Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 905–909, February 1985
Medical Sciences

Anti-peripheral myelin antibody in patients with demyelinating neuropathy: Quantitative and kinetic determination of serum antibody by complement component 1 fixation

(Guillain–Barré syndrome/demyelination/peripheral neuropathy)

CAROL L. KOSKI*, RICHARD HUMPHREY†, AND MOON L. SHIN‡

Departments of *Neurology and ‡Pathology, University of Maryland School of Medicine, Baltimore, MD 21201; and †Department of Oncology, The Johns Hopkins Medical School, Baltimore, MD 21205

Communicated by Solomon H. Snyder§, October 3, 1984

ABSTRACT **The role of anti-peripheral nerve myelin antibody (anti-PNM Ab) in the pathogenesis of acquired demyelination of peripheral nerve is unclear, in part, due to the poor correlation between antibody and disease activity. Previous studies show that only 27–50% of patients with acute demyelinating neuropathy or Guillain–Barré syndrome (GBS) had serum Abs to peripheral nerve or PNM as demonstrated by consumption of hemolytic activity of serum complement. In the present study by the use of a complement component 1 (C1) fixation and transfer assay, quantitative determinations of anti-PNM Ab showed significantly high titers in the serum of patients with GBS, chronic and recurrent polyneuritis, and paraproteinemia associated with peripheral neuropathy. All 11 patients with acute-phase GBS had Ab titers 6–56 times higher than controls. In 6 GBS patients, serial Ab determinations showed that titers were highest on admission, fell rapidly the first week, and became undetectable or barely detectable by the third week. Declining Ab titers coincided with cessation of clinical progression. In 3 GBS patients, depletion of serum IgM lowered anti-PNM Ab titers significantly, whereas IgG depletion failed to produce a similar effect. This study shows that the C1 fixation and transfer assay is a sensitive method to detect anti-PNM Ab in the serum of patients with a variety of demyelinating neuropathies and provides good correlation between Ab level and the clinical course of GBS patients. It may provide important information about the pathogenesis of the demyelinating neuropathies.**

The primary demyelination of peripheral nerve seen in patients with Guillain–Barré Syndrome (GBS) or chronic and recurrent polyneuropathy is thought to represent immunologically mediated myelin destruction (1, 2). The role of the cellular and humoral immune system in initiation of myelin breakdown has not been defined. Antibodies (Abs) against peripheral nerve (3, 4), peripheral nerve myelin (PNM) (4–6), dorsal root ganglia (7), spinal cord tissue components (3), and neuroblastoma cells (8) have been identified in the serum of some GBS patients. However, it is not known whether these Abs actively participate in myelin destruction or whether they are an epiphenomenon reflecting a host response to PNM antigen(s) following myelin destruction. Recently a putative function for anti-PNM Abs in demyelination has been advanced by findings that some patients with monoclonal gammapathy develop demyelination and the monoclonal immunoglobulins are directed against PNM (6, 9). Serum from GBS patients as well as serum from patients with paraproteinemia associated with peripheral neuropathy can induce segmental demyelination following intraneural injection into rat and cat sciatic nerves (10–13).

A reasonable hypothesis states that complement-activating, anti-PNM Abs may participate in peripheral nerve demyelination, particularly in light of the growing information on the membrane attack function of activated C5b-9 (14) and the C5b-9 requirement for Ab-mediated demyelination of mouse cerebellum explant cultures (15). To test this hypothesis, it was necessary to determine the frequency of anti-PNM Abs present in patients with demyelinating peripheral neuropathy, the kinetic dynamics of such Ab titer during the course of illness, and finally the possible relationship between Ab titer and disease severity.

In two previous studies (3, 6), serum Abs to peripheral nerve and PNM were measured by a conventional complement fixation method that involves a differential measurement between complement activity in the control and the test proper. In this method, a test result can be taken as positive only when the difference is substantial, which creates a blind zone with respect to detection of minute amounts of antigen–Ab complex. By contrast, the complement component 1 (C1) fixation and transfer (C1FT) assay measures fixation of C1 directly (16), which eliminates the blind zone. Thus, the C1FT assay is superior for detecting small quantities of Ab in patients' sera, and this is the reason the C1FT test was used in the present studies for assaying specific Abs to PMN. We studied the prevalence of such Ab in the serum of patients with various types of demyelinating neuropathies. It was possible to quantitate the anti-PNM activity and follow the dynamic kinetics of this Ab in GBS patients during the course of illness.

