Journal of Clinical Laboratory Analysis 9:308–319 (1995)

Myelin- and Microbe-Specific Antibodies in Guillain-Barré Syndrome

Jeff Terryberry,[1] Made Sutjita,[1] Yehuda Shoenfeld,[2] Boris Gilburd,[2] David Tanne,[2] Margalit Lorber,[3] Iyad Alosachie,[1] Noori Barka,[1] Hun-Chi Lin,[1] Pierre Youinou,[4] and James B. Peter[1]

[1]Specialty Laboratories, Santa Monica, California; [2]Research Unit of Autoimmune Diseases, Department of Medicine "B", Sheba Medical Center, Tel-Hashomer and [3]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [4]Laboratoire d'Immunologie, Centre Hospitalier Regional & Universitaire, Brest, France

We surveyed the frequency of reported infections and target autoantigens in 56 Guillain Barré syndrome (GBS) patients by detecting antibodies to myelin and microbes. Sulfatide (43%), cardiolipin (48%), GD_{1a} (15%), SGPG (11%), and GM_3 (11%) antibodies were the most frequently detected heterogenous autoantibodies. A wide spectrum of antimicrobial IgG and IgM antibodies were also detected; mumps-specific IgG (66%), adenovirus-specific IgG (52%), varicella-zoster virus-specific IgG (46%), and *S. pneumoniae* serotype 7-specific IgG (45%) were the most prevalent. Our results indicate that polyclonal expansion of physiologic and pathologic antibodies and/or molecular mimicry likely occurs following infection and is related to other autoimmune factors in the etiology of GBS. Although no single definitive myelin-specific autoantibody was identified, our results suggest a unique pattern of reactivity against autoantigens.

Key words: autoantibodies, autoimmunity, gangliosides, myelin, neuropathy

INTRODUCTION

Guillain-Barré syndrome (GBS) is a transient neurological disorder characterized by areflexic motor paralysis with mild sensory disturbances in conjunction with an acellular rise of total protein in the cerebrospinal fluid associated with an inflammatory demyelination of peripheral nerves (1,2). Although the pathogenesis of GBS has not been fully elucidated, there is increasing evidence pointing to an autoimmune etiology (3,4). Autoantibodies to various myelin-associated glycoconjugates are described in GBS patients (5,6). However, the primary autoantigen in GBS is not yet clearly identified. Moreover, it is not yet fully determined whether the autoantibodies associated with GBS are directly involved in the pathogenesis of nerve damage or induce demyelination and release of autoantigens via a T-cell-mediated inflammatory reaction (7,8). Successful treatment of some GBS patients by plasmapheresis and intravenous gammaglobulin (IVIG) supports the notion that circulating myelin autoantibodies could play a direct role in pathogenesis. Myelin-specific antibodies are potentially useful for diagnosis and predicting treatment success with either plasmapheresis or IVIG (9–11).

Prior infections are often associated with GBS (12,13), although the identity of the pathogen(s) and the relationship to subsequent onset of neurological disease remain uncertain. Pathogens reportedly associated with GBS include herpes viruses (HSV) (14,15), Epstein-Barr virus (EBV) (16,17), cytomegalovirus (CMV) (18,19), human herpesvirus-6 (HHV-6) (20), varicella-zoster virus (VZV) (21,22), human T-cell lymphotrophic viruses (HTLV) (23,24), human immunodeficiency viruses (HIV) (25,26), measles (27,28), coxsackievirus (29–31), rubella (32,33), mumps (34), influenza (35,36), hepatitis viruses (37,38), *Mycoplasma pneumoniae* (39,40), *Borrelia burgdorferi* (41), respiratory syncytial virus (42), *Campylobacter jejuni* (43,44) echovirus (45), coronavirus (46), parainfluenza (47), streptococcus (48), enterovirus (EV-70) (49), and parvovirus (50).

In this study, we surveyed sera and CSF of GBS patients for the presence of antibodies against 18 myelin autoantigens including gangliosides, galactosyl cerebroside, sulfated glycolipids, Forssman antigen, myelin basic protein (MBP), and cardiolipin. Because sequence similarities exist between bacteria, viruses, and neuronal autoantigens (51–53), this study analyzes the association

Received January 24, 1995; accepted February 27, 1995.

Address reprint requests to James B. Peter, M.D., Ph.D., Specialty Laboratories, Inc., 2211 Michigan Avenue, Santa Monica, CA 90404-3900.

between the presence of myelin autoantibodies and antibodies to 18 common infectious agents known to be associated with GBS. This study also attempts to identify a possible pattern of autoantibody and pathogen-specific antibody reactivity. Additionally, we used a novel flow cytometry assay to detect neuronal antibodies.

MATERIALS AND METHODS

Serum and CSF Specimens

Sera were obtained from 56 GBS patients (National Institute of Neurological Disease and Stroke diagnostic criteria; 137–139) (29 white males and 27 white females), aged 8–47 years, at diagnosis within a median period of 1 week of onset of neurologic symptoms. Age-, gender-, and race-matched normal and disease control sera were collected from 70 healthy subjects, 10 patients with clinically definite multiple sclerosis (MS) (Poser's criteria), 10 patients with idiopathic transverse myelitis (TM) (Jeffery's criteria), 10 patients with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) (Uncini's criteria), 15 patients with polyneuropathy (PN) (Pestronk's criteria), and 16 patients with chronic fatigue and immune dysfunction syndrome (CFIDS) (CDC criteria). CSF specimens were obtained from 12 GBS patients and 10 control individuals without evidence of neuroborreliosis.

Determination of Antibodies to Myelin Glycoconjugates

Glycolipid antigens (galactosyl cerebroside [Gal-Cer], sulfatide [ceramide galactoside 3-sulfate], SGPG [sulfoglucuronyl paragloboside], sphingomyelin, cardiolipin, Forssman antigen, monosialogangliosides [GM_1, GM_2, GM_3], disialogangliosides [GD_{1a}, GD_{1b}, GD_2, GD_3], trisialoganglioside [GT_{1b}] and asialo-GM_1), and myelin glycoprotein antigens (myelin basic protein [MBP], myelin-associated glycoprotein [MAG]) were used to determine autoantibodies in sera and CSF. SGPG, MBP, and MAG antigens were prepared by extraction and chromatography (132–134). All other myelin antigens were purchased from Matreya (Pleasant Gap, PA).

Antibodies to myelin glycoconjugates were determined by enzyme-linked immunosorbent assay (EIA); 250 ng/well of the appropriate myelin antigen was dissolved in chloroform:methanol (1:3 v/v) and coated by evaporation in flatbottom 96-well plates (Immulon I). MBP and MAG antigens in 50 mM $KHCO_3$ pH 9.6 (2.5 ng/well) were coated for 24 h. Uncoated wells (background) were filled only with coating solution. The wells were blocked with phosphate-buffered saline-1% bovine serum albumin (PBS-1% BSA) for 30 min at RT and washed with PBS-T (50 mM sodium phosphate, 150 mM NaCl and 0.05% Tween-20, pH 7.4). A total of 100 µL of test sera and positive controls (diluted 1:300 in PBS-1% BSA) or undiluted CSF specimens were added to the wells and incubated for 1 h at RT. Following repeated washing, bound antibodies were detected by adding goat antihuman IgM and/or antihuman IgG conjugated to alkaline phosphatase (1:2,000 dilution) and paranitrophenyl phosphate. The optical density (OD) was read at 405 nm on an automatic microplate reader.

Determination of Microbial Antibodies

Antibodies specific for *Campylobacter jejuni* (IgG), human herpesvirus 6 (HHV-6) (IgG & IgM), influenza viruses A & B (IgG), *Streptococcus pneumoniae* (strains 3, 7, 9, 14), (IgG), *Legionella pneumophila* (IgG, IgM & IgA), varicella-zoster virus (VZV) (IgG & IgM), cytomegalovirus (CMV) (IgG & IgM), herpes simplex viruses (HSV-1/2) (IgG & IgM), human immunodeficiency viruses (HIV-1/2) (IgG), *Mycoplasma pneumoniae* (IgM), Epstein-Barr virus (EBV) (VCA IgM), and Japanese encephalitis virus (JEV) (IgM) were determined. Commercial kits were used to detect specific antibodies to HSV-1/2 (Pharmacia, Parsippany, NJ), *M. pneumoniae* (BioWhittaker, Walkansville, MD), and HIV-1/2 (AccuSpot™, Specialty Biosystems, San Diego, CA) according to the manufacturer's instructions.

