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Anti-phospholipid antibodies in serum from patients with Guillain-Barré syndrome

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Abstract *Objective:* The Guillain-Barré syndrome (GBS) is an acute inflammatory polyneuropathy related to autoimmunity. However, no conclusive etiological concept has yet been found. We examined the variation in autoantibodies to lipids in serum of GBS patients in response to the course of the disease but investigated titer modifications during treatment with γ -globulin. *Design and setting:* Prospective clinical study in a 14-bed general ICU. *Patients:* Nine patients with GBS and nine controls were included in the study. *Measurements and results:* Four blood samples were obtained before and after treatment. Serum samples, diluted 1:60, were tested by enzyme-linked immunosorbent assay for IgM, IgA, and IgG antibodies to phosphatidylcholine, phosphatidylinositol, cardiolipin, phosphatidic acid, phosphatidylserine, phosphatidylglycerol, phosphatidylethanolamine, sphingomyelin, and gangliosides. Anti-phospholipid antibod-

ies of the IgM, IgA, and IgG families were detected in all GBS patients but in none of the controls. Phosphatidylinositol, cardiolipin, phosphatidylcholine, and phosphatidic acid were the main antigens. All patients developed anti-phosphatidylinositol antibodies of the IgM family and anti-cardiolipin antibodies of the IgA and IgG families. A decrease in the level of anti-phospholipid autoantibodies was observed after 1 day of treatment with γ -globulin. Two days after ending γ -globulin administration the IgG antibodies increased again. *Conclusions:* Our findings suggest that in GBS there is an extensive immune reaction, which is altered after γ -globulin treatment. Anti-cardiolipin and anti-phosphatidylinositol antibodies could be useful markers for the response to treatment.

Keywords Guillain-Barré syndrome · Demyelination · Antiphospholipid antibodies · Autoantibodies · γ -Globulin

Introduction

Guillain-Barré syndrome (GBS) is a clinical entity characterized by progressive areflexic weakness of more than one limb with greatest progression in less than 4 weeks [1]. There are many variants of GBS with more or less regional involvement of the peripheral nervous system, including paraparesis alone, ophthalmoplegia, pure sensory GBS, pharyngeal-cervical-brachial paralysis, and others. Although most patients have a good recovery,

overall mortality is about 5–10%, and more than one-half retain some damage to their peripheral nervous system [1, 2, 3, 4].

Two forms of the syndrome's pathology have been described, the demyelinating and the axonal. The demyelinating form, the more common one, is characterized by demyelination and inflammatory infiltrates of the peripheral nerves and roots. In the axonal form the nerves show wallerian degeneration with absence of inflammation. Discrimination between axonal and demyelinating

Table 1 Demographic data of patients with GBS (AIDP acute inflammatory demyelinating polyneuropathy)

| No. | Age (years) | Sex | Form | Intubation | Symptom duration ^a | Outcome |
|-----|-------------|-----|------|------------|-------------------------------|---------|
| 1 | 65 | F | AIDP | No | 7 | Alive |
| 2 | 37 | M | AIDP | Yes | 12 | Alive |
| 3 | 63 | F | AIDP | Yes | 6 | Alive |
| 4 | 76 | F | AIDP | Yes | 5 | Alive |
| 5 | 75 | M | AIDP | No | 8 | Alive |
| 6 | 60 | F | AIDP | No | 10 | Alive |
| 7 | 30 | F | AIDP | Yes | 15 | Dead |
| 8 | 65 | M | AIDP | No | 4 | Alive |
| 9 | 48 | M | AIDP | Yes | 7 | Alive |

^a Days from initiation of symptoms until the first sampling day

forms relies mainly on electrophysiological methods.

There is a close association between GBS and a preceding infection, suggesting the immune basis of the syndrome. The most common infections are due to *Cambylobacter jejuni*, cytomegalovirus, and Epstein-Barr virus [5, 6, 7, 8, 9, 10]. Approximately 15–50% of patients with GBS develop anti-gangliosidic antibodies [11, 12, 13, 14, 15]. These antibodies are targeted against a wide range of glycolipids including GM1, GM1b, GalNAc-GM1b, GD1a, GalNAc-GD1a, GD1b, 9-*o*-acetyl-GM1b, GD₃, GT1b, GQ1b LMI, galactocerebroside, and sulfated glucuronyl paragloboside. The anti-GQ1b IgG antibodies have been found to be associated with Miller-Fisher syndrome, a variant of GBS. Lipopolysaccharides of *C. jejuni* share structural similarity with epitopes in gangliosides, and therefore a cross-reaction of the antibodies against micro-organisms could recognize gangliosides of the nerves. This aspect of molecular mimicry has extensively been investigated [6, 7, 8, 9, 10]. It is not established, however, whether apart from gangliosides other lipid antigens are also recognized as targets for antibodies in GBS.