MATERIALS AND METHODS

Collection of Clinical Material. Serum was collected from 11 patients meeting the criteria of the Ad Hoc Committee for GBS (17) during the acute phase of their illness. Neurologic symptoms of these GBS patients developed over a 2- to 4-week period, plateaued, and then slowly resolved over several months, as generally observed in GBS patients. Clinical presentations were varied and included a classic ascending paralysis with respiratory involvement, symmetrical multiple cranial neuropathy, moderate proximal weakness, and the Miller–Fisher syndrome of opthalmoplegia associated with ataxia. None was treated with either plasmapheresis or immunosuppression. Serum was stored at −70°C. Prior to use (as a source of antibody) the serum was heat-inactivated at 56°C for 60 min. Serum was also obtained from 5 patients with monoclonal gammapathy with demyelinating peripheral neuropathy, 3 with IgM and 2 with IgG paraproteinemia, and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Ab, antibody; C1, C2, etc., complement components; C2D-HS, human serum congenitally deficient in C2; GBS, Guillain–Barré syndrome; PNM, peripheral nerve myelin.
§Communication of this paper was initiated by Manfred Mayer.

patients with chronic and recurrent forms of peripheral neuritis. Electrodiagnostic studies were performed to confirm clinical diagnosis. Control sera were obtained from normal laboratory workers and from patients with systemic lupus erythematosis, rheumatoid arthritis, cancer, paraproteinemia without neuropathy, sarcoidosis, poliomyelitis, carpal tunnel syndrome, and alcoholic neuropathy. Seven of the 12 disease controls had clinically significant peripheral neuropathies resulting in their referral for neurological evaluation.

Buffers. Barbital-buffered saline (pH = 7.4; μ = 0.15) (barbital/NaCl) was prepared by diluting a stock solution (18) 5-fold with water. G/barbital/NaCl was barbital/NaCl with 0.1% gelatin, 0.15 mM $CaCl_2$, and 1.0 mM $MgCl_2$. EDTA/barbital/NaCl was prepared by mixing 9 vol of barbital/NaCl with 1 vol of 0.1 M EDTA.

Complement and Complement Components. Guinea pig C2 was purchased from Cordis (Miami, FL). Human serum congenitally deficient in C2 (C2D-HS) was the gift of Jerry Winkelstein (The Johns Hopkins Medical School).

Preparation of Sheep Erythrocytes Carrying C4b and C3b. Sheep erythrocytes were sensitized with anti-Forssman IgM (EA) and incubated with fresh serum that had been treated with K-76 monocarboxylic acid to inactivate C5 (19–21). The resulting complement-cellular intermediates, EAC-1,4b,2a,3b, were further incubated at 37°C for 2 hr in EDTA/barbital/NaCl to obtain EAC4b3b by removing C2a and C1 (22).

Isolation of Myelin. Myelin was prepared from human sciatic nerves obtained at autopsy within 6 hr of death according to the procedure of Norton (23). The degree of purity of isolated PNM was assessed by $NaDodSO_4$/PAGE analysis. The myelin membrane was stored at −70°C under nitrogen until use. Membranes were standardized according to lyophilized weight as well as the protein content, which was determined by a modified Lowry assay (24).

Determination of C1-Fixing Anti-PNM Abs in Patients' Serum. In a standard assay, a fixed amount of PNM was incubated overnight with varying dilutions of heated human serum (at 56°C for 60 min) in a total volume of 500 μl at 4°C. The myelin was washed three times with G/barbital/NaCl by centrifugation in a Beckman Microfuge, model B, for 4 min, and the pellet was resuspended in 300 μl of C2D-HS, as a source of excess C1, diluted 1:500 with G/barbital/NaCl, and then incubated for 30 min at 37°C. Unlike the original procedure (16), 0.15 μ buffer was used throughout. The myelin was washed again three times in G/barbital/NaCl. The myelin slurry now carrying anti-PNM Abs and C1 was incubated with 250 μl of EAC4b,3b (1.0×10^8 per ml) for 15 min at 30°C. In brief, this mixture was further incubated for 10 min at 30°C with excess guinea pig C2, which was followed by the final incubation for 60 min at 37°C with 2 ml of guinea pig serum diluted 1:40 with EDTA/barbital/NaCl as a source of C5–C9. Hemolysis of EAC4b,3b was determined spectrophotometrically at 412-nm wavelength.

Depletion of Serum Immunoglobulins. Selective depletion of serum IgG or IgM was performed to examine its effect on anti-PNM activities in patients' serum. Previously determined optimal amounts of heat-inactivated serum were mixed with 250 μl of rabbit anti-human IgM (μ-chain specific; Cappel Laboratories, Cochranville, PA) and the final volume was adjusted to 500 μl with G/barbital/NaCl. Following an overnight incubation at 4°C, the precipitates were pelleted in a Beckman Microfuge and the supernates were tested for anti-PNM activity. For IgG depletion, 100 μl of serum was mixed with an equal volume of packed staphylococcal slurry (The Enzyme Center, Boston) overnight at 4°C. The supernates were also tested for anti-PNM activity. Serum IgG and IgM concentrations of the pre- and post-depletion samples were measured by radial immunodiffusion as described by Mancini (25).

RESULTS

C1 Fixation by Anti-PNM Ab. Antibody binding to antigen activates the complement system by fixing and activating the first component of complement, C1. A single molecule of IgM or two molecules of IgG forming an effective duplex in the immune complex is required to activate one molecule of C1 (26). To demonstrate the feasibility of using this system to detect small numbers of Ab molecules against PNM, varying amounts of PNM were incubated overnight at 4°C with a 1:100 dilution of heat-inactivated rabbit antiserum to human PNM in a total volume of 500 μl of G/barbital/NaCl. The washed PNM pellet with bound Ab was incubated with a previously determined optimal dilution (1:500) of C2D-HS as a source of excess nascent C1. The PNM antigen–Ab complexes can now bind and activate C1 to express C1 esterase activity, which can be detected by the ability to lyse EAC4b cells together with C2 and C3–C9 (16). As shown in Fig. 1, C1 fixation increased as a function of increasing myelin concentration in the presence of a single dose of specific Ab. Ab in the absence of PNM did not fix and transfer C1, as shown at the origin of the curve. Up to 200 μg of PNM, in the absence of Ab, did not fix C1, unlike central nervous system myelin that activates C1 in the absence of specific Ab (27).

Determination of Anti-PNM Ab Titers in Human Serum. Varying dilutions of human serum were incubated with 50 μg of PNM in a total volume of 500 μl. Ab bound to the myelin antigen(s) was detected by its ability to fix C1, which then transfer to EAC4b3b cells and lyse them. Anti-PNM Ab titer was arbitrarily determined by taking the reciprocal serum dilution that can lyse 30% of 2.5×10^7 EAC4b cells in each assay (see Fig. 2). When the straight linear portion of the hemolytic curve did not cross the 30% point, the intercept was determined by extrapolating the straight linear portion of the dose–response curve. Titer fluctuation on different days was normalized on the basis of a single patient serum that was used as a standard in each assay. This standard serum was aliquoted and stored at −70°C. Following normal-