Specific antibodies to the other infectious agents were detected by the following generalized EIA procedure. Antigens (250 ng/well) in 50 mM carbonate buffer, pH 9.6 were coated onto Immulon I (Dynatech, Chantilly, VA) or Polysorp (NUNC, Naperville, IL) polystyrene microtiter plates overnight at 4°C, decanted, washed with PBS-T, and blocked with PBS-1% BSA for 30 min at RT. Standards, controls, and test sera (diluted 1:300 in PBS-1% BSA) were pipetted in duplicate into wells and incubated for 2 h at RT on a shaker. Following repeated washing, bound antibodies were detected by adding goat antihuman IgM or antihuman IgG conjugated to alkaline phosphatase (1:2,000 dilution) and paranitrophenyl phosphate. The optical density (OD) was read at 405 nm on an automatic microplate reader.

Thin-layer Chromatography (TLC) With Immunofixation

Representative sera positive by EIA for myelin component reactivity were analyzed by TLC. A total of 5 µL each of GM_1, GM_3, GD_{1a}, GT_{1b}, and Gal-Cer were aliquoted from 1 mg/mL stock solutions to make glycolipid cocktail #1; 5 µL each of GM_2, GD_{1b}, GD_3, asialo-GM_1, sulfatide, and SGPG were aliquoted from 1 mg/mL stock solutions to make glycolipid cocktail #2; 5 µL of each cocktail were spotted in duplicate on 10 × 10 cm aluminum-backed silica gel plates (Aldrich, Milwaukee, WI), air dried, and developed with 50:40:5 (v:v) chloroform:methanol:0.2% KCl. The plates were air dried and briefly immersed in 0.05% polyisobutylmethacrylate in n-hexane. After thorough drying, the fixed plates were blocked in PBS-1% BSA for 30 min, washed for

10 min in PBS, immersed in a 1:100 dilution of the appropriate serum specimen in PBS-1% BSA, and incubated overnight on a rocker. The plates were washed three times for 10 min with PBS and incubated with 1:2,000 goat antihuman IgG-biotin and 1:2,000 goat antihuman IgM-biotin conjugates (Chemicon, Temecula, CA) for 2 h at RT. After washing, the plates were incubated with 1:1,000 streptavidin-horseradish peroxidase for 1 h at RT, washed again, and developed with 1:50 diaminobenzidine substrate in 50 mM Tris-HCl, pH 7.4, and 0.01% H_2O_2. For TLC validation of glycolipid migration values (Rf), parallel plates with individual glycolipids per lane were colorimetrically stained with orcinol (gangliosides) or azure A (sulfoglycolipids).

Flow Cytometric Determination of Neuronal Antibodies

The human neuroblastoma cell lines SK-N-MC and SK-N-SH (American Type Culture Collection (ATCC), Rockville, MD) were used as a source of antigens. The neuronal cell lines were maintained in RPMI medium supplemented with 10% fetal calf serum in 5% CO_2/95% air. Confluent cells were harvested and washed twice with isotonic-buffered saline: EDTA solution (BSS). After resuspension, the cells were counted and the cell concentration was adjusted to an optimal concentration determined by block titration. Nonspecific binding sites were blocked by incubating the cells with BSS containing 10% goat serum. After incubation, cells were repeatedly washed and resuspended in BSS; 50 µl aliquots of the cell suspension were dispensed into incubation tubes and 50 µL of diluted sera were added to the tubes. The cells were incubated, repeatedly washed, and stained with FITC-labeled goat antihuman IgG conjugate. After further washing, the cells were resuspended in BSS and analyzed in an EPICS Profile flow cytometer. The mean intensity of fluorescence (MIF) using a logarithmic scale was determined for each sample. Cutoff values were defined as the mean + 2 standard deviations of MIF units using the frequency distribution curve of healthy control sera at the 95% confidence limit (5 MIF units).

Statistical Analyses

The statistical analyses included testing for normal distribution, Student's t-test and Spearman rank correlation. Accuracy and precision of each EIA run was estimated using 70 normals to establish the cutoff (mean + 3 SD). Corrected values of $P < 0.05$ were considered significant for t-test analyses, as were Rs values ≥ 0.49 in the Spearman rank correlation tests.

RESULTS

The interassay coefficient of variation (CV) of the cutoff value was ≥15% for each glycolipid antibody EIA and ≤5% for each microbe-specific antibody EIA. Various autoantibodies were detected in the sera and CSF of GBS patients (Table 1); none of the antibodies alone could be considered diagnostic (<50% reactivity). Cardiolipin (48% [27/56]), sulfatide (43% [24/56]), and GD_{1a} (14% [8/56]) were the most frequently detected antibodies in GBS sera followed by GM_1, GM_3, and SGPG antibodies (11% [6/56]). None of the healthy controls had antibodies to these autoantigens. Neuronal antibodies were not detected in any of the GBS patient sera. Overall, 52% (29/56) of the GBS patient sera had at least one antibody against one of the myelin autoantigens; reactivity against more than five autoantigens was noted in at least six sera (up to 14 autoantigens) (Table 2). Multireactivity to 10 or more autoantigens was found in all CSF specimens from GBS patients (Table 1). Of the 12 GBS CSF specimens, 100% (12/12) reacted with GD_{1a} antigen and 92% (11/12) reacted with each of 10 other autoantigens. The specificity of CSF reactivity was confirmed with the control CSF from individuals without evidence of neuroborreliosis. None of these control specimens (0/10) were reactive with any myelin component.

Representative sera positive for autoantibodies to myelin components by EIA were confirmed by TLC with immunofixation. The sera tested were multireactive by EIA; this multireactivity was confirmed by TLC with up to 10 bands detected (r = 0.99) (Fig. 1); no bands were detected in normal control sera.

Specific IgG antibodies to *S. pneumoniae* (34–45%), adenovirus (52% [29/56]), mumps (66% [37/56]), VZV antibodies (46% [26/56]), and influenza A/B antibodies (23% [13/56]) were commonly detected in GBS patient sera (Table 3). Specific IgM antibodies to *Campylobacter jejuni* (20% [11/56]), CMV (13% [7/56]), HHV-6 (7% [4/56]), VZV (11% [6/56]), mumps (16% [9/56]), HSV-1 (18% [10/56]), *M. pneumoniae* (11% [6/56]), HSV-2 (9% [5/56]), EBV-VCA (4% [2/56]), and JEV (4% [2/56]) were also detected in GBS patient sera. A multireactive pattern of both IgG and IgM microbe-specific antibodies were also observed in GBS sera; some patients had antibodies to as many as 15 infectious agents (Table 4). Only eight patients lacked antibodies to any of the above microorganisms.

Various levels of significance between the sera multireactive for autoantigens and microbes were established by Spearman rank correlation (Table 5). A significant relation of mumps IgG and sulfatide antibodies is illustrated (see Fig. 3) by linear regression analysis. One patient with 10 glycolipid autoantibodies had antibodies against *C. jejuni* only, whereas another patient had one autoantibody and antibodies to eight different microorganisms (Table 6). The majority of autoantibodies were of low titer polyreactivity (Fig. 2), although some higher affinity autoantibodies of perhaps increased specificity and pathogenicity were also detected (54,55).

To determine whether transient sulfatide autoantibodies (56) were present in mumps IgG-positive sera from individuals without GBS or other clinical autoimmune diseases, control mumps IgG-positive sera were tested for sulfatide

TABLE 1. Frequency of Autoantibodies in 56 GBS Patients and Controls

Autoantigens (n)	GBS sera (56)	GBS CSF (12)	MS sera (10)	TM sera (10)	CIDP sera (10)	CFIDS sera (16)	PN sera (15)
GM_1	6	10	0	0	0	0	0
GM_2	5	11	0	ND	0	3	1
GM_3	6	11	0	ND	0	0	0
GD_{1a}	8	12	0	ND	0	1	0
GD_{1b}	4	11	0	ND	0	3	1
GT_{1b}	4	11	0	ND	0	0	0
Gal-Cer	5	11	1	0	0	2	0
SGPG	6	11	0	0	1	3	1
Sulfatide	24	9	0	2	1	1	1
Asialo-GM_1	4	11	3	ND	0	4	1
Forssman	3	ND	ND	ND	ND	ND	ND
MBP	5	ND	ND	ND	ND	ND	ND
Sphingomyelin	0	11	2	0	0	6	1
GD_3	4	11	0	ND	0	1	0
Neuronal	0	2	ND	2	ND	ND	ND
Cardiolipin	27	11	0	ND	0	0	0
MAG	1	ND	ND	ND	ND	ND	ND
GD_2	4	ND	ND	ND	ND	ND	ND

ND = not determined.

antibodies. Eighteen percent (2/11) of control mumps IgG-positive sera were positive for sulfatide antibodies (data not shown). Preincubation of up to 1 μg of sulfatide with GBS and control mumps-positive sera did not inhibit binding to mumps antigen-coated plates.