Autoimmune diseases such as graft-vs.-host disease, systemic lupus erythematosus, scleroderma, and sicca syndrome are sometimes associated with GBS [16]. Some of these are also characterized by the presence of anti-phospholipid antibodies. This relationship between the above autoimmune diseases and GBS raises the question of whether anti-phospholipid antibodies are encountered in patients with the syndrome. To address this question we investigated whether anti-phospholipid antibodies are present in serum samples of GBS patients without known autoimmunity disorders. Furthermore, we examined the fluctuation in auto-antibodies to lipids in serum samples of GBS patients in response to the course of the disease and also the sequential modifications of titers during treatment with γ -globulin.

Materials and methods

Patients and protocol

The study included nine patients with the acute inflammatory demyelinating polyneuropathy type of GBS and nine postoperative patients without neurological disease, matched in terms of in age, sex and race with patients. Clinical and electrophysiological criteria of GBS diagnosis were used [17]. The clinical criteria were: progressive motor weakness of more than one limb and areflexia. All of our patients fulfilled the electrophysiological criteria of diagnosis for acute GBS demyelinating polyneuropathy: They had motor nerve conduction velocities in at least three nerves (mostly median, ulnar, and peroneal) less than 70% of the lower normal limit, temporal dispersion of M wave and conduction block, mostly in the ulnar nerve across the elbow, absent F waves, impersistence and prolongation of these (70 ms and 85 ms, distal median). None of our patients had spontaneous activity on the 3 weekly electromyographic examinations in the clinically weak limbs; therefore an acute deterioration of a preexisting illness or an axonal form of GBS was excluded.

Patients' demographic data are shown in Table 1. Patients received γ -globulin (IgG, Flebogamma 5%, Grifols, Can Guasch, Barcelona, Spain) at a total dose of 2 g/kg administered on two consecutive days after diagnosis (for review see [18]). Serum samples were obtained before the infusion of γ -globulin and on the 1st, 2nd, 5th, and 8th days. To investigate whether γ -globulin infusion affected the titers of the anti-phospholipid antibodies additional serum samples were obtained 1 h after the infusion on the 1st and 2nd days. The variation in anti-phospholipid antibodies over time was followed for 9 consecutive days in serum samples from two patients.

The presence of anti-phospholipid antibodies of IgG, IgA, and IgM families in the administered γ -globulin (Flebogamma) was also explored as a control. The dilution was made up to 1:300, assuming that this corresponds to the final dilution of serum samples. Value equal to the absorbance of γ -globulin was subtracted from the corresponding value of absorbance of serum. Antibodies against *C. jejuni*, *Mycoplasma pneumoniae*, cytomegalovirus, and Epstein-Barr and other viruses were investigated.

The protocol was approved by the Ethics Committee of the University Hospital of Ioannina, Greece, and the patients or the next of kin gave an informed consent to the study.

Detection of anti-phospholipid autoantibodies

The presence of anti-lipid antibodies was investigated by enzyme-linked immunosorbent assay in serum samples diluted 1:60, dilution in which none of the control group showed any positive antibody reaction. Phospholipids were coated on the microtiter plates at a concentration of 5 μ g/well. We investigated autoantibodies of the IgM, IgA, and IgG families against phosphatidylcholine (PC),

Table 2 Number of patients who developed of IgM, IgA, and IgG anti-phospholipid antibodies on the four sampling days on the four sampling days ($n=9$) (PC phosphatidylcholine, PI phosphatidylinositol, PS phosphatidylserine, PE phosphatidylethanolamine, PA phosphatidic acid, PG phosphatidylglycerol; Sph sphingomyelin, Gang gangliosides, CL cardiolipin, Total total number of patients who developed the respective anti-phospholipid antibodies at least once during the course of GBS, ND nondetectable)

| IgM | 1st | 2nd | 5th | 8th | Total |
|------|-----|-----|-----|-----|-------|
| PC | 0 | 0 | 0 | 0 | 0 |
| PI | 6 | 5 | 5 | 5 | 9 |
| PS | ND | ND | ND | ND | ND |
| PE | 2 | 2 | 2 | 2 | 2 |
| PA | 2 | 2 | 3 | 3 | 3 |
| PG | ND | ND | ND | ND | ND |
| Sph | ND | ND | ND | ND | ND |
| Gang | ND | ND | ND | ND | ND |
| CL | 3 | 2 | 2 | 3 | 4 |
| IgA | | | | | |
| PC | 0 | 0 | 0 | 0 | 0 |
| PI | 3 | 3 | 5 | 3 | 6 |
| PS | ND | 1 | 3 | 2 | 3 |
| PE | 1 | 1 | 3 | 2 | 2 |
| PA | 3 | 3 | 5 | 3 | 6 |
| PG | ND | ND | 2 | ND | 2 |
| Sph | 1 | ND | 2 | ND | 2 |
| Gang | ND | ND | 2 | 2 | 2 |
| CL | 7 | 6 | 5 | 6 | 9 |
| IgG | | | | | |
| PC | 1 | 3 | 1 | 1 | 4 |
| PI | 5 | 5 | 3 | 6 | 9 |
| PS | 2 | 2 | 6 | 3 | 6 |
| PE | 1 | 1 | 3 | 3 | 3 |
| PA | 1 | 3 | 6 | 3 | 7 |
| PG | 1 | 1 | ND | 2 | 2 |
| Sph | 1 | 3 | ND | 3 | 3 |
| Gang | 1 | ND | 2 | 3 | 4 |
| CL | 8 | 6 | 6 | 4 | 9 |

phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidic acid (PA), sphingomyelin, cardiolipin (CL), and gangliosides (containing monosialoganglioside, GM1, ganglioside GT1B, and galactocerebroside). This was by peroxidase/*o*-phenylene diamine/ H_2O_2 reaction [19]. Absorbance was measured at 496 nm in an automated microplate reader.

Dose response curve

To identify whether a specific antigen-antibody reaction occurs IgG antibody binding was measured as a function of increasing PI concentrations to determine the linear part of the curve. PI was selected because anti-PI antibodies were detected in all patients with GBS.

Statistical analysis

Data are presented as mean \pm standard deviation. The four consecutive serum levels in the study group were compared by Friedman's test, which is the nonparametric equivalent of one-way repeated-measures design. Correlations between values were analyzed by linear regression. The level of significance was defined as a p value of less than 0.05.

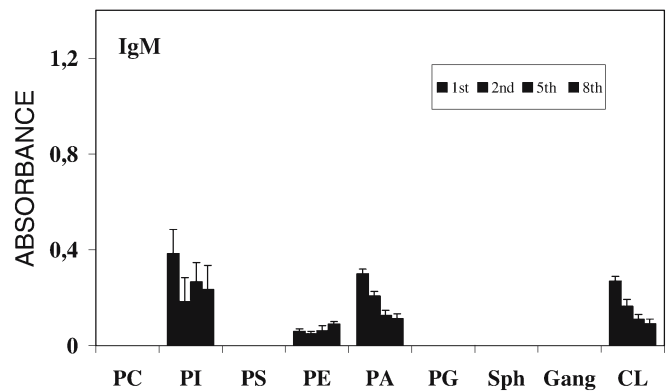


Fig. 1 IgM anti-phospholipid antibodies in serum samples from each patient obtained in different dates. Antibodies were measured by enzyme-linked immunosorbent assay in triplicate samples. Columns Mean absorbance \pm standard deviation; different columns serum samples obtained on the 1st, 2nd, 5th, and 8th days. PC Phosphatidylcholine; PI phosphatidylinositol; PS phosphatidylserine; PE phosphatidylethanolamine; PA phosphatidic acid; PG phosphatidylglycerol; Sph sphingomyelin; Gang gangliosides; CL cardiolipin

Results

Patient data

Antibodies against *C. jejuni* and cytomegalovirus were negative; in one patient antibodies against Epstein-Barr virus and in another one antibodies against *M. pneumoniae* were detected. In serum samples (diluted 1:60) from patients with GBS we detected anti-phospholipid autoantibodies (Table 2, Figs. 1, 2, 3) but in none of the controls. The mean absorbance values and standard deviations representing the antibodies concentrations are shown in Table 3. There was no significant difference in the presence or the titers of antibodies between intubated and nonintubated patients, men and women, or survivors and nonsurvivors.

IgM autoantibodies

We detected anti-PI, anti-PE, anti-PA, and anti-CL antibodies of the IgM family. The highest response was observed for anti-PI antibodies (Fig. 1). Furthermore, we found that all nine of the patients developed IgM anti-PI antibodies during the course of the disease: four (44.5%) anti-CL, three (33.3%) anti-PA, and two (22.2%) anti-PE antibodies (Table 2). Seven patients developed IgM antibodies, at least against one phospholipid tested, in the first or second sample and two patients in the third or fourth sample. Five patients had detectable IgM antibodies levels against PI at all four sampling times.

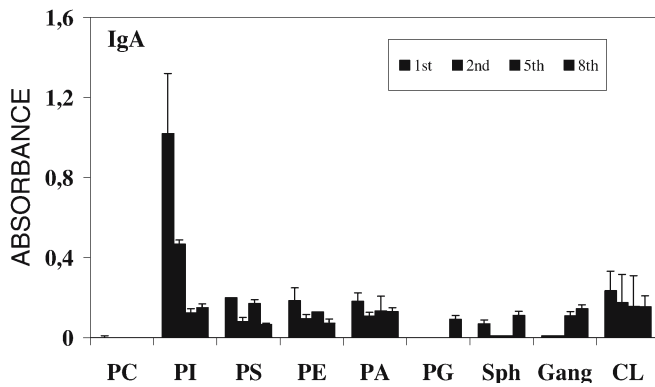


Fig. 2 IgA anti-phospholipid antibodies in serum samples from each patient obtained in different dates. Antibodies were measured by enzyme-linked immunosorbent assay in triplicate samples. Columns Mean absorbance \pm standard deviation; different columns serum samples obtained on the 1st, 2nd, 5th, and 8th days. PC Phosphatidylcholine; PI phosphatidylinositol; PS phosphatidylserine; PE phosphatidylethanolamine; PA phosphatidic acid; PG phosphatidylglycerol; Sph sphingomyelin; Gang gangliosides; CL cardiolipin

IgA autoantibodies

We detected anti-phospholipid antibodies against all the examined phospholipids. The highest response was observed for anti-PI antibodies. The levels of anti-PI antibodies of the first sample of the patients were significantly higher than those of the second, third, and fourth ones ($p < 0.01$; Fig. 2). All nine patients developed IgA anti-CL antibodies during the course of the disease; six (67%) IgA anti-PI and anti-PA antibodies, two (22%) anti-PE, anti-PG, anti-sphingomyelin, and anti-ganglioside antibodies, three (33%) anti-PS antibodies, and two (22%) anti-PC antibodies (Table 2). Eight of the nine patients developed IgA antibodies, at least against one phospholipid, in the first or second sample, and one patient in the fourth sample. Five patients had detectable IgA antibodies levels against CL at all four sampling times.

IgG autoantibodies

We detected anti-phospholipid antibodies to all tested phospholipids. The concentrations of all anti-phospholipid antibodies in the first samples were significantly higher than those in the second ($p < 0.01$; Fig. 3). All patients nine (100%) developed IgG anti-PI and anti-CL antibodies during the course of the disease; seven (78%) IgG anti-PA, six (78%) IgG anti-PS, four (44%) anti-PC, and anti-ganglioside, three (33%) anti-PE and anti-sphingomyelin antibodies, and two (22%) anti-PG (Table 2). Five patients developed IgG antibodies against PI in the first sample and four in the third or fourth sample. Eight patients developed IgG antibodies against CL in the

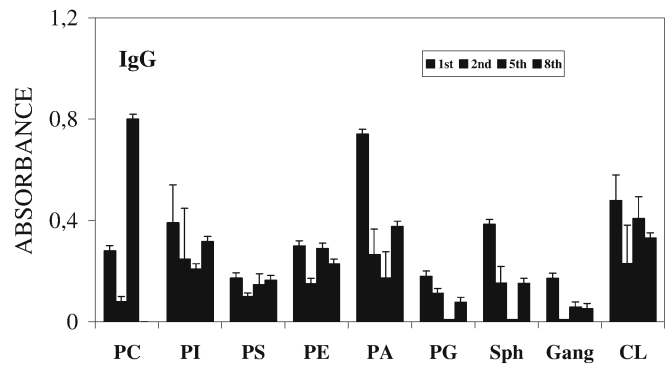


Fig. 3 IgG anti-phospholipid antibodies in serum samples from each patient obtained in different dates. Antibodies were measured by enzyme-linked immunosorbent assay in triplicate samples. Columns Mean absorbance \pm standard deviation; different columns serum samples obtained on the 1st, 2nd, 5th, and 8th days. PC Phosphatidylcholine; PI phosphatidylinositol; PS phosphatidylserine; PE phosphatidylethanolamine; PA phosphatidic acid; PG phosphatidylglycerol; Sph sphingomyelin; Gang gangliosides; CL cardiolipin

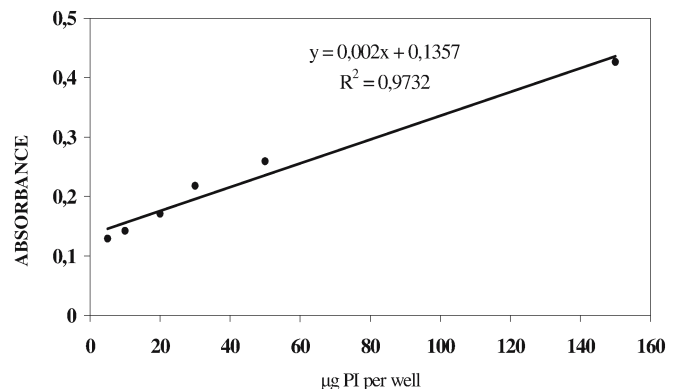


Fig. 4 Kinetics of IgG anti-phosphatidylinositol (PI) antibodies in response to PI concentration. Serum samples from GBS patients were diluted 1:60. Results represent mean values of three experiments

first sample and one in the third sample. Three patients had detectable IgG antibodies levels against PI and four against CL at all four sampling times.

Dose response curve

The binding response of serum samples from GBS patients increased proportionally with PI concentrations ($R^2 = 0.97$; Fig. 4), confirming the specificity of the antibodies to PI.

Table 3 Mean absorbance values of IgM, IgA, and IgG anti-phospholipid antibodies on the four sampling days ($n=9$) (*PC* phosphatidylcholine, *PI* phosphatidylinositol, *PS* phosphatidylserine, *PE* phosphatidylethanolamine, *PA* phosphatidic acid, *PG* phosphatidylglycerol; *Sph* sphingomyelin, *Gang* gangliosides, *CL* cardiolipin. *Total* total number of patients who developed the respective anti-phospholipid antibodies at least once during the course of GBS)

| | 1st | 2nd | 5th | 8th |
|------|-------------|-------------|-------------|-------------|
| IgA | | | | |
| PC | 0.05±0.01 | 0 | 0 | 0 |
| PI | 1.02±0.3 | 0.468±0.02 | 0.124±0.02 | 0.149±0.02 |
| PS | 0 | 0.081±0.02 | 0.17±0.02 | 0.066±0.007 |
| PE | 0.084±0.065 | 0.095±0.02 | 0.128±0.0 | 0.073±0.02 |
| PA | 0.181±0.042 | 0.107±0.02 | 0.134±0.07 | 0.13±0.019 |
| PG | 0 | 0 | 0 | 0.091±0.021 |
| Sph | 0.069 | 0.01±0.00 | 0.01±0.00 | 0.111±0.019 |
| Gang | 0.009±0.02 | 0.01±0.00 | 0.11±0.00 | 0.144±0.02 |
| CL | 0.135±0.098 | 0.175±0.142 | 0.256±0.154 | 0.195±0.054 |
| IgG | | | | |
| PC | 0.09±0.01 | 0 | 0 | 0 |
| PI | 0.391±0.15 | 0.248±0.02 | 0.209±0.02 | 0.317±0.019 |
| PS | 0.173±0.02 | 0.1±0.014 | 0.147±0.042 | 0.164±0.02 |
| PE | 0.3±0.02 | 0.151±0.02 | 0.29±0.019 | 0.228±0.018 |
| PA | 0.741±0.18 | 0.266±0.011 | 0.173±0.103 | 0.377±0.02 |
| PG | 0.18±0.022 | 0.112±0.021 | 0.01±0.017 | 0.077±0.018 |
| Sph | 0.385±0.019 | 0.153±0.065 | 0.01±0.00 | 0.152±0.02 |
| Gang | 0.172±0.02 | 0.01±0.00 | 0.058±0.02 | 0.052±0.02 |
| CL | 0.479±0.1 | 0.231±0.152 | 0.408±0.086 | 0.331±0.024 |
| IgM | | | | |
| PC | 0 | 0 | 0 | 0 |
| PI | 0.385±0.1 | 0.184±0.1 | 0.267±0.08 | 0.235±0.011 |
| PS | 0 | 0 | 0 | 0 |
| PE | 0.06±0.01 | 0.05±0.009 | 0.063±0.02 | 0.09±0.012 |
| PA | 0.3±0.02 | 0.207±0.021 | 0.127±0.018 | 0.113±0.02 |
| PG | 0 | 0 | 0 | 0 |
| Sph | 0 | 0 | 0 | 0 |
| Gang | 0 | 0 | 0 | 0 |
| CL | 0.17±0.021 | 0.165±0.028 | 0.111±0.019 | 0.091±0.21 |

Variation over time

The variation in IgG, IgA, and IgM anti-phospholipid antibodies against PI during the course of the disease is shown for two representative cases in Fig. 5. The levels of all classes of anti-phospholipid antibodies were significantly lower 1 day after the administration of γ -globulin than before administration (first day). The mean reduction in IgG anti-PI antibodies was $42\pm 28\%$ ($p<0.01$) while that of IgM and IgA anti-PI antibodies was $52\pm 23\%$ ($p<0.01$) and $54\pm 32\%$ ($p<0.01$) respectively. The levels of IgM and IgA anti-PI antibodies remained low for all the following study period. However, the levels of IgG increased again to the initial levels 2 days after the discontinuation of γ -globulin and then started to decrease again on the 9th day (Fig. 5).

The initial effect of γ -globulin in the level of antibodies to phosphatidyl-choline, phosphatidyl-inositol, phosphatidic acid and cardiolipin

The levels of IgG antibodies against PC, PI, PA, and CL increased significantly 1 h after both doses of γ -globulin (data not shown). This increase ranged from 20% to 38% ($p<0.05$) compared to the levels before administration. In

contrast, no significant change was observed in the levels of IgA or IgM anti-phospholipid antibodies.

Anti-phospholipid antibodies in γ -globulin

The presence anti-phospholipid antibodies of IgG, IgA, and IgM families were examined in a 1:300 γ -globulin dilution as a control. IgG anti-phospholipid antibodies were detected only against PC, PI, and CL. No IgA or IgM anti-phospholipid antibodies were detected in the γ -globulin solution. The relevant values were subtracted from the serum samples.

Discussion

In our study we detected a wide range of anti-phospholipid antibodies in patients with idiopathic GBS. All nine GBS patients developed anti-phospholipid antibodies directed against at least one lipid during the course of the disease, whereas none of the controls demonstrated any anti-phospholipid activity under our experimental conditions. More striking is the fact that the IgG and IgM anti-PI antibodies as well as IgA and IgG anti-CL antibodies were detected in all patients at least in one sampling time.

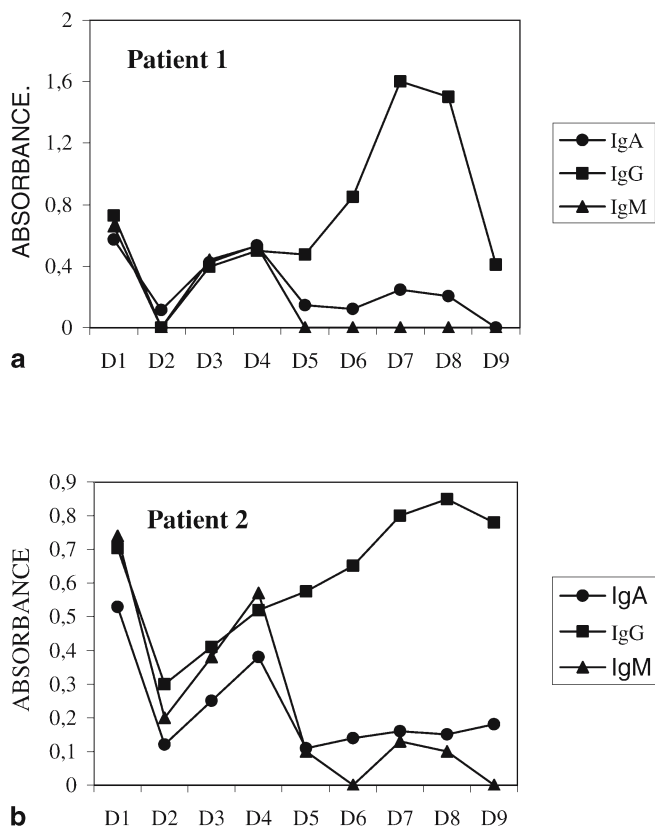


Fig. 5 Daily variation in anti-phosphatidylinositol (PI) anti-phospholipid antibodies of the IgA, IgM, and IgG families in two representative cases. *D* Sampling day

The linear relationship between absorbance (IgG binding) and the coated PI confirms the specificity of the antibodies detected towards this phospholipid. If this relationship did not exist, the reaction could be due to other nonspecific interactions generated even in the absence of anti-PI antibodies. It should be noted that the concentration indicated represents the actual quantity of lipid coating; it has been shown that under the conditions used more than 95% of the lipid deposited remained attached to the plate until the end of the test [20].

Many investigators [21, 22, 23] consider that anti-phospholipid autoantibodies are targeted only against anionic phospholipids (PA, PS, PG, PI, and especially CL) but not to zwitterionic ones (PC, PE). Our results confirm those of a previous study [24] which found that these antibodies are directed against all the phospholipids, including PE and PC.

The association of GBS and certain autoimmune diseases, including systemic lupus erythematosus, is well recognized [25]. High levels of anti-phospholipid antibodies were expressed in a patient with lupuslike syndrome who also developed secondary GBS [26]. It is thought that whenever polyneuropathy occurs in the context of autoimmune diseases, mainly in systemic lupus

erythematosus, where anti-phospholipid activity already exists, these antibodies can cross-react with phospholipids and mediate damage in neural structures containing the particular phospholipids [27]. It has been reported that 15% of patients with inflammatory demyelinating polyradiculoneuropathy also suffer from autoimmune diseases. Many studies have evaluated the clinical relevance of the anti-phospholipid antibodies but with conflicting results [28, 29, 30].

In our study polyneuropathy should be considered as primary since none of the patients suffered from any kind of autoimmune disease, nor did any clinical or laboratory findings indicate anti-phospholipid syndrome. Soon after (1 h) the administration of γ -globulin IgG antibodies to PC, PI, PA, and CL were significantly increased. This increase could be related to the antiphospholipid activity in the infused γ -globulin in which significant levels of IgG anti-phospholipid antibodies were detected. This finding is supported by those of other investigators who found high levels of anti-phospholipids antibodies in several commercial preparations of intravenous γ -globulin [25]. An increase in anti-phospholipid antibodies has been reported after plasma-exchange treatment [31]. However, the authors did not give any explanation for this phenomenon.

Interestingly, 1 day after the administration of γ -globulin the levels of anti-phospholipid antibodies were significantly reduced. A possible interpretation is that γ -globulin blocked anti-phospholipid antibodies and thus reduced or abolished their activity. Indeed, γ -globulin preparations exert activity against anti-phospholipid autoantibodies [18, 25]. The increase in IgG anti-phospholipid antibodies 2 days after discontinuation of γ -globulin may indicate inefficient blocking of anti-phospholipid antibodies by γ -globulin. In this case a second infusion of γ -globulin should be justified, as some investigators have suggested (for review see [18]). The most extensively studied antibodies in GBS are those directed against a number of different glycolipids. A large number of data demonstrate anti-ganglioside antibodies in the acute phase of GBS. In particular, anti-GQ1b antibodies are consistently associated with the Miller-Fisher syndrome, a variant of GBS [5]. However, other GBS variants show no significant relationship between anti-ganglioside antibodies and the clinical or electrophysiological findings. In addition, the electrophysiological phenomenon of conduction blockage which has been attributed to these antibodies has been debated (for review see [24]). In our study we detected anti-ganglioside antibodies, mainly in the IgG fraction, in 44.0% of patients. Our data are compatible with those of other researchers [14].

It is not well understood whether these anti-phospholipid antibodies play a role in the pathogenesis of the polyneuropathy or represent a part of a more extensive immunoreaction that takes place in the GBS. However, immunopathology in autopsies suggests that antibody-

mediated injury is a predominant disorder in the demyelinating form of GBS [32]. The immune attack is directed against components of Schwann cell membrane and is accompanied by the characteristic feature of vesicular demyelination [33, 34]. Therefore it is crucial to investigate how anti-phospholipid antibodies are related to specific antigens in Schwann cell membrane.

What is the prospective benefit of the anti-phospholipid antibodies evaluation in the GBS even in the case of their being an epiphenomenon? First, we could possibly use anti-phospholipid antibodies as an early additional marker of diagnostic significance given the fact that both electromyographic techniques and cerebrospinal fluid biochemical findings lag for days or even weeks in the

early diagnosis of the syndrome. Secondly, alteration in anti-phospholipid antibodies titer could be of some prognostic significance for GBS, as it may reflect the response to treatment and indicate the need for a second dose. Of note is that in our cases there was no relationship between the presence of anti-phospholipid antibodies and outcome, nor to the severity of the disease, probably due to the limited number of patients and variety of GBS. Therefore further research, with a larger number of patients with different GBS variants is required.

Our findings suggest that in GBS there is a more extensive immune reaction, beyond the well known anti-ganglioside production, which has been related to the demyelination of the peripheral nerves.

References

- Hahn AF (1998) Guillain-Barré syndrome. *Lancet* 352:635–641
- Asbury AK (2000) New concepts of Guillain-Barré syndrome. *J Child Neurol* 15:183–191
- Gande AR, Taylor IR, Nolan KM (1999) Autonomic instability and hypertension resulting in subarachnoid haemorrhage in the Guillain-Barré syndrome. *Intensive Care Med* 25:1432–1434
- Vargas F, Hilbert G, Gruson D, Valentino R, Gbikpi-Benissan G, Cardinaud JP (2000) Fulminant Guillain-Barré syndrome mimicking cerebral death: case report and literature review. *Intensive Care Med* 26:623–627
- Odaka M, Koga M, Yuki N, Susuki K, Hirata K (2003) Longitudinal changes of anti-ganglioside antibodies before and after Guillain-Barré syndrome onset subsequent to *Campylobacter jejuni* enteritis. *J Neurol Sci* 210:99–103
- Vriesendorp FJ, Mishu B, Blaser MJ, Koski CL (1993) Serum antibodies to GMT, GD1b, peripheral nerve myelin, and *Campylobacter jejuni* in patients with Guillain-Barré syndrome and controls: correlation and prognosis. *Ann Neurol* 34:130–135
- Dowling PC, Cook SD (1981) Role of infection in Guillain-Barré syndrome: laboratory confirmation of herpesviruses in 41 cases. *Ann Neurol* 9 [Suppl]:44–45
- Neisser A, Schwerer B, Bernheimer H, Moran AP (2000) Ganglioside-induced antiganglioside antibodies from a neuropathy patient cross-react with lipopolysaccharides of *Campylobacter jejuni* associated with Guillain-Barré syndrome. *J Neuroimmunol* 102:85–88
- Rees JH, Soudain SE, Gregson NA, Hughes RAC (1995) *Campylobacter jejuni* infection and Guillain-Barré syndrome. *N Engl J Med* 333:1374–1379
- Ang CW, Laman JD, Willison HJ, Wagner ER, Endtz HP, De Klerk MA, Tio-Gillen AP, Van den Braak N, Jacobs BC, Van Doorn PA (2002) Structure of *Campylobacter jejuni* lipopolysaccharides determines anti-ganglioside specificity and clinical features of Guillain-Barré and Miller Fisher patients. *Infect Immun* 333:1202–1208
- Hughes RAC, Hadden RD, Gregson NA, Smith KJ (1999) Pathogenesis of Guillain-Barré syndrome. *J Neuroimmunol* 100:74–97
- Carpo M, Pedotti R, Allaria S, Lolli F, Mata S, Cavaletti G, Protti A, Pomati S, Scarlato G, Nobile-Orazio E (1999) Clinical presentation and outcome of Guillain-Barré and related syndromes in relation to anti-ganglioside antibodies. *J Neurol Sci* 168:78–84
- Ogawara K, Kuwabara S, Mori M, Hattori T, Koga M (2000) Axonal Guillain-Barré syndrome: relation to anti-ganglioside antibodies and *Campylobacter jejuni* infection in Japan. *Ann Neurol* 48:624–631
- Press R, Mata S, Lolli F, Zhu J, Andersson T, Link H (2001) Temporal profile of anti-ganglioside antibodies and their relation to clinical parameters and treatment in Guillain-Barré syndrome. *J Neurol Sci* 190:41–47
- Bulsara KR, Bron PW, Tuttle-Newhall JE, Clavien PA, Morgenlander J (2001) Guillain-Barré syndrome in organ and bone marrow transplant patients. *Transplantation* 71:1169–1172
- Paparounas K (2004) Anti-GQ1b ganglioside antibody in peripheral nervous system disorders: pathophysiologic role and clinical relevance. *Arch Neurol* 6:1013–1016
- Asbury AK, Cornblath DR (1990) Assessment of current diagnostic criteria for Guillain-Barré syndrome. *Ann Neurol* 27 [Suppl]:S21–S24
- Dalakas MC (2004) The use of intravenous immunoglobulin in the treatment of autoimmune neuromuscular diseases: evidence-based indications and safety profile. *Pharmacol Ther* 102:177–193
- Maneta-Peyret L, Kitsiouli Ei, Lekka ME, Nakos G, Cassagne C (2001) Autoantibodies to lipids in bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome. *Crit Care Med* 29:1950–1954
- Maneta-Peyret L, Previsani C, Sultan Y, Beziau JH, Cassagne C (1991) Autoantibodies against all the phospholipids: a comparative systematic study with systemic lupus erythematosus and healthy sera. *Eur J Clin Chem Clin Biochem* 29:39–43
- Hanly JG (2003) Anti-phospholipid syndrome: an overview. *Can Med Assoc J* 168:1675–1682
- Gaipl US, Beyer TD, Baumann I, Voll RE, Stach CM, Heyder P, Kalden JR, Manfredi A, Herrmann M (2003) Exposure of anionic phospholipids serves as anti-inflammatory and immunosuppressive signal—implications for anti-phospholipid syndrome and systemic lupus erythematosus. *Immunobiology* 207:73–81
- Caso V, Panarelli P, Albi E, Viola-Magni MP, Parnetti L, Gallai V (2002) Phospholipid autoantibodies: time for a new immuno-assay? *Clin Exp Hypertens* 24:511–516