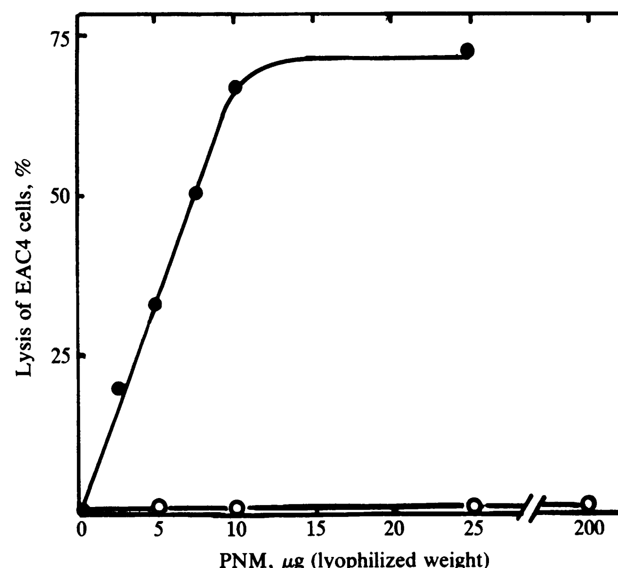

FIG. 1. Quantitation of PNM antigen used to detect C1-fixing anti-PNM Ab by C1 fixation and transfer assay. Varying amounts of PNM suspended in G/barbital/NaCl were incubated with a polyclonal rabbit anti-human PNM antiserum, washed thoroughly, and then incubated with C2D-HS as a source of C1. The PNM, Ab, C1 complexes thus obtained were incubated with EAC43 cells. The erythrocytes were then lysed with excess C2 and C-EDTA. In the absence of specific Ab, no C1 fixation was noted with PNM alone (up to 200 μg) (○), whereas Ab-mediated C1 fixation was linearly correlated with increasing amounts of PNM (●).

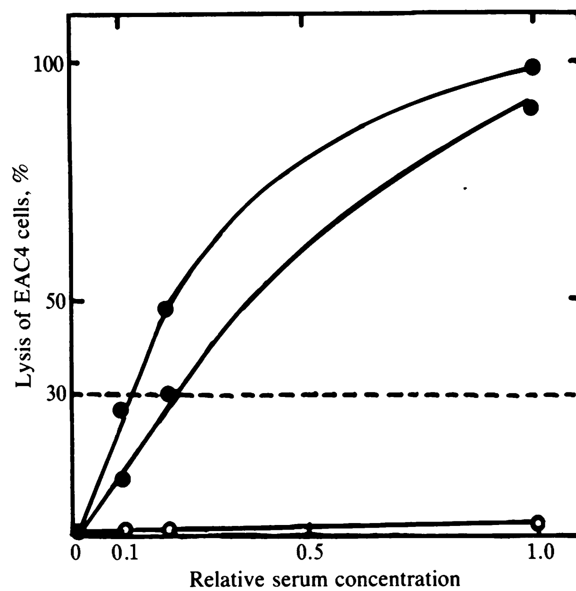

FIG. 2. Titration of Cl-fixing anti-PNM Ab in patient serum. A dose–response curve of Cl fixation was obtained with each patient or control serum. The Ab titer was arbitrarily determined by taking the reciprocal serum dilution at which the linear portion of the dose–response curve met the 30% lysis point of EAC43 cells. Representative serum from 2 GBS patients (●) are illustrated with a typical curve from 1 control individual (○). Relative serum concentration of 1 = 1:10 dilution.

ization, determinations of a serum titer would vary 5% between assays.

Titers of C1-Fixing Anti-PNM Ab in Patients with Demyelinating Neuropathies Compared with Controls. Titers of anti-PNM Abs were determined in 12 patients with GBS. Serum in 11 obtained during the acute phase of the disease showed significantly high titers of anti-PNM Ab, which ranged from 35 to 265 (Fig. 3). In 1 patient, serum obtained during the plateau phase of GBS in the fourth week of disease showed no significant Ab. Ab titers were also elevated in 3 of 4 patients with the chronic and recurrent forms of polyneuritis and in 5 of 5 patients with paraproteinemia associated with demyelinating neuropathies.

Control serum was obtained from 9 laboratory personnel without known disease. In this group, 7 had titers of zero (no lysis of EAC4b,3b cells with undiluted serum). Two others had low but measurable titers of Ab, 4 and 5, respectively. Ab was also measured in serum of patients with a variety of other diseases, including 2 patients with systemic lupus erythematosis, 3 with rheumatoid arthritis, 1 with sarcoid, 1 with alcoholic neuropathy, 2 with paraproteinemia without neuropathy, 1 carpal tunnel syndrome, 1 with poliomyelitis with quadriparesis, and 1 with wound botulism. Among these controls, 4 with measurable titers were those patients with carpal tunnel syndrome, wound botulism, and 2 of the 3 rheumatoid arthritis; titers were 8, 10, 2, and 4, respectively. Seven of 12 patients, 1 with systemic lupus erythematosis, 1 with sarcoidosis, 1 rheumatoid arthritis, 1 with alcoholic neuropathy, 2 with cancer, and 1 with carpal tunnel syndrome had clinically significant neuropathies. Among these, only the carpal tunnel patient had any measurable anti-PNM Abs.

Kinetics of Serum Anti-PNM Ab in 6 GBS Patients. Abs were measured three or more times during the course of disease in 6 patients. Titers were all highest on admission, fell precipitously over the first 2 weeks, and then became either unobtainable or very low by the third and fourth weeks (Fig. 4). Low but measurable titers could still be demonstrated in serum of some patients up to 4 months after the monophasic

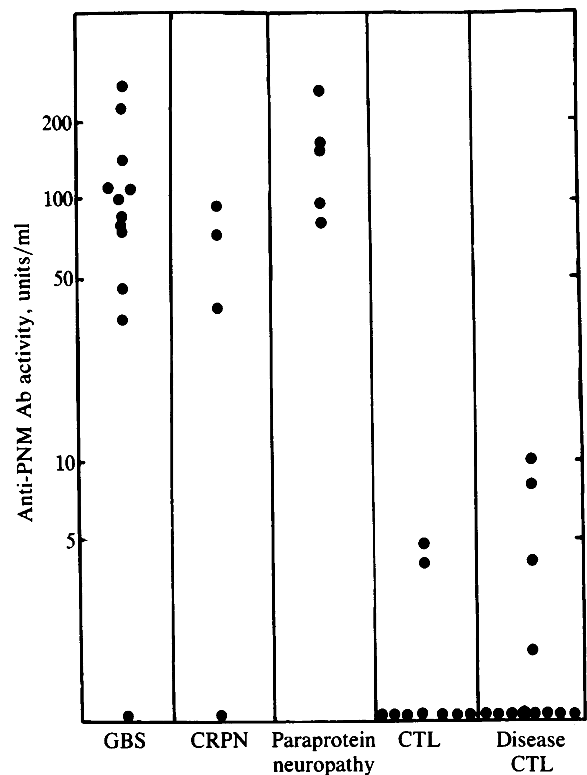

FIG. 3. Anti-PNM Ab fixing Cl in serum of patients with demyelinating neuropathy. The anti-PNM Ab activity, expressed as a logarithm, is reported as units/ml of serum (i.e., 100 units/ml indicates that a 1:100 dilution of the serum will activate enough Cl to lyse 30% of EAC43 indicator cells) in this compound figure. Data from 12 GBS patients, 4 patients with chronic and recurrent forms of polyneuritis (CRPN), and 5 patients with paraproteinemia and peripheral neuropathy are given in the three columns indicated. Nine healthy individuals and 11 patients with various diseases, including systemic lupus erythematosis and rheumatoid arthritis, are shown in the two right-hand columns. CTL, control.

illness. Serial examination of serum over a 5-week period in 1 patient (Fig. 4 *Inset*) showed highest anti-PNM Ab titers 2 days after the initial onset of parasthesias. The Ab titer then

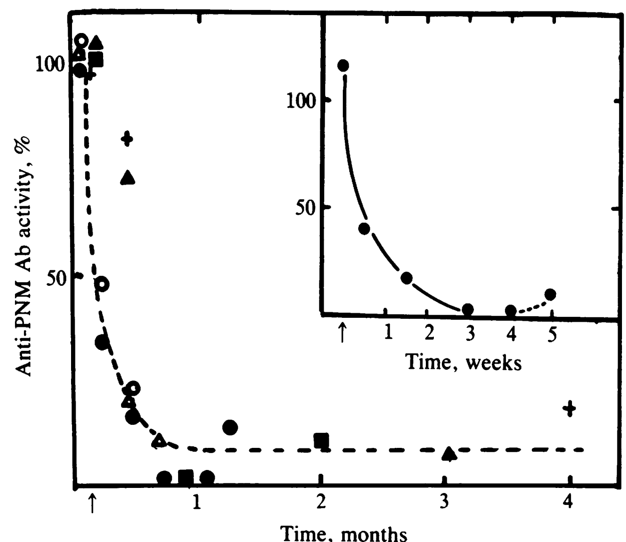

FIG. 4. Kinetics of serum anti-PNM Ab in 6 GBS patients. Antibody titers were measured on three or more samples for each GBS patient and are expressed as a percent of the initial titer obtained on admission (↑). Titers fell precipitously over 2 weeks and became unobtainable or very low by 3–4 weeks. (*Inset*) Serial determinations of anti-PNM Ab in a single GBS patient.

Table 1. Effect of Ig isotype depletion on anti-PNM Ab titers

Demyelinating neuropathy	Pre-depletion			Post-depletion: Anti-μ precipitation			Post-depletion: *Staphylococcus* A absorption		
	Titer	Ig isotype, mg/dl IgM	Ig isotype, mg/dl IgG	Titer	Ig isotype, mg/dl IgM	Ig isotype, mg/dl IgG	Titer	Ig isotype, mg/dl IgM	Ig isotype, mg/dl IgG
GBS									
1	60	320	1095	7	20	1123	65	320	111
2	88	450	1095	9	20	1126	90	425	250
3	116	512	1337	56	132	1375	112	500	850
IgG monoclonal gammapathy									
1	257	335	2625	257	36	2400	48	315	1050
2	154	510	2875	156	40	2870	81	310	750

fell rapidly and became undetectable by the third week, when his condition had clinically plateaued. During clinical recovery, the patient died suddenly from pulmonary emboli. The postmortem sample obtained at fifth week revealed a measurable Ab activity of 14.

To correlate the C1-fixing anti-PNM activities with specific isotypes of serum Ig, anti-PNM Ab titers were measured prior to and after depletion of IgM or IgG in serum obtained on admission from 3 of the 6 GBS patients included in Fig. 4. As shown in Table 1, the immunoprecipitation with anti-μ antiserum resulted in a depletion of IgM concentrations by 75%, 94%, and 95% with a concomitant decrease of anti-PNM activity by 50%, 89%, and 90%, respectively. Depletion of up to 90% of the IgG in the same serum by *Staphylococcus* A absorption failed to lower anti-PNM Ab titers. In 2 patients with IgG monoclonal gammapathy and associated demyelinating neuropathy, absorption of 56% and 74% of serum IgG resulted in a 48% and 81% drop of Ab titers, respectively, whereas titers were unchanged following precipitation of IgM. In patient 2, *Staphylococcus* A treatment reduced both IgG (75%) and IgM (40%) levels for an unknown reason. The decreased Ab titers in this case were still considered to be due to IgG loss since the titer was unchanged when IgM was depleted 92% by immunoprecipitation.

DISCUSSION

The present study compared the prevalence and titer of anti-PNM Abs in the serum of patients with a variety of acquired demyelinating neuropathies and other immunopathologic conditions and normal individuals. Purified PNM vesicles were used as a target in this assay to identify all Abs that would react with any antigenic determinant on the surface of PNM and that could activate C1.

The study demonstrated anti-PNM Ab in the serum of patients with a variety of demyelinating neuropathies (Fig. 3) and showed that Ab levels in these patients are greatly increased compared with control groups, which included patients with neuropathies secondary to collagen vascular disease, sarcoidosis, nutritional deficiency, and compression. Specifically, in 11 GBS patients in acute phase of illness, the titers of anti-PNM Ab were 6- to 56-fold greater than that found in normal controls without known disease. In 3 of 4 cases of chronic and recurrent polyneuritis and in all 5 paraproteinemia patients with neuropathy Ab titers were increased 9- to 50-fold over the controls.