DISCUSSION

GBS is an acute condition that usually resolves within a few days to a few weeks and responds to immunomodulation with IVIG and IgG-clearing modalities such as plasmapheresis. During the last three decades, ample information indicates that GBS could have an autoimmune etiopathogenesis (57–59).

Wide discrepancies and variations are reported in both the type and binding characteristics of the autoantibodies associated with GBS and the potential infectious causes of the disease. Autoantibodies reported in GBS include mainly antibodies to gangliosides such as GM_1 (60,61), GD_{1b} (62), asialo-GM_1 (63), GQ_{1b} (64,65), and 3′-LM1 (66) as well as antibodies to galactosylcerebroside (67,68) and sulfated structures such as SGPG and sulfatides (69,70), the Forssman-like antigen (71), and to myelin proteins such as P_0 (72), myelin oligodendrocyte glycoprotein (MOG) (73) and P_2 (74). Furthermore, specific T-cell reactions to P_0 and P_2 antigens were recently described (75,76). Autoantibodies to phospholipids such as cardiolipin and phosphatidylcholine (77,78), as well as β-tubulin (79), neuron-specific enolase (NSE), *S*-100b protein (80), antineutrophil cytoplasmic antibodies (ANCA) (81), and double-stranded DNA (82) are also described. The frequencies reported for the appearance of these various autoantibodies in GBS range from 5–50%.

TABLE 2. Polyreactivity of GBS Sera Against 18 Myelin Antigens

# of antigens	# of sera 29/56
1	3
2	16
3	2
4	1
5	1
6	1
8	1
9	1
10	1
13	1
14	1

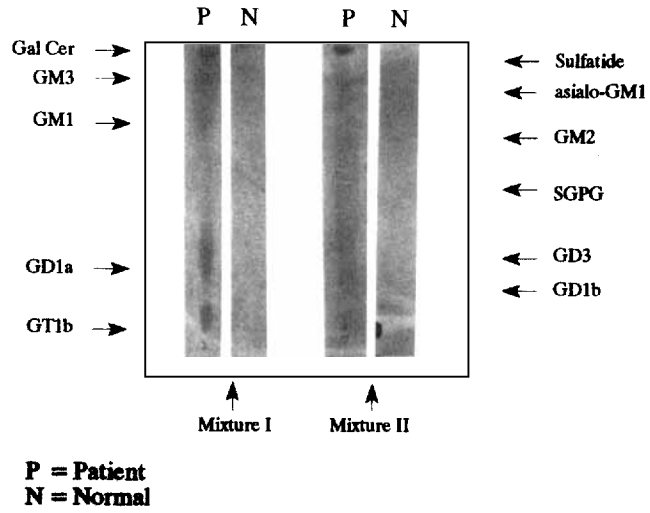


Fig. 1. TLC of representative glycolipid-reactive GBS serum vs. normal control.

TABLE 3. Frequency of Antibodies to Infecting Agents in 56 Sera of Patients With GBS

Infectious agent	Isotype	# Positive sera
C. jejuni	IgM	11
Influenza A/B	IgG	13
S. pneumoniae-3	IgG	19
S. pneumoniae-7	IgG	25
S. pneumoniae-9	IgG	21
S. pneumoniae-14	IgG	23
M. pneumoniae	IgM	6
L. pneumophilia	IgG + IgM + IgA	2
CMV	IgG	19
CMV	IgM	7
HHV-6	IgG	4
HHV-6	IgM	4
Adenovirus	IgG	29
VZV	IgG	26
Mumps	IgG	37
HSV-1	IgM	10
HSV-2	IgM	5
HIV-1/2	IgG	1
Mumps	IgM	9
EBV-VCA	IgM	2
VZV	IgM	6
JEV	IgM	2

Conserved epitopes could also be distributed in organ systems other than the nervous system (83–88,106).

Current evidence supports the notion that there is no single GBS antigen. Likewise, there are multiple antecedent factors (i.e., infectious agents) for the disease, and the different myelin antigens might be related in terms of sequential and conformational homology to the various pathogens (molecular mimicry) (89–93). GBS can also occur in association with other autoimmune diseases (neurologic overlap syndrome) (130).

Although some autoantibodies (cardiolipin, GD_{1a}, sulfatide, GM_3, and SGPG) were detected at a higher frequency in sera of GBS patients, none of these autoantibodies can be exclu-

TABLE 4. Number of Sera of GBS Patients Having Multiple Antibodies Against Infecting Agents

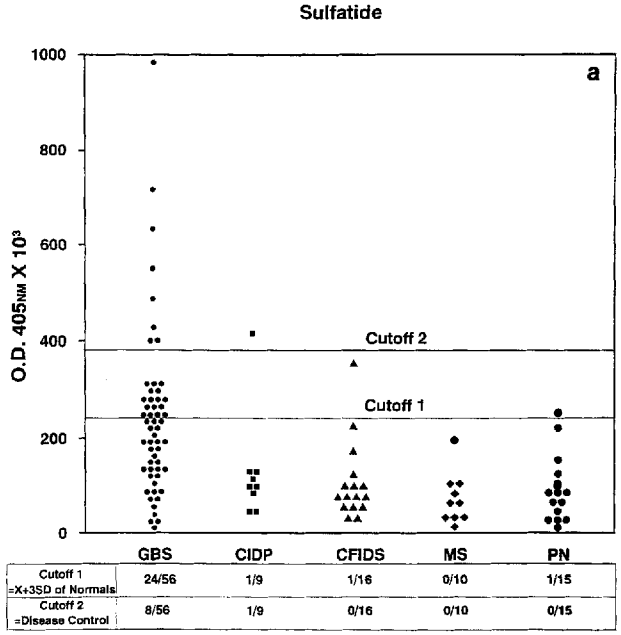
# Antibodies	# Positive sera
1	9
2	5
3	3
4	2
5	3
6	5
7	2
8	8
9	1
10	1
11	1
12	2
13	1
14	1
15	1

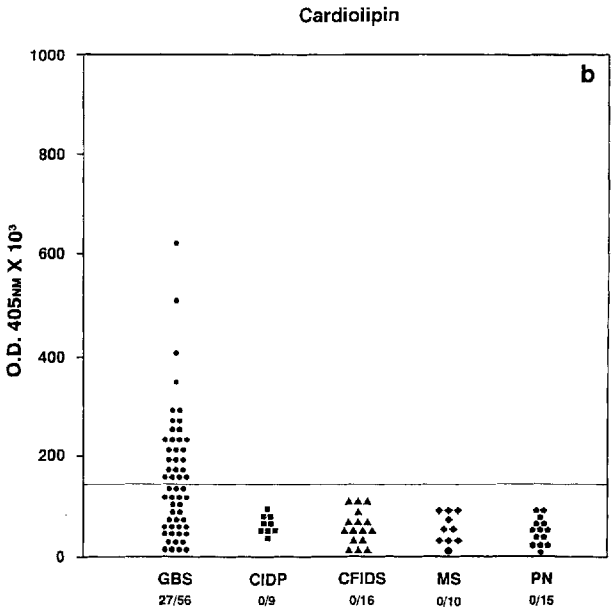
TABLE 5. Significant Associations Between Antimyelin and Antipathogen Reactivities

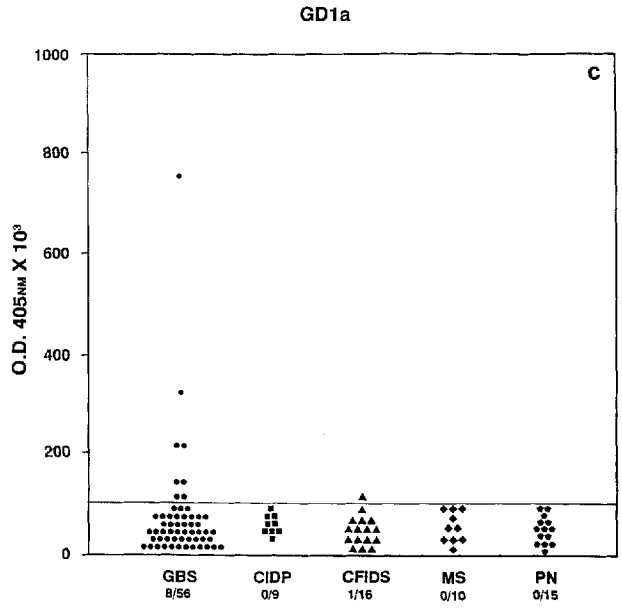
Spearman rank correlation	*P* value	Rs value
Mumps IgG vs. Sulfatide	0.00003	0.809
Mumps IgG vs. asialo-GM_1	0.00004	0.756
Mumps IgG vs. GT1b	0.00004	0.746
S. pneu 7 IgG vs. cardiolipin	0.00004	0.726
Mumps IgG vs. cardiolipin	0.00005	0.698
Mumps IgG vs. GD1b	0.00005	0.694
Mumps IgG vs. GM3	0.00005	0.684
Mumps IgG vs. SGPG	0.00005	0.683
Mumps IgG vs. GM2	0.00005	0.681
Mumps IgG vs. GM1	0.00005	0.662
HSV 2 IgM vs. asialo-GM1	0.00006	0.640
S. pneumoniae 7 IgG vs. sulfatide	0.00006	0.638
Mumps IgG vs. GalCer	0.00006	0.636
CMV IgM vs. SGPG	0.00006	0.627
CMV IgM vs. GM2	0.00006	0.617
CMV IgM vs. sulfatide	0.00006	0.611
CMV IgM vs. GD1b	0.00007	0.598
CMV IgM vs. asialo-GM1	0.00007	0.595
S. pneumoniae 7 IgG vs. SGPG	0.00007	0.593
CMV IgM vs. GM3	0.00007	0.589
CMV IgM vs. GD1b	0.00008	0.579
Mumps IgG vs. GD1a	0.00008	0.572
CMV IgM vs. GD1a	0.00008	0.563
HSV2 IgM vs. GM2	0.00009	0.556
Influenza A/B IgG vs. asialo-GM1	0.00009	0.556
CMV IgM vs. GM1	0.00009	0.549
Adv IgG vs. sulfatide	0.0001	0.542
CMV IgM vs. GalCer	0.0001	0.540
HSV2 IgM vs SGPG	0.0001	0.539
L. pneumophila (total) vs. GD1a	0.0001	0.538
HSV 2 IgM vs. GD1b	0.0001	0.535
Mumps IgG vs. GD3	0.0001	0.534
HSV 2 IgM vs. GT1b	0.0001	0.524
HSV 2 IgM vs. GM3	0.0001	0.518
Adv IgG vs. cardiolipin	0.0001	0.517
HSV 2 IgM vs. GM1	0.0002	0.505
Influenza A/B IgG vs. GM1	0.0002	0.500
L. pneumophila (total) vs. asialo-GM1	0.0002	0.495
Adv IgG vs. GalCer	0.0002	0.495
Influenza A/B IgG vs. GT1b	0.0003	0.493

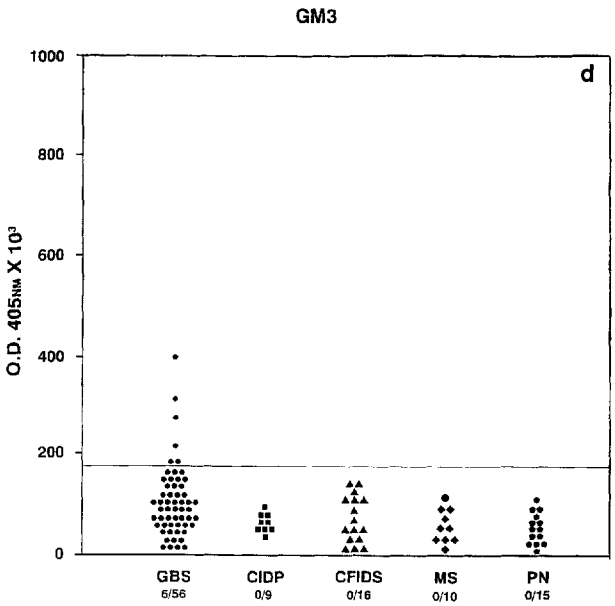
TABLE 6. Comparison of Representative Sera With Multiple Autoantibodies vs. Multiple Antibodies to Infecting Agents

Patient	# Autoantibodies	# Infecting agents
6A	10	1
10	14	12
14	13	11
6B	9	5
8	5	4
32	6	12
18	2	8
3	1	8









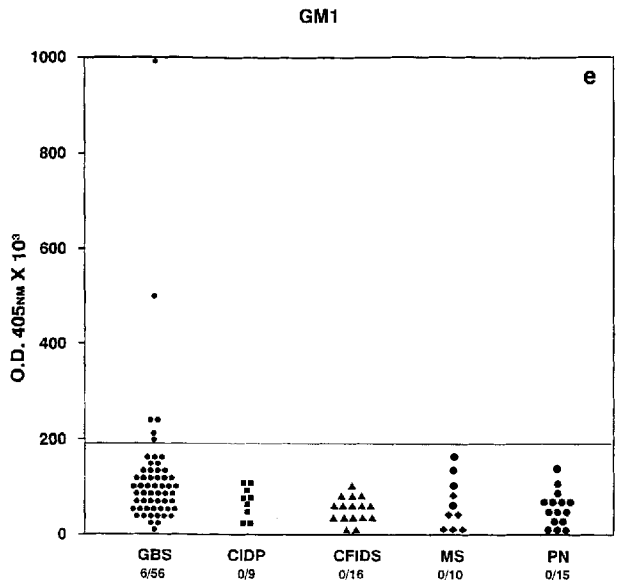


Fig. 2. Titers of antibodies to myelin sheath components as measured by EIA: **a** = sulfatide, **b** = cardiolipin, **c** = GD_{1a}, **d** = GM_3, **e** = GM_1.

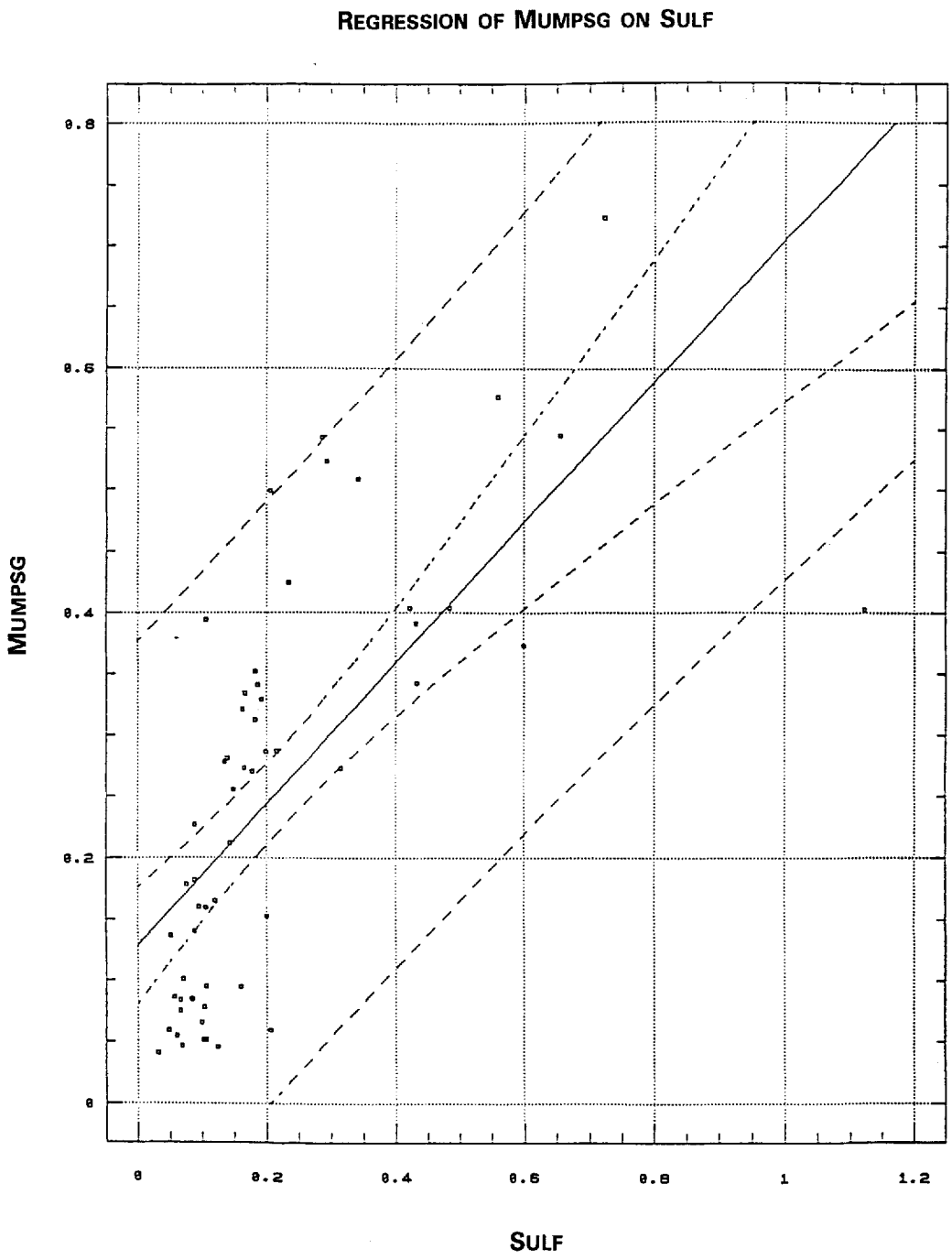


Fig. 3. Regression analysis of mumps IgG (OD405) vs. sulfatide (OD405).

sively used as a diagnostic parameter because they were detected in <50% of the patients. Interestingly, 11% of the patient sera reacted with more than five structurally diverse autoantigens (MBP, gangliosides, and sulfatide). Patterns of glycolipid cross- and/or poly-reactivity are also noted in IgM paraproteinemia associated with polyneuropathy (94) and amyotrophic lateral sclerosis (95). Of all GBS patient sera, 52% reacted with one or more of the myelin antigens; the highest frequencies of reactivity were observed with cardiolipin (48%) and sulfatide (43%). The finding that 100% and 92% of GBS CSF specimens were reactive with GD_{1a} and each of 10 other autoantigens, respectively, indicates that CSF might be a more appropriate specimen for evaluation of GBS and suggests possible sequestration of circulating autoantibodies in the central nervous system (96) and/or intrathecal synthesis of immunoglobulins (97,98). However, most CSF myelin antibody titers were low and the specimens were polyreactive. Elevated CSF myelin antibodies (relative to sera) were previously described in neuroborreliosis, chronic inflammatory demyelinating polyneuropathy, and GBS (131).

GBS sera also showed a wide spectrumof microbe-specific reactivity. Indeed, IgM antibodies were detected against both common as well as infrequent infections in these patients. For example, *C. jejuni*-specific IgM was detected in 20% of patients, whereas IgM antibodies to HSV-1/2, mumps, VZV, HHV-6, CMV, and *M. pneumoniae* were detected in 11–18% of patients. Furthermore, high titers of IgG antibodies were detected in some patients. The high correlation of mumps and sulfatide reactivities, as well as the specificity of mumps reactivity, suggests polyclonal activation of lymphocytes by mumps infection as a pathogenic mechanism rather than true cross-reactivity (99). Further work with immunoaffinity fractionated mumps-positive sera is in progress.

There is currently much discrepancy in the literature concerning the clinical and analytical (i.e., endpoint titration versus concentration [AU/L]) specificity and sensitivity of sulfatide antibodies. Depending on the method of detection, sulfatide antibodies are reported in 5 to >50% of GBS patients (6,100). The prevalence of sulfatide antibodies in other neurologic, immunologic, and autoimmune diseases and healthy controls emphasizes the current uncertainty of the clinical utility of sulfatide antibody detection (85,101). Definitive cohort studies with acute and convalescent specimens are needed to assess whether detectable "natural" sulfatide autoantibodies predispose patients to neuropathy and autoimmune disease and whether low titers progress to elevated and pathological levels. Antibody avidity studies (55) are also required to discriminate between "natural" and pathogenic sulfatide antibodies.

Similar problems of interlaboratory assay standardization, definition of assay cutoffs, and differences in diagnostic categorization as well as patient and control selection criteria are reported for GM_1 antibodies in motor neuron disease (102). However, high titer and/or high activity sulfatide, cardiolipin, and other glycolipid antibodies are usually confirmatory for neuropathy, nervous system inflammation, and neuropsychiatric involvement. High titer sulfatide antibodies (defined to give ≥ 98% clinical specificity; cut-off = 2) were detected in 14% of the GBS patients in this study. The high specificity of CSF GD_{1a} antibodies supports the diagnostic utility of GD_{1a} antibodies in "severe GBS" (103). Further evaluation of the clinical specificity of GD_{1a} antibodies is in progress. The reactivity of the GBS sera to the 18 myelin autoantigens evaluated suggests a unique pattern of myelin-specific antibodies.

Our results suggest a multi-infectious etiology of GBS or an increased susceptibility of GBS patients to infections and illustrate the difficulties in determining whether infection by a specific organism triggers the disease in individual patients. Interestingly, a recent report describes a patient in whom each recurrence of GBS was preceded by different infections (104). The reported predominance of upper respiratory or gastrointestinal tract infections in GBS patients was not confirmed in our studies because pathogens with various tissue specificities were detected in most of the GBS patients evaluated.

Our results indicate that autoantibody screening is potentially useful for early detection of autoimmune disease onset following certain infections, especially mumps, CMV, HSV, *S. pneumoniae*, VZV, adenovirus, *C. jejuni*, and Influenza A/B (Table 6), and that patients with certain autoimmune disorders should be tested for appropriate infectious diseases (105–108). Appropriate autoantigen and infectious agent testing is advised in insulin-dependent diabetes mellitus (mumps, CMV, coxsackieviruses [109,110]), Syndenham's chorea, Chagas disease, and myocarditis (*Trypanosoma cruzi* and CMV [85,111,129]), reactive and rheumatoid arthritis (*Streptococcus, Yersinia*, and *Klebsiella* [48,112,113]) and in multiple sclerosis (measles, rubella, varicella-zoster [MRZ reaction] [135]).

We believe that GBS is both a cellular and humoral autoimmune disease induced by infection with multiple microorganisms and that the presence of microbe-specific antibodies and T cells with cross-reactivity to various nerve-sheath components initiate inflammatory demyelination and shedding of peripheral nerve autoantigens (114). Infection can result in polyclonal expansion and elevation of physiological autoantibody concentration (54,115,116). In the presence of cytokine-induced antigen presenting cells (e.g., Schwann cells) (117), autoantigens are presented to the immune system leading to secondary T-cell mediated activity, which together with loss of tolerance, B-cell proliferation, and autospecific affinity maturation could be responsible for the progressive and sometimes chronic changes and dysfunction in GBS patients (118). Autoantibodies can also cause proliferation of activated T cells in a dose-dependent manner through the idiotype network (119). Tertiary suppressor T-cell responses are noted in spontaneous recovery from

experimental demyelination (120,121), as well as apoptosis of secondary and primary T cells (122), the induction of anti-idiotypic antibodies (123,124), and ganglioside-induced immunosuppression of lymphocytes (136). If instituted early, both plasmapheresis and IVIG can interfere with the progression of the disease and promote recovery, although in some instances plasmapheresis and IVIG are contraindicated (125,126,128). Corticosteroid treatment is effective in some cases (127) and can work synergistically with IVIG (128).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the contributions of Dr. A.R. Bradwell, Dr. Madhumita Patnaik, Dr. Shu-Guang Li, Dr. Ravi Pottathil, Ramin Nekoukar, Jocelyn Mangosing, Mehran Ahrablo, and Susan Pintado. We also thank Dr. Herminio Reyes for a critical review of the manuscript, and Nancy Campman for assistance in manuscript preparation.

REFERENCES

1. Ropper AII: The Guillain-Barré syndrome. *N Engl J Med* 326: 1130–1136, 1992.
2. Ropper AH, Wijdicks EFM, Truax BT, eds: Guillain-Barré syndrome. FA Davis, Philadelphia, 1991, p 18–24.
3. Rostami AM: Pathogenesis of immune mediated neuropathies. *Pediatr Res* 33:590–594, 1993.
4. Lampert PW: Autoimmune and virus-induced demyelinating diseases: A review. *Am J Pathol* 91:176–208, 1978.
5. Svennerholm L, Fredman P: Antibody detection in Guillain-Barré syndrome. *Ann Neurol* 27(suppl):S35–S40, 1990.
6. Fredman P, Vedeler CA, Hyland H, et al.: Antibodies in sera from patients with inflammatory demyelinating polyradiculoneuropathy react with ganglioside LM1 and sulfatide of peripheral nerve myelin. *J Neurol* 238:75–79, 1991.
7. Koski CL: Characterization of complement-fixing antibodies to peripheral nerve myelin in Guillain-Barré syndrome. *Ann Neurol* 27(suppl):544–547, 1990.
8. Yu R, Ariga T, Kohriyama T, Kusunoki S, Maeda Y, Miyatani N: Autoimmune mechanisms in peripheral neuropathies. *Ann Neurol* 27(suppl):S30–S35, 1990.
9. van Doorn PA, Brand A, Vermeulen N: Anti-neuroblastoma cell line antibodies in inflammatory demyelinating polyneuropathy: Inhibition in vitro and in vivo by IV immunoglobulin. *Neurology* 38:1592–1595, 1988.
10. The Guillain-Barré Syndrome Study Group: Plasmapheresis and acute Guillain-Barré syndrome. *Neurology* 335:1096–1104, 1985.
11. Gerken G, Trautmann F, Kohler H, et al.: Rare association of herpes simplex virus IgM specific antibodies and Guillain-Barré syndrome treated with plasma exchange and immunosuppression. *Klin Wochenschr* 63:468–474, 1985.
12. Melnick SC: Role of infection in the Guillain-Barré syndrome. *J Neurol Neurosurg Psychiatry* 27:395–407, 1964.
13. Pepose JS: A theory of virus-induced demyelination in the Landry-Guillain-Barré syndrome. *J Neurol* 227:93–97, 1982.
14. Dowling PC, Cook SD: Role of infection in Guillain-Barré syndrome: Laboratory confirmation of herpesviruses in 41 cases. *Ann Neurol* 9(suppl):44–55, 1981.
15. Menonna J, Goldschmidt B, Haidri N, et al.: Herpes simplex virus IgM-specific antibodies in Guillain-Barré syndrome and encephalitis. *Acta Neurol Scand* 56:223–231, 1977.
16. Smith MS, Laguna JF: Neurologic complications of infectious mononucleosis. *Pediatr Clin North Am* 26:315–326, 1979.
17. Wahren B, Link H: Antibodies to Epstein-Barr virus and cytomegalovirus in Guillain Barré syndrome. *J Neurol Sci* 28:129–138, 1976.
18. Schmitz H, Enders G: Cytomegalovirus as a frequent causeof Guillain-Barré syndrome. *J Med Virol* 1:21–27, 1977.
19. Harada T, Kohriyama T, Ishizaki F, et al.: Guillain-Barré syndrome and disturbance in multiple organs associated with cytomegalovirus infection. *No To ShinKei* 42:245–251, 1990.
20. Merelli E, Sola P, Faglioni P, et al.: Newest human herpesvirus (HHV-6) in the Guillain-Barré syndrome and other neurological diseases. *Acta Neurol Scand* 85:334–336, 1992.
21. Welch RG: Chicken-pox and the Guillain-Barré syndrome. *Arch Dis Child* 37:557–559, 1962.
22. Leeming RD: Varicella-zoster virus and facial palsy. *J Laryngol Otol* 90:365–371, 1976.
23. Lolli F, Fredrikson S, Kam-Hansen S, Link H: Increased reactivity to HTLV-I in inflammatory nervous system diseases. *Ann Neurol* 22:67–71, 1987.
24. Cornblath DR, McArthur JC, Kennedy PG, et al.: Inflammatory demyelinating peripheral neuropathies associated with human T-cell lymphotropic virus III infection. *Ann Neurol* 21:32–40, 1987.
25. Gross FJ, Mindel JS: Pseudotumor cerebri and Guillain-Barré Syndrome associated with human immunodeficiency virus infection. *Neurology* 41:1845–1846, 1991.
26. Dalakas MC, Pezeshkpour GH: Neuromuscular diseases associated with human immunodeficiency virus infection. *Ann Neurol* 23(suppl):S38–48, 1988.
27. Drueke TB, Pujade-Lauraine E, Persson M, et al.: Measles virus and Guillain-Barré syndrome during long-term hemodialysis. *Am J Med* 60:444–446, 1976.
28. Grose C, Spigland I: Guillain Barré syndrome following administration of live measles vaccine. *Am J Med* 60:441–443, 1976.
29. Usui T, Hamada Y, Arita M: A case of Guillain-Barré syndrome associated with Coxsackie B-5 virus infection. *Tokushima J Exp Med* 21:17–19, 1974.
30. Estrada Gonzalez, Mas P: Virological studies in acute polyradiculoneuritis-LGBS type. Various findings in relation to Coxsackie A4 virus. *Neurol Neurocir Psiquiatr* 18(suppl 2)3:527–531, 1977.
31. Gear JH: Nonpolio causes of polio-like paralytic syndromes. *Rev Infect Dis* 6(suppl 2):S379–S384, 1984.
32. Saeed AA, Lange LS: Guillain-Barré syndrome after rubella. *Postgrad Med J* 54:333–334, 1978.
33. Atkins MC, Esmonde TF: Guillain-Barré syndrome associated with rubella. *Postgrad Med J* 67:375–376, 1991.
34. Dussaix E, Lebon P, Ponsot G, et al.: Intrathecal synthesis of different alpha-interferons in patients with various neurologic diseases. *Acta Neurol Scand* 71:504–509, 1985.
35. Seneca H: Influenza: Epidemiology, etiology, immunization and management. *J Am Geriatr Soc* 28:241–250, 1980.
36. Stevens D, Burman D, Clarke SKR, et al.: Temporary paralysis in childhood after influenza B. *Lancet* 2:1354–1355, 1974.
37. Lin SM, Ryu SJ, Liaw YF: Guillain-Barré syndrome associated with acute delta hepatitis virus superinfection. *J Med Virol* 22:144–145, 1989.
38. Tabor E: Guillain-Barré syndrome and other neurologic syndromes in hepatitis A, B, and non-A, and non-B. *J Med Virol* 21:207–216, 1987.
39. Goldschmidt B, Menonna J, Fortunato J, et al.: Mycoplasma antibody in Guillain-Barré syndrome and other neurological disorders. *Ann Neurol* 7:108–112, 1980.
40. Dowling PC, Bosch VV, Cook SD, Chmel H: Serum immunoglobulins in Guillain-Barré syndrome. *J Neurol Sci* 57:435–440, 1982.
41. Garcia-Arenzana JM: Antibodies to Borrelia burgdorferi in Guillain-Barré syndrome. *Lancet* 335:1168, 1990.
42. Boucquey D, Sindic CJ, Lamy M, et al.: Clinical and serological stud-

ies in a series of 45 patients with Guillain-Barré syndrome. *J Neurol Sci* 104:56–63, 1991.
43. Gruenewald R, Ropper AH, Lior H, et al.: Serological evidence of Camphylobacter jejuni and *E. coli* enteritis in patients with Guillain-Barré syndrome. *Arch Neurol* 48:1080–1082, 1991.
44. Kuroki S, Saida T, Nukina M, et al.: Camphylobacter jejuni strains from patients with Guillain-Barré syndrome belong mostly to Penner serogroup 19 and contain β-N-Acetylglucosamine residues. *Ann Neurol* 33:243–247, 1993.
45. Samantray SK, Johnson SC, Mathai KV, et al.: Landry-Guillain-Barré-Strohl syndrome: A study of 302 cases. *Med J Aust* 2:84–91,1977.
46. Zimprich F, Winter J, Wege H, Lassmann H: Coronavirus induced primary demyelination: Indications for the involvement of a humoral immune response. *Neuropathol Appl Neurobiol* 17:469–484, 1991.
47. Roman G, Phillips CA, Poser CM: Parainfluenza virus type 3: isolation from CSF in a patient with Guillain Barré-syndrome. *JAMA* 240:1613–1615, 1978.
48. Bronze MS, Dale JB: Epitopes of streptococcal M proteins that evoke antibodies that cross-react with human brain. *J Immunol* 151: 2820–2828, 1993.
49. Chopra A, Rana PV, Narayanaswamy AS, et al.: Neurological complications following acute viral conjunctivitis: A new profile. *Trop Geogr Med* 38:197–202, 1986.
50. Winer JB, Hughes RA, Anderson MJ, et al.: A prospective study of acute idiopathic neuropathy. II Antecedent Events. *J Neurol Neurosurg Psychiatry* 51:616–618, 1988.
51. Serratrice G: Polyneuritis, polyradiculoneuritis, polyneuro-pathies: Development of a concept. *Rev Prat* 42:9–17, 1992.
52. Weise MF, Canegie RR: An approach to search protein sequences for superfamily relationships or chance similarities relevant to the molecular mimicry hypothesis: Application to the basic proteins of myelin. *J Neurochem* 51:1267–1273, 1988.
53. Plotz PH: Autoantibodies are anti-idiotype antibodies to antiviral antibodies. *Lancet* 2:824–826, 1983.
54. Rose NR, Bona C: Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol Today* 14:426–430, 1993.
55. Ternynck T, Avrameas S: Murine natural monoclonal autoantibodies: A study of their polyspecificities and their affinities. *Immunol Rev* 94:99–112, 1986.
56. Nina Y, Sakane T, Kanoh T, et al.: Transient autoantibodies with elevated complement levels in common viral diseases. *J Clin Lab Immunol* 13:183–188, 1984.
57. Mithen FA, Ilyas AA, Birchem R, Cook SD: Effectsof Guillain-Barré sera containing antibodies against glycolipids in cultures of rat Schwann cells and sensory neurons. *J Neurol Sci* 112:223–232, 1992.
58. Brinkmeier H, Wollinsky KH, Hulser PJ, et al.: The acute paralysis in Guillain-Barré syndrome is related to the Na^+ channel blocking factor in the cerebrospinal fluid. *Pflugers Arch* 421:552–557, 1992.
59. Sawant-Mane S, Clark MB, Koski CL: In vitro demyelination by serum antibody from patients with Guillain-Barré syndrome requires terminal complement complexes. *Ann Neurol* 29:397–404, 1991.
60. Ilyas AA, Mithen FA, Chen IW, Cook SD: Anti-GM_1 IgA antibodies in Guillain-Barré syndrome. *J Neuroimmunol* 36:69–76, 1992.
61. Simone IL, Annunziata P, Majnone D, Liguori M, Leante R, Livrea P: Serum and CSF anti-GM1 antibodies in patients with Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy. *J Neurol Sci* 114:49–55, 1993.
62. Gregson NA, Kollar S, Hughes RAC: Antibodies to gangliosides in Guillain-Barré syndrome: specificity and relationship to clinical features. *Quart J Med* 86:111–117, 1993.
63. Gregson NA, Jones D, Thomas PR, Willison HJ: Acute motor neuropathy with antibodies to GM1 ganglioside. *J Neurol* 238:447–451, 1991.
64. Chiba A, Kusunoki S, Obata H, et al.: Serum anti-GQ1b antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain Barré syndrome: Clinical and immunohistochemical studies. *Neurology* 43:1911–1917, 1993.
65. Willison HJ, Veitch J, Paterson G, Kennedy PGE: Miller Fisher syndrome is associated with serum antibodies with GQ_{1b} gangliosides. *J Neurol Neurosurg Psychiatry* 56:204–206, 1993.
66. Ilyas AA, Mithen FA, Dalakas MC, et al.: Antibodies to acidic glycolipids in Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy. *J Neurol Sci* 107:111–121, 1992.
67. Enders U, Karch H, Toyka KV, et al.: The spectrum of immune responses to Campylobacter jejuni and glycoconjugates in Guillain-Barré syndrome and in other neuroimmunological disorders. *Ann Neurol* 34:136–144, 1993.
68. Winer JB, Gray IA, Gregson NA, et al.: A prospective study of acute idiopathic neuropathy. III Immunological studies. *J Neurol Neurosurg Psychiatry* 51:619–625, 1988.
69. Ilyas AA, Mithen FA, Dalakas MC, et al.: Antibodies to sulfated glycolipids in Guillain-Barré syndrome. *J Neurol Sci* 105:108–117, 1991.
70. van den Berg LH, Marrik J, de Jager AFJ, Van Inshoff GW, Oator N, Sadig SA: Anti-GM1 antibodies in patients with Guillain-Barré syndrome. *J Neurol Neurosurg Psychiatry* 55:8–11, 1992.
71. Koski CL, Chou DKH, Jungalwala FB: Anti-peripheral nerve myelin antibodies in Guillain-Barré syndrome bind a neutral glycolipid of peripheral myelin and cross-react with Forssman antigen. *J Clin Invest* 84:280–287, 1989.
72. Quarles RH, Ilyas AA, Willison HJ: Antibodies to gangliosides and myelin proteins in Guillain-Barré Syndrome. *Ann Neurol* 27(suppl): S48–S58, 1990.
73. Piddlesden SJ, Lassmann H, Zimprich F, et al.: The demyelinating potential of antibodies to myelin oligodendrocyte glycoprotein is related to their ability to fix complement. *Am J Pathol* 143:555–564, 1993.
74. Zweiman B, Rostami A, Lisak R, et al.: Immune reactions to P2 protein in human demyelinative neuropathies. *Neurology* 33:234–237, 1983.
75. Khalili-Shirazi A, Atkinson P, Gregson N, Hughes RAC: Antibody responses to P0 and P2 myelin proteins in Guillain-Barré syndrome and chronic idiopathic demyelinating polyradiculo-neuropathy. *J Immunol* 46:245–252, 1993.
76. Wang WZ, Olsson T, Kostulas V, et al.: Myelin antigen reactive T cells in cerebrovascular disease. *Clin Exp Immunol* 88:157–162, 1992.
77. Gilburd B, Stein M, Tomer Y, et al.: Autoantibodies to phospholipids and brain extract in patients with the Guillain-Barré syndrome: Cross-reactive or pathogenic. *Autoimmunity* 16:23–27,1993.
78. Frampton G, Winer JB, Cameron JS, Hughes RA: Severe Guillain Barré syndrome: An association with IgA anti-cardiolipin antibody in a series of 92 patients. *J Neuroimmunol* 19:133–139, 1988.
79. Connolly AM, Pestronk A, Trotter JL, Fellman EL, Cornblath DR, Olney RK: High titer selective serum anti-β tubulin antibodies in chronic inflammatory demyelinating polyneuropathy. *Neurology* 43:557–562, 1993.
80. Mokuno K, Yasuda T, Sugimura K, et al.: Cerebrospinal fluid neuron-specific enolase (NSE) and S-100b protein in Guillain-Barré syndrome—Their relations to prognosis. *Rinsho Shinkeigaku (Japan)* 32:535–537, 1992.
81. Chalk CH, Homburger HA, Dyck PJ: Anti-neutrophil cytoplasmic antibodies in vasculitic peripheral neuropathy. *Neurology* 43: 1826–1827, 1993.
82. Feutren G, Gerbal JL, Allinquant B, Schuller E: Association of Guillain Barré syndrome and B-virus hepatitis: Simultaneous presence of anti-DS-DNA antibodies and HBs antigen in cerebrospinal fluid. *J Clin Lab Immunol* 11:161–164, 1983.
83. Olbricht CJ, Stark E, Helmchen U, et al.: Glomerulonephritis associ-

ated with inflammatory demyelinating polyradiculoneuropathy: A case report and review of the literature. *Nephron* 64:139–141, 1993.
84. Costallat LTL, de Oliveira RM, Santiago MB, et al.: Neuropsychiatric manifestations of systemic lupus erythematosis: The value of anticardiolipin, antigangliosides and antigalactocerebrosides antibodies. *Clin Rheumatol* 9:489–497, 1990.
85. Avila JL, Rojas M, Carrasco H: Elevated levels of antibodies against sulfatide are present in all chronic chagasic and dilated cardiomyopathy sera. *Clin Exp Immunol* 92:460–465, 1993.
86. Zanone MM, Peakman M, Purewal T, et al.: Autoantibodies to nervous tissue structures are associated with neuropathy in Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 36:564–569, 1993.
87. Khalili A, Cooper RC: A study of immune responses to myelin and cardiolipin in patients with systemic lupus erythematosus (SLE). *Clin Exp Immunol* 85:365–372, 1991.
88. McFarlane I: Autoimmunity and hepatotropic viruses. *Semin Liver Dis* 11:223–233, 1991.
89. Adelmann M, Linington C: Molecular mimicry and the autoimmune response to the peripheral nerve myelin PO glycoprotein. *Neurochem Res* 17:887–881, 1992.
90. Rothbard JB, Taylor WR: A sequence pattern common to T cell epitopes. *EMBO J* 7:93–100, 1988.
91. Fujinami RS, Oldstone MBA: Amino acid homology between the encephalitogenic site of myelin basic protein and virus: Mechanism for autoimmunity. *Science* 230:1043–1045, 1985.
92. Shoenfeld Y, Isenberg D, eds: The Mosaic of Autoimmunity. Elsevier, Amsterdam, 1989, pp. 343–387.
93. Hojeberg B, Ingemarsson R, Kristensson K, et al.: A monoclonal antibody against HSV type 1 ribonucleotide reductase cross reacts with the P0 protein of peripheral nerve myelin. *J Neurol Sci* 106:91–95,1991.
94. Arai M, Yoshino H, Kusano Y, et al.: Ataxic polyneuropathy and anti-Pr2 IgMκ M proteinemia. *J Neurol* 106:91–95, 1991.
95. Stevens A, Weller M, Wietholter H: A characteristic ganglioside antibody pattern in the CSF of patients with amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 56:361–364, 1993.
96. Teplizki H, Buskila D, Argov S, et al.: Low serum antimycobacterial glycolipid antibody titers in patients with systemic lupus erythematosus associated with central nervous system involvement. *J Rheumatol* 14:507–511, 1987.
97. McLean BN, Luxton RW, Thompson EJ: A study of immunoglobulin G in the cerebrospinal fluid of 1007 patients with suspected neurological disease using isoelectric focusing and the log IgG-index. *Brain* 113:1269, 1990.
98. Link H, Wahren B, Norrby E: Pleocytosis and immunoglobulin changes in cerebrospinal fluid and herpes-virus serology in patients with Guillain Barré syndrome. *J Clin Microbiol* 9:305–316, 1979.
99. Ratzmann KP: Autoimmunity and the development of diabetes mellitus in relation to mumps infection. *Diabetologia* 29:673–674, 1986.
100. van den Berg LH, Lankamp CLAM, deJager AEJ, et al.: Anti-sulphatide antibodies in peripheral neuropathy. *J Neurol Neurosurg Psychiatry* 56:1164–1168, 1993.
101. Alosachie I, Shoenfeld Y, Mevorach D, et al.: Central nervous system (CNS) involvement in SLE: The diagnostic role of antibodies to neuronal antigens. *Arthritis Rheum* submitted, 1993.
102. Willison HJ, Kennedy PGE: Gangliosides and bacterial toxins in Guillain-Barré Syndrome. *J Neuroimmunol* 46:105–112, 1993.
103. Yuki N, Yamada M, Sato S, et al.: Association of IgG anti-GD1a antibody with severe Guillain-Barré syndrome. *Muscle Nerve* 16:642–647, 1993.
104. Hayashi H, Park-Matsumoto YC, Yuki N, Itoh T, Amakawa T, Muneyuki T: A case of current Guillain Barré syndrome preceded by different infections. *J Neurol* 240 (3):196–197, 1993.
105. Barthalomaeus WN, O'Donoghue H, Foti D, et al.: Multiple autoantibodies following cytomegalovirus infection: Virus distribution and specificity of autoantibodies. *Immunology* 64:397–405, 1988.
106. Hendricksc MT, Triger DR: Autonomic and peripheral neuropathy in primary biliary cirrhosis. *J Hepatol* 19:401–407, 1993.
107. Schattner A, Rager Z: Virus-induced autoimmunity. *Rev Infect Dis* 12:204, 1990.
108. Sharma KR, Sriram S, Fries T, et al.: Lumbosacral radiculoplexopathy as a manifestation of Epstein-Barr virus infection. *Neurology* 43:2550–2554, 1993.
109. Ratzmann KP, Strese J, Rjasanowski I, et al.: Metabolic, hormonal and immunological alterations in subjects with antecedent mumps infections. *Exp Clin Endocrinol* 86:323–334, 1985.
110. Nicoletti F, Scalia G, Lunetta M, et al.: Correlation between islet cell antibodies and anti-cytomegalovirus IgM and IgG antibodies in healthy first degree relatives of type I (insulin-dependent) diabetic patients. *Clin Immunol Immunopathol* 55:139–147, 1990.
111. Price P, Olver SD, Gibbons AE, Shellam GR: B-cell activation following murine cytomegalovirus infection: implications for autoimmunity. *Immunology* 78:14–21, 1993.
112. Hmama Z, Lina G, Normier G, et al.: Role of acyl residues in polyclonal murine B cell activation by acylpoly (1,3) galactosides form *Klebsiella pneumoniae*. *J Immunol* 151:5440–5449, 1993.
113. Toivanen A, Lahesmaa-Rantala R, Vuento R, Granford K: Association of persisting IgA response with yersinia triggered reactive arthritis: A study of 104 patients. *Ann Rheum Dis* 46: 898–901, 1987.
114. Abu-Shakra M, Shoenfeld Y: Natural Autoantibodies. Shoenfeld Y, Isenberg D, eds. CRC Press, Boca Raton, 1993, p 15–33.
115. Sun J-B: Autoreactive T and B cells in nervous system diseases. *Acta Neurol Scand* 87(S143):5–55, 1993.
116. Jones BM, Cheng KLP, Wong RWS, Kung AWC: CD5-positive and CD5-negative Rheumatoid factor-secreting B cells in IgA nephropathy, rheumatoid arthritis and Grave's disease. *Scand J Immunol* 38:575–580, 1993.
117. Wekerle H, Schwab M, Linington C, Meyerman R: Antigen presentation in the peripheral nervous system: Schwann cells present endogenous myelin autoantigens to lymphocytes. *Eur J Immunol* 16:1551–1557, 1986.
118. Simone IL, Annunziata P, Maimone D, et al.: Serum and CSF anti-GM1 antibodies in patients with Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy. *J Neurol Sci* 114:49–55, 1993.
119. Yi Q, Bergenbrant S, Osterborg A, et al.: T cell stimulation induced by idiotypes on monoclonal immunoglobulins in patients with monoclonal gammopathies. *Scand J Immunol* 38:529–534, 1993.
120. Khalili-Shirazi A, Hughes RAC, Brostoff SW, et al.: T cell responses to myelin proteins in Guillain-Barré syndrome. *J Neurol Sci* 111:200–203, 1992.
121. Kumar V, Sercarz EE: The involvement of T cell receptor peptide specific regulatory CD4+ T cells in recovery from antigen-induced autoimmune disease. *J Exp Med* 178:909–916, 1993.
122. Schmied M, Breitschopf H, Gold R, et al.: Apoptosis of T lymphocytes in experimental autoimmune-encephalomyelitis. *Am J Pathol* 143:446–452, 1993.
123. Lundkuist I, van Doorn PA, Vermeulen M, et al.: Spontaneous recovery from Guillain-Barré syndrome is associated with anti-idiotypic antibodies recognizing a cross-reactive idiotype on anti-neuroblastoma cell line antibodies. *Clin Immunol Immunopathol* 67:192–198, 1993.
124. Sutjita M, Hohmann A, Comacchio R, et al.: A common anti-cardiolipin antibody idiotype in autoimmune disease: Identification using a mouse monoclonal antibody directed against a naturally-occurring anti-phospholipid antibody. *Clin Exp Immunol* 75:211–216, 1989.
125. Castro LH, Ropper AH: Human immune globulin infusion in Guillain-Barré syndrome: Worsening during and after treatment. *Neurology* 43:1034–1036, 1993.
126. Ross MA, Silbert P, Knezevic W, et al.: Immunoglobulins and stroke. *Neurology* 42:1847–1848, 1992.
127. Guillain-Barré Syndrome Steroid Trial Group: Double-blind trial of

intravenous methylprednisolone in Guillain-Barré syndrome. *Lancet* 341:586–590, 1993.

128. van der Meche FGA, Ng KKP, Bleck TP, et al.: Intravenous immunoglobulin versus plasma exchange in Guillain Barré syndrome. *Neurology* 43:2729–2731, 1993.
129. Gea S, Ordonez P, Cerban F, et al.: Chagas' disease cardioneuropathy: association of anti-trypanosoma cruzi and anti-sciatic nerve antibodies. *Am J Trop Med Hyg* 49:581–588, 1993.
130. Nitsche A, Leiguarda RC, Maldonado JA, et al.: Neurological features in overlap syndrome. *Clin Rheumatol* 10:5–9, 1991.
131. Weller M, Stevens A, Sommer N, Witholter H: Are CSF or serum ganglioside antibodies related to peripheral nerve demyelination in neuroborreliosis, Guillain-Barré syndrome or chronic inflammatory demyelinating polyradiculoneuropathy? *Eur Arch Psych Clin Neurosci* 242:122–126, 1992.
132. Quarles RH, Barbarash GR, Figlewicz DA, McIntyre LJ: Purification and partial characterization of the myelin associated glycoprotein from adult rat brain. *Biochim Biophys Acta* 757:140–143, 1983.
133. Giegerich G, Pette M, Wekerle H, et al.: Rapid method based on RP-HPLC for purification of human myelin basic protein and its thrombic and endoproteinase Lys-C peptides. *J Chromatog* 528:79–90, 1990.
134. Ariga T, Kohriyama T, Freddo L, et al.: Characterization of sulfated glucuronic acid-containing glycolipids reacting with IgM M-proteins in patients with neuropathy. *J Biol Chem* 262:848–853, 1987.
135. Felgenhauer K, Reiber H: The diagnostic significance of antibody specificity indices in multiple sclerosis and herpesvirus-induced diseases of the nervous system. *Clin Invest* 70:28–37, 1992.
136. Ladisch S, Becker H, and Ulsh L: Immunosuppression by human gangliosides: I. Relationship of carbohydrate structure to the inhibition of T cell responses. *Biochim Biophys Acta* 1125:180–188, 1992.
137. National Institute of Neurological Communicative Disease and Stroke, Ad Hoc Committee: Diagnostic Criteria for GBS. *Ann Neurol* 3:565–566, 1978.
138. National Institute of Neurological Disease and Stroke, Guillain-Barré Syndrome, Publication #922902, 1992.
139. Magi S, Sabatelli M, Mignogna T, et al.: Acute axonal idiopathic polyneuropathy: a Guillain-Barré Syndrome variant? *Ital J Neurol Sci* 13:481–486, 1992.