivocal (24%), and 7 had inexcitable motor nerves (5%). The median ages for AMAN, AIDP, equivocal, and inexcitable were 13, 18, 22, and 7 years, respectively.

### Anti-Glycolipid Serology

IgG anti-GD1a, GM1, GD1b, and GA1 antibody titers for the different groups are shown in the Figure.

**Anti-GD1A.** This antibody was the most specific for the AMAN group, with good sensitivity (see Table 1). With a low cutoff, 60% of AMAN patients compared with 4% of AIDP patients were positive for IgG anti-GD1a antibodies ($p = 0.0001$; odds ratio, 38; 95% confidence interval, 5–297). With a higher cutoff, the sensitivity for AMAN decreased to 24%, but specificity was 100% ($p = 0.005$). Only 1 of the family controls showed a low titer (1:390) of IgG anti-GD1a antibodies. None of the other controls had detectable anti-GD1a antibodies.

**Anti-GM1.** Like anti-GD1a, anti-GM1 antibodies were statistically more common in the GBS patients than in the control groups. Only 3 of the village controls ($p = 0.0001$) and 1 of the family controls ($p = 0.002$) had low-titer anti-GM1 antibodies compared with GBS patients. When analyzed by GBS subtypes, IgG anti-GM1 antibodies were more common in the AMAN group than in the AIDP group. With the low cutoff, 57% of the AMAN patients versus 35% of AIDP patients were positive for IgG anti-GM1 antibodies (not significant [NS]). With a higher cutoff, 24% versus 8% were positive for IgG anti-GM1 antibodies (NS). Thirty-eight percent of AMAN and 4% of AIDP patients were positive for both IgG anti-GD1a and anti-GM1 antibodies with the lower cutoff.

**OTHER ANTI-GLYCOLIPID ANTIBODIES.** IgG anti-GA1 antibodies were frequently detected in both GBS patients and in control groups and did not differ significantly (see Fig. D). The levels of other classes of anti-glycolipid antibodies (IgA and IgM) did not reach statistical significance (results not shown).

### Comparison with US GBS Patients

Forty-three US GBS patients were tested for the presence of anti-glycolipid antibodies; 36 exhibited demyelinating electrophysiology and 2 had axonal electrophysiology. Five more met clinical criteria for Fisher syndrome. IgG anti-GD1a antibodies at greater than 1:1,000 were detected only in 1 of the 2 axonal patients and in none of the other 36 AIDP patients (NS). In addition, 2 AIDP and 1 Fisher patient had low titers (<1:400) of IgG anti-GD1a antibodies. IgG GM1...
antibodies were present in 3 patients, of which 1 was an AMAN case.

**Relationship with Anti–C. jejuni Serology**
With positive defined as an ODR 1 or more in two or more immunoglobulin classes, 74% of Chinese patients with GBS (81% of patients with AMAN and 50% of patients with AIDP) and 16% of Chinese village control subjects were seropositive. With the more stringent definition of positive (ODR $\geq 2.0$ in two or more immunoglobulin classes), 45% of patients with GBS (47% of patients with AMAN and 27% of patients with AIDP) and 4% of Chinese village controls were seropositive.

Anti-glycolipid antibodies were common in both *C. jejuni*–positive and *C. jejuni*–negative groups (see Table 2). However, *C. jejuni*–positive patients were more likely to have anti-ganglioside antibodies (at low-titer cutoff, $p = 0.02$, not significant after Bonferroni correction; $p = 0.005$, at high-titer cutoff). In a similar manner, IgG anti-GM1 antibodies were slightly more frequent in the *C. jejuni*–positive group than in the *C. jejuni*–negative group (NS). No significant difference was observed for IgG anti-GD1a antibodies.

**Discussion**
In this prospective study, high-titer IgG anti-GD1a antibodies were strongly correlated with Chinese AMAN but not with AIDP patients or control subjects. In the US patients studied, high titers of IgG anti-GD1a antibodies were found in 1 of the 2 axonal cases but in none of the AIDP cases. Although case reports have suggested that IgG anti-GD1a can occur in GBS,32–36 most studies have not found this association.13,14,16,37 We suggest the association between AMAN and IgG anti-GD1a was not recognized, as most studies involved GBS populations consisting predominantly, if not exclusively, of AIDP patients.