The prevalence of anti-PNM Ab in GBS serum shown here contrasts sharply with previously reported incidences of 27% and 50% (3, 6). Several explanations could account for such discrepancies. The previous studies used assays that measured complement consumption by inhibition of hemolysis of sheep erythrocytes. In addition to the general limitations of the classical assay cited in the Introduction, other factors play a role in the present case. Thus, it can also reflect processes such as activation of the alternative pathway or the presence of aggregated IgG and IgA (28) in patient serum. Indeed, we have observed that PNM consumes significant amounts of human serum complement via the alternative pathway in the absence of C1 fixation (29). These factors all contribute to the high background, thus reducing the ability to detect a specific increment contributed by the PNM–Ab complexes. An additional factor is the time of serum collection in relation to chronology of disease. Serial determinations of Ab in 6 GBS patients demonstrated a rapid clearance of anti-PNM Ab from the serum (Fig. 4). Thus, the dynamic decline of anti-PNM titers during illness points out the importance of early serum sampling in GBS. The precipitous fall in serum Ab may reflect the decline of an IgM response as suggested by the isotype depletion studies on 3 GBS samples (Table 1) or clearance by immune complex formation or both. The nature of the IgM isotype and the correlation of Ab decline with clinical improvement suggest that these Abs are involved in the pathogenesis of demyelination and are not a secondary response to myelin destruction. In 1 GBS patient in the plateau phase of disease, we were unable to detect C1-fixing anti-PNM Ab (Fig. 2). The inability to show C1 fixation in 1 patient with chronic recurrent polyneuropathy could reflect absence of anti-PNM Ab but might also indicate the presence of IgG isotype recognizing an antigen with very low density (26). IgG isotype appears to be responsible for Ab titers in 2 patients who had IgG monoclonal gammapathy. At present, we do not know whether the abnormal IgG in these patients is responsible for the Ab activities. Regardless, it should also be considered that all demyelination neuropathies may not have a common pathogenic mechanism in spite of clinical similarities.

Patients with rheumatoid arthritis, systemic lupus erythematosis, and paraproteinemia without neuropathy were included as disease controls to evaluate the specificity of the assay for PNM antigens. Circulating immune complexes, various autoantibodies, and aggregated immunoglobulins did not appear to interfere with our assay. Low and probably insignificant titers (2 and 4) were found in 2 of 3 rheumatoid patients, all of whom had high titers of rheumatoid factor (Fig. 3). Two patients with systemic lupus erythematosis and 2 with paraproteinemia did not show measurable C1 activation.

For Ab to be a factor in the initial phases of demyelination, it must penetrate a blood–nerve barrier, bind to presumably a surface antigen determinant on PNM or Schwann cells, and then mediate myelin destruction through activation of complement, Ab-dependent cytotoxic lymphocytes, or mac-

rophages. With the C1FT assay, we have demonstrated the presence of complement-activating Ab reacting with surface PNM determinants in the serum of patients with actively demyelinating neuropathies. Demyelinating activity of GBS serum *in vivo* is reported highest early during the acute phase and falls with time (10, 12) in a manner similar to the Ab curve in 6 GBS patients followed serially in this study (Fig. 4). The clearance of Ab in these patients correlated with a plateau in disease activity and clinical improvement. Although this may not prove Ab involvement in disease initiation, it clearly shows availability of a potentially myelin-destructive agent in the patient's serum. Other factors that stimulate Ab formation and allow access of Ab to PNM are unknown but obviously important in understanding the pathogenesis of immunologically mediated demyelination of peripheral nerve. Regardless, the significantly high titers of anti-PNM, C1-fixing Ab demonstrated in all patients with acute-phase GBS, and in most patients with chronic and recurrent polyneuritis and paraproteinemia with peripheral neuropathy, suggest that the Ab may be a marker for primary peripheral demyelinating diseases.

We express deep appreciation to Drs. S. Cherry, T. Paula, J. Rosenthal, and R. F. Mayer for their help in obtaining clinical material and to Drs. K. P. Johnson, R. F. Mayer, and M. M. Mayer for critical review of the manuscript. This work was supported by National Institutes of Health Grant 1 PO1, NS 20022 to C.L.K. and by Grant RO1 NS 15662 and National Multiple Sclerosis Society Grant RG 1374-B-2 to M.L.S. This is publication no. 1736 of the Department of Pathology, University of Maryland, School of Medicine.