24. Maneta-Peyret L, Biron C, Previsani C, Moreau P, Cassagne JH, Cassagne C (1993) Demonstration that anti-phospholipid auto-antibodies react with both anionic and zwitterionic phospholipids. *Immunol Lett* 35:141–146
25. Krause I, Blank M, Shoenfeld Y (1998) Anti-DNA and antiphospholipid antibodies in IVIG preparations: in vivo study in naïve mice. *J Clin Immunol* 18:52–60
26. Willison HJ, Yuki N (2002) Peripheral neuropathies and anti-glycolipid antibodies. *Brain* 125:2591–2625
27. Harris EN, Gharavi AE, Mackworth-Young CG, Patel BM, Derue G, Hughes GR (1985) Lupoid sclerosis: a positive pathogenetic role for anti-phospholipid antibodies. *Ann Rheum Dis* 49:281–283
28. Favaloro EJ, Silvestrini R (2002) Assessing the usefulness of anticardiolipin antibody assays: a cautious approach is suggested by high variation and limited consensus in multilaboratory testing. *Am J Clin Pathol* 118:548–557
29. Korn-Lubetzki I, Abramsky O (1986) Acute and chronic demyelinating inflammatory polyradiculopathy: association with autoimmune diseases and lymphocyte response to human neurotogenic protein. *Arch Neurol* 43:604–608
30. Hughes RA, Gray IA, Gregson NA, Kadlubowski M, Kennedy M, Leibowitz S, Thompson H (1984) Immune responses to myelin antigens in Guillain-Barré syndrome. *J Neuroimmunol* 6:303–312
31. Mata S, Avanzi G, Lombardo R, Cepparone F, Pinto F, Lolli F (1998) Anti-GM1, anti-central myelin proteins, and anti-cardiolipin autoantibodies during plasma-exchange in Guillain-Barré syndrome (GBS). *J Clin Apheresis* 13:155–162
32. Gilburd B, Stein M, Tomer Y, Tanne D, Abramski O, Chapman Y, Ahiron A, Blank M, Shoenfeld Y (1993) Autoantibodies to phospholipids and brain extract in patients with the Guillain-Barré syndrome: cross-reactive or pathogenic? *Autoimmunity* 16:23–27
33. Hafer-Macko C, Hsieh S-T, Li CY, Ho TW, Sheikh K, Cornblath DR, McKhann GM, Asbury AK, Griffin JW (1966) Acute motor axonal neuropathy: an antibodies mediated attack on axolemma. *Ann Neurol* 40:635–644
34. Hafer-Marcko C, Sheikh K, Li CY, Ho TW et al (1966) Immune attack on the Schwann cell surface in acute inflammatory demyelinating poloneuropathy. *Ann Neurol* 39:635–644