Forty percent of AMAN patients were negative for anti-GD1a antibodies. We did not find any statistically significant differences between the anti-GD1a–positive or anti-GD1a–negative AMAN cases in terms of the time when the first blood samples were taken, age, season, or prior *C. jejuni* infection. It is possible that other antigens or other pathogenetic mechanisms may be involved in these patients. A more sensitive assay, however, to test for anti-GD1a antibodies may be needed.

IgG anti-GM1 antibodies were found more frequently in AMAN than in AIDP patients. The differ-

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**Fig.** IgG anti-ganglioside antibodies in different groups. AMAN = acute motor axonal neuropathy; AIDP = acute inflammatory demyelinating polyneuropathy; Equiv. = equivocal; Inexcit. = inexcitable; OND = other neurological disease; C.J. = C. jejuni enteritis; $\bullet$ = 5 patient samples; $\triangle$ = US axonal Guillain-Barré syndrome (US GBS).
ence is greatest when the high-titer cutoff is used as previously reported by Kornberg and colleagues.27 Similar to results from our previous study, however, IgG anti-GM1 antibodies are neither as specific nor as sensitive as IgG anti-GD1a antibodies.5 Enders and colleagues12 and Vriesendorp and associates11 found no correlation between anti-GM1 antibodies and GBS subtypes as determined electrophysiologically. Rees and co-workers16 detected IgG anti-GM1 antibodies in 25% of their AIDP cases versus 57% in AMAN/acute motor sensory axonal neuropathy (AMSAN) (p = 0.07). These studies suggest that anti-GM1 antibodies are only modestly correlated with different subtypes of GBS.

The epitope(s) involved in AIDP remains elusive. Anti-GM1, GD1b, and GA1 antibodies can occur in both axonal and demyelinating patients. Although there may be many different forms of AIDP, each with its own antibodies, the presence of many of these anti-glycoconjugate antibodies in axonal and in demyelinating cases of GBS and in controls suggests questions about their specificity and their biological significance.

Antecedent C. jejuni infection is common in all subtypes of GBS including both AIDP and AMAN.5,38,39 In the 10 strains of C. jejuni we have isolated from GBS patients, two are from patients with AIDP, seven are from patients with AMAN, and one is from a patient with Fisher syndrome.49 Rees and colleagues14 reported that in GBS patients from England, 76% of C. jejuni–positive patients had AIDP and 24% had AMAN/AMSAN. AMAN/AMSAN patients were frequently C. jejuni positive (6 of 7 patients), but because of the greater frequency overall of AIDP patients, most of their C. jejuni–positive patients had AIDP. In a similar manner, studies in Europe and the United States, by Enders and associates,12 by Vriesendorp and collaborators,11 and by Jacobs and colleagues,41 have shown that GBS associated with C. jejuni is predominantly associated with AIDP.

We did not find any relationship between anti-ganglioside antibody and C. jejuni infection. In a single strain of C. jejuni, the terminal sugar structure in the lipopolysaccharide contains many ganglioside-like epitopes, mimicking among others GM1, GM1b, and GD1a.17,19 Therefore, it is not surprising that many GBS patients with prior C. jejuni infection have raised antibodies against these epitopes. Anti-ganglioside antibodies are common in both C. jejuni–infected and C. jejuni–noninfected patients, suggesting that other inciting factors can also elicit anti-ganglioside antibody responses.

In conclusion, IgG anti-GD1a antibodies are strongly associated with the AMAN subtype of GBS. Our study suggests that antibody against a GD1a-like epitope is important in the pathogenesis of axonal GBS. Our previous studies have shown that the axolemma at motor nodes of Ranvier is the most likely target for antibody-mediated immune attack.21 The present results suggest that a GD1a-like epitope may be present on the node of Ranvier and subject to immune attack in axonal GBS. Further studies are needed to investigate the localization of GD1a in peripheral nerve and to determine the pathogenetic significance of IgG anti-GD1a antibodies in animal models of GBS.

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