1. Arnason, B. G. W. (1975) in *Peripheral Neuropathy*, eds. Dyck, P. J., Thomas, P. K. & Lambert, E. H. (Saunders, Philadelphia), pp. 1137–1148.
2. Zito, G., Cook, S. D. & Dowling, P. C. (1982) *Clin. Immunol. Allergy* **2**, 309–331.
3. Melnick, S. C. (1963) *Br. Med. J.* **1**, 368–373.
4. Cook, S., Murray, M. R., Whitaker, J. N. & Dowling, P. (1969) *Neurology* **19**, 284 (abstr.).
5. Tse, K. S., Arbesman, C. E., Tomasi, J. B. & Tourville, D. (1971) *Clin. Exp. Neurol.* **8**, 881–887.
6. Latov, N., Gross, R. B., Kastelman, J., Flanagan, T., Lamme, S., Alkaitis, D. A., Olarte, M. R., Sherman, W. H., Chess, L. & Penn, A. S. (1981) *Neurology* **31**, 1530–1534.
7. Dowling, P. & Cook, S. D. (1973) *Neurology* **23**, 423 (abstr.).
8. Rosenberg, R. N., Aung, M. H., Tindall, R. S. A., Molenick, S., Baskin, F., Capra, J. D. & Toben, H. R. (1975) *Neurology* **25**, 1101–1110.
9. Latov, N., Sherman, W. H., Nemni, R., Galassi, G., Shyong, J. S., Penn, A. S., Chess, L., Olarte, M. R., Rowland, L. P. & Osserman, F. F. (1980) *N. Engl. J. Med.* **303**, 618–621.
10. Feasby, T. E., Hahn, A. F. & Gilbert, J. J. (1982) *Neurology* **32**, 1159–1167.
11. Saida, T., Saida, K., Lisak, R. P., Brown, M. J., Silverberg, D. H. & Asbury, A. K. (1982) *Ann. Neurol.* **11**, 69–75.
12. Sumner, A. J., Lisak, R. P., Brown, M. J. & Asbury, A. K. (1983) *Neurology* **33** Suppl. 2, 81.
13. Hays, A. P., Takatsu, M., Latov, N. & Sherman, W. H. (1983) *J. Neuropathol. Exp. Neurol.* (1983) **42**, 349 (abstr.).
14. Mayer, M. M., Michael, D. W., Ramm, L. E., Whitlow, M. B., Willoughby, J. B. & Shin, M. L. (1981) *Crit. Rev. Immunol.* **2**, 133–165.
15. Liu, W. T., Vanguri, P. & Shin, M. L. (1983) *J. Immunol.* **131**, 778–782.
16. Borsos, T. & Rapp, H. J. (1965) *J. Immunol.* **95**, 559–572.
17. Asbury, A., Arnason, B., Karp, H. & McFarlin, D. E. (1978) *Ann. Neurol.* **3**, 565–566.
18. Kabat, E. A. & Mayer, M. M. (1964) in *Experimental Immunochemistry* (Thomas, Springfield, IL), pp. 149–153.
19. Hong, K., Kinoshita, T. & Inoue, K. (1981) *J. Immunol.* **127**, 109–114.
20. Hong, K., Kinoshita, T., Miyazaki, W., Izawa, T. & Inoue, K. (1979) *J. Immunol.* **122**, 2418–2423.
21. Hong, K., Kinoshita, T., Kitajima, H. & Inoue, K. (1981) *J. Immunol.* **127**, 104–108.
22. Stroud, R. M., Mayer, M. M., Miller, J. A. & McKenzie, A. T. (1966) *Immunochemistry* **3**, 163–176.
23. Norton, W. T. (1975) *Methods Enzymol.* **31**, 435.
24. Markwell, M. A. K., Haas, S. M., Bieber, L. L. & Tolbert, N. E. (1978) *Anal. Biochem.* **87**, 206–210.
25. Mancini, G., Carbonara, A. O. & Heremans, J. F. (1965) *Immunochemistry* **2**, 235–254.
26. Ishizaka, T., Ishizaka, K., Borsos, T. & Rapp, H. J. (1966) *J. Immunol.* **97**, 716–726.
27. Vanguri, P., Koski, C. L., Silverman, B. & Shin, M. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3290–3294.
28. Iida, K., Fujita, T., Inai, S., Sasaki, M., Kato, T. & Kobayashi, K. (1976) *Immunochemistry* **13**, 747–752.
29. Koski, C. L., Vanguri, P. & Shin, M. L. (1985) *J. Immunol.*, in press.