

SHORT REPORT

Cytomegalovirus infections and anti-GM2 antibodies in Guillain-Barré syndrome

B C Jacobs, P A van Doorn, J H M Groeneveld, A P Tio-Gillen, F G A van der Meché

Abstract

To investigate whether antecedent cytomegalovirus (CMV) infections in patients with Guillain-Barré syndrome are associated with the presence of specific antiganglioside antibodies, acute phase serum samples from 130 patients with Guillain-Barré syndrome and 200 controls were tested. Anti-GM2 IgM antibodies were found more often in patients with Guillain-Barré syndrome with CMV infection (22%) than in patients without the infection (2%) ($P = 0.003$). CMV infections may elicit anti-GM2 antibodies in susceptible patients, which may contribute to the pathogenesis of Guillain-Barré syndrome associated with CMV.

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Antecedent infections and antiganglioside antibodies are related to various clinical patterns in the Guillain-Barré syndrome.¹ *Campylobacter jejuni* infections are associated with anti-GM1 antibodies and a more severe and pure motor variant of Guillain-Barré syndrome.^{2,3} Some *C jejuni* strains express GM1-like epitopes and may induce anti-GM1 antibodies.⁴ Cytomegalovirus (CMV) infections are the most frequent viral infections preceding Guillain-Barré syndrome,⁵ and are associated with severe sensory loss, cranial nerve involvement, and respiratory insufficiency.⁶ Recently, three patients with Guillain-Barré syndrome were reported with a CMV infection and antibodies against the ganglioside GM2.⁷ Such antibodies may contribute to the pathogenesis of Guillain-Barré syndrome, as GM2 is found in peripheral nerves.⁸ In the present study we investigated in a large group of patients with Guillain-Barré syndrome whether CMV infections are associated with anti-GM2 antibodies in the acute phase of the disease.

Patients and methods

PATIENTS

Pretreatment serum samples, obtained within two weeks of onset of weakness, were available

for serological testing in 130 of the 147 patients with Guillain-Barré syndrome who participated in the Dutch Guillain-Barré syndrome trial.⁹ The 17 excluded patients did not differ from the other patients in clinical manifestations and course of disease. Serum samples were also tested from patients with other neurological diseases ($n = 50$), controls infected with CMV ($n = 50$), and controls infected with *C jejuni* ($n = 50$) both without neurological involvement, and normal controls ($n = 50$). The group of other neurological diseases comprised inflammatory polyneuropathy ($n = 24$), non-inflammatory polyneuropathy ($n = 12$), and neurological diseases other than polyneuropathy ($n = 14$).

DETECTION OF ANTIBODIES AGAINST GLYCOLIPIDS

IgM and IgG antibodies against GM2 were tested by enzyme linked immunosorbent assay (ELISA) and thin layer chromatography overlay, as described previously.³ Serum samples, tested at a dilution of 1:100 in ELISA, with an optical density (OD) of more than 3 SD above the mean value of 50 normal control serum samples were tested by thin layer chromatography overlay. Positive samples were tested again by ELISA, using serial dilutions starting at 1:100. The reciprocal of the highest dilution that resulted in an OD higher than the cut off value was then taken to be the titre. The patients with Guillain-Barré syndrome with anti-GM2 antibodies were also tested for IgM and IgG antibodies against GM1, GM3, GD1b, GD2, asialo-GM1 (GA1), and asialo-GM2 (GA2).

All patients were serologically tested for CMV and *C jejuni* infection. IgM antibodies against CMV indicated a recent CMV infection,⁶ and a recent *C jejuni* infection was indicated by IgA, IgM, or high titres of IgG antibodies against *C jejuni*.³

STATISTICAL ANALYSIS

Differences in proportions were tested with Fisher's exact test. A P value < 0.05 was considered significant.

Results

Anti-GM2 IgM antibodies were detected in six (5%) of 130 patients with Guillain-Barré

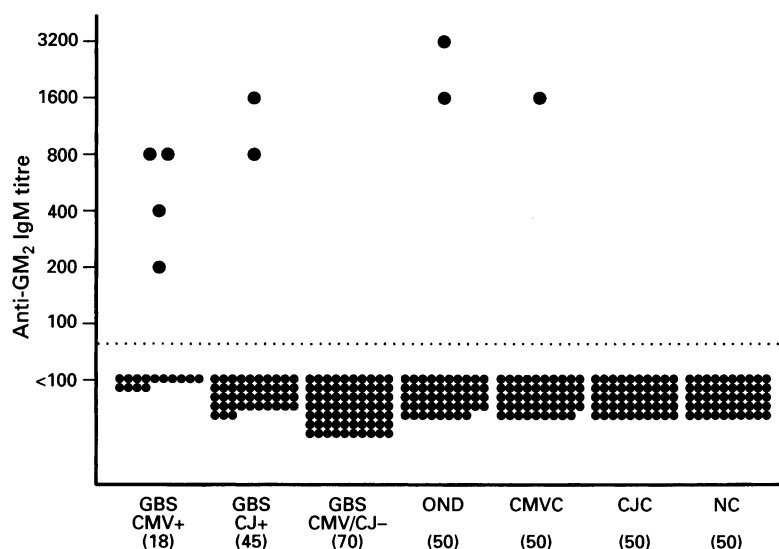
Department of Neurology

B C Jacobs
P A van Doorn
J H M Groeneveld
A P Tio-Gillen
F G A van der Meché

Department of Immunology,
University Hospital
Dijkzigt and Erasmus
University,
Rotterdam, The
Netherlands
B C Jacobs

Correspondence to:
Dr B C Jacobs, Department
of Neurology, Erasmus
University, Ee 2222, PO Box
1738, 3000 DR Rotterdam,
The Netherlands.

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Titre of anti-GM2 IgM antibodies in patients with Guillain-Barré syndrome (GBS), other neurological diseases (OND), cytomegalovirus (CMV) infected controls (CMVC), and *C jejuni* infected controls (CJC) both without neurological involvement, and normal controls (NC). Patients with Guillain-Barré syndrome were subdivided into patients with CMV infection (CMV+), *C jejuni* infection (CJ+), and those without CMV and *C jejuni* infection (CMV/CJ-). Three patients with Guillain-Barré syndrome had a dual infection with CMV and *C jejuni* and were negative for anti-GM2 antibodies.

syndrome, two (4%) of 50 patients with other neurological diseases, one (2%) of 50 control patients with CMV, and in none of the 50 control patients with *C jejuni* infection or 50 normal controls (figure). These antibodies were found in four (22%) of 18 patients with Guillain-Barré syndrome with a recent CMV infection. This frequency of anti-GM2 IgM antibodies was higher than in patients with Guillain-Barré syndrome without CMV infection (2%) ($P = 0.003$), and patients with other neurological diseases ($P = 0.04$), CMV controls ($P = 0.02$), control patients with *C jejuni* infection ($P = 0.004$), and normal controls ($P = 0.004$). Anti-GM2 IgM antibodies were found in two (4%) of 45 patients with Guillain-Barré syndrome with a recent *C jejuni* infection, but this frequency was lower than in patients with Guillain-Barré syndrome with a CMV infection ($P = 0.04$). Three patients with Guillain-Barré syndrome had a dual infection with CMV and *C jejuni*, but did not have anti-GM2 antibodies. In all patients with Guillain-Barré syndrome the titre of the anti-GM2 IgM antibodies decreased with clinical improvement. Anti-GM2 IgG antibodies could not be found in pretreatment serum. Isotype switch to anti-GM2 IgG or IgA antibodies during follow up of the anti-GM2 positive patients with Guillain-Barré syndrome did not occur.

Anti-GM2 IgM antibodies were found in two patients with other neurological diseases. One patient had a chronic pure motor and demyelinating polyneuropathy with paraproteinaemia. The other patient had paraesthesiae and a chronic motor and demyelinating polyneuropathy without paraproteinemia. In these patients no evidence for CMV infections was found.

The four patients with Guillain-Barré syn-

drome and CMV with anti-GM2 antibodies had additional IgM antibodies against GA2, and in one patient against GD2, but not against GM1, GM3, GD1b, or GA1. In these patients IgG antibodies against glycolipids were not found. Interestingly, the two patients with Guillain-Barré syndrome and *C jejuni* infection with anti-GM2 antibodies also had IgM antibodies against GM1, GD1b, and GA1, but not against GA2 and GM3. In these two patients IgG antibodies against GM1 and AGM1 were also found.

The clinical characteristics of the patients with Guillain-Barré syndrome with anti-GM2 antibodies were found to be related to the antecedent infections. The four CMV positive patients were relatively young females who had an antecedent upper respiratory infection. Thereafter, they had globally distributed moderate to severe weakness with facial palsy, sensory loss, and paraesthesiae, and in three patients, respiratory insufficiency. The two patients infected with *C jejuni* had antecedent diarrhoea, followed by a severe and predominantly distal weakness without involvement of cranial and sensory nerves, and without respiratory insufficiency. These two patients slowly recovered to independent locomotion (125 days and more than 181 days). The clinical symptoms of the patients with Guillain-Barré syndrome with anti-GM2 antibodies did not differ significantly from those of other patients with Guillain-Barré syndrome with CMV or *C jejuni* infections.

Discussion

The association between CMV infections and anti-GM2 antibodies described in this study further supports the concept that antecedent infections are related to antiglycolipid antibodies and clinical subgroups in Guillain-Barré syndrome.¹ It parallels the previously reported associations between *C jejuni* infections and anti-GM1 antibodies,^{2,3} and between *Mycoplasma pneumoniae* infections and antigalactocerebroside antibodies.¹⁰

Our results are partly in accordance with those published by Irie *et al.*⁷ They found anti-GM2 antibodies in all three patients with Guillain-Barré syndrome and CMV infection and in none of the patients with Guillain-Barré syndrome without CMV infection. In a large group of patients with Guillain-Barré syndrome we showed the presence of anti-GM2 antibodies in a lesser percentage (22%) of patients infected with CMV. However, serum samples in the study of Irie *et al.* were obtained at rather a long time after neurological onset (mean 24, range two to 180 days) compared with our study (mean six, range one to 14 days) which may lead to an underestimation of CMV infections in their patients. In addition, we also found anti-GM2 antibodies in some patients with Guillain-Barré syndrome with *C jejuni* infections, but the frequency was significantly less than in patients infected with CMV.

Antiganglioside antibodies in patients with Guillain-Barré syndrome are predominantly

IgG, although IgM and IgA antibodies are also found. Remarkably, the anti-GM2 antibodies in our study were all IgM. This is probably not related to specific antecedent infections, as the anti-GM2 antibodies in the patients infected with *C jejuni* were also IgM, and others found anti-GM2 IgG antibodies in patients infected with CMV.⁷ As serum with these antibodies show no activity against similar gangliosides such as GM3, the anti-GM2 IgM antibodies found in our study are not aspecific.

Instead, two specific patterns of antibody activity against GM2 may be present in patients with Guillain-Barré syndrome. Firstly, some patients have additional antibodies that bind to GA2 or GD2 but not to GM1, which may suggest that the antibodies recognise the shared GalNAc(β 1-4)Gal terminal in GM2, GA2, and GD2.⁷ The three patients in the study of Irie *et al* and the four patients in our study with this pattern of antibody activity all had a CMV infection. Secondly, other patients show additional antibody activity against GM1 but not against GA2 or GD2, indicating that these antibodies bind with the GalNAc(β 1-4)[NeuAc α 2-3Gal](β 1-4)Glc moiety which GM2 and GM1 have in common.¹² We found this pattern in two patients with antecedent *C jejuni*.¹¹ These antibodies may be produced initially during the antecedent infection, as lipopolysaccharides from several *C jejuni* strains are recognised by monoclonal antibodies against GM1 and GM2,¹² and have a terminal tetrasaccharide identical to that of GM2.¹³ However, the number of anti-GM2 positive patients is too small to conclude that these two antibody patterns are associated with specific antecedent infection.

The clinical characteristics of the patients with Guillain-Barré syndrome with anti-GM2 antibodies were related to the antecedent infection and did not differ from those of other patients with Guillain-Barré syndrome with CMV or *C jejuni* infections. In previous studies we found that CMV infections are associated with severe sensory loss, cranial nerve involvement, and respiratory insufficiency,⁶ and *C jejuni* infections with severe and predominantly distal weakness without sensory or cranial nerve involvement.³ In the six anti-GM2 positive patients with Guillain-Barré syndrome, these clinical differences may be related to the fine specificity of the anti-GM2 antibodies.

At present, the role of CMV in the pathogenesis of Guillain-Barré syndrome is unknown. Direct infection of peripheral nerves is unlikely, as the CMV genome was not detected in sural nerve biopsies of patients

infected with CMV with Guillain-Barré syndrome.¹⁴ Serum anti-GM2 antibodies have been shown to bind with CMV infected cells,⁷ indicating that these antibodies in Guillain-Barré syndrome may be induced by antecedent CMV infection. Only one patient with a CMV infection without neurological involvement had anti-GM2 antibodies. This suggests that CMV infections are not the only factor determining the production of anti-GM2 antibodies and the development of immune mediated polyneuropathy. Further research is needed to investigate whether anti-GM2 antibodies are induced by CMV infections, and whether they are involved in the pathogenesis of CMV related Guillain-Barré syndrome.

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