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Pleocytosis and Immunoglobulin Changes in Cerebrospinal Fluid and Herpesvirus Serology in Patients with Guillain-Barré Syndrome

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In a follow-up study of 24 patients with Guillain-Barré syndrome, 55% developed a cerebrospinal fluid (CSF) mononuclear pleocytosis, which persisted for 4 months or more. Raised index values of CSF-immunoglobulin G (IgG), CSF-IgA, and CSF-IgM, indicating synthesis of the immunoglobulin in question in the central nervous system, were found in 63, 35, and 25%, respectively. Agarose gel electrophoresis revealed oligoclonal immunoglobulin in CSF in 21% and in both CSF and serum in another 21% of the patients. Twenty-five percent had abnormally low kappa/lambda ratios of CSF and/or serum, indicating synthesis of oligoclonal immunoglobulin, mainly of the lambda light-chain type. The inflammatory reaction in the central nervous system, as reflected by pleocytosis, immunoglobulin synthesis, and oligoclonal immunoglobulin, was not correlated to the severity or course of Guillain-Barré syndrome. A raised CSF-IgM index and oligoclonal immunoglobulin were found more often in the Guillain-Barré syndrome patients who displayed pleocytosis. All patients had or developed antibodies to Epstein-Barr virus. Three patients had serology indicating a primary infection, 11 patients had antibody changes indicating a reactivated infection, and 10 had serology indicating previous exposure. Two patients showed serological evidence for a primary cytomegalovirus infection, 2 had serology indicative of a reactivated infection, 12 had titers as caused by previous exposure, and 8 remained seronegative. Virus-specific IgM was measurable in all cases of primary infection. Neither primary or reactivated Epstein-Barr virus nor cytomegalovirus infections were obviously related to CSF pleocytosis.

The etiology of the Guillain-Barré syndrome (GBS) is largely unknown. It occurs after immunization with, e.g., inactivated influenza vaccine (3) or after acute viral infections caused by, e.g., cytomegalovirus (CMV) (9, 11, 26, 29) or Epstein-Barr virus (EBV) (7). After herpes simplex or zoster virus infections it seems to be much less frequent (2, 10, 38). Since symptoms of GBS may appear after an interval of around 2 weeks, it has been proposed that the disease may be due to an immune reaction. Elevated immunoglobulin levels in cerebrospinal fluid (CSF), probably due to immunoglobulin synthesis within the central nervous system (CNS), and oligoclonal immunoglobulin in both CSF and serum have been demonstrated in this disease (13).

Herpesviruses may be reactivated during various diseases, and it has been pointed out that one may detect not only acute but also reactivated herpesvirus infections during GBS (37). Nothing is yet known about how a reactivated herpesvirus infection may affect the pathogenesis of GBS.

Our repeated CSF and serum sampling has yielded evidence of recent and reactivated CMV and EBV infections, CSF pleocytosis of long duration, and oligoclonal immunoglobulin synthesis within the CNS. In some cases immunoglobulin M (IgM) with specificity for CMV or EBV has been demonstrated in both serum and CSF, indicating primary infection.

MATERIALS AND METHODS

Materials. CSF and serum samples were obtained from 24 consecutive patients with GBS at the Department of Neurology, University Hospital, Linköping. The diagnostic criteria proposed by Masucci and Kurtzke (22) were used. Specimens were taken repeatedly during the course of disease from each patient. Slight symptoms indicate ambulatory patients, moderate symptoms indicate that the patient could

not walk, and severe symptoms indicate that ventilatory support was required. The present investigations were carried out on the first of one or more specimens obtained up to 10 days after onset of neurological disease (specimen I), 11 days to 1 month after onset (specimen II), 1 to 2 months after onset (specimen III), 2 to 4 months after onset (specimen IV), and later than 4 months after onset (specimen V).

Cell counts and determinations of total protein, IgG, and albumin were carried out immediately after lumbar puncture on all CSF specimens. After cell counting by phase-contrast microscopy, CSF cells were sedimented by centrifugation. The erythrocyte count in CSF did not exceed 100×10^6 cells per liter. Immunoglobulin and albumin were determined simultaneously in CSF and serum, and the corresponding CSF-immunoglobulin index (see below) was determined.

A portion of the CSF was concentrated by ultrafiltration in collodium bags (Sartorius Membranfilter, Göttingen, West Germany) at 4°C to an IgG concentration of about 3 g/liter and then analyzed by agarose gel electrophoresis in parallel with serum. An aliquot of each specimen was stored at −20°C. Specimens from individual patients were then investigated simultaneously for IgA, IgM, kappa and lambda light chains, and viral antibodies.

Methods. Immunochemistry. The total protein concentration of CSF was determined according to Lowry et al. (21), and the age-dependent normal values calculated by Tibbling et al. (33) were adopted (Table 1).

Determinations of albumin, IgG, IgA, and kappa and lambda light chains were carried out on unconcentrated CSF and on serum by an automatic immunoprecipitation technique utilizing nephelometric analyses of antigen-antibody complexes in a continuous-flow system (Auto-Analyzer II, Technicon, New York, N.Y.). IgM was determined by a modified single-radial immunodiffusion technique (20). Antisera to albumin, IgG and IgA heavy chains, and kappa and lambda light chains were purchased from Dakopatts (Copenhagen, Denmark), and that to IgM heavy chains was from Organon (Oss, Holland).

The reference values for albumin and IgG, IgA, and IgM are given in Table 1. These values refer to a material defined according to Tibbling et al. (33), based on 56 individuals with functional neurological disorders but with normal findings at clinical examination (5). The CSF/serum albumin ratio, which is claimed to be a better measure of the blood-brain barrier function than the total protein concentration of CSF (17), increases with age (Table 1), the upper normal values being 5.7 at 30 years and 9.0 at 60 years.

TABLE 1. *Normal ranges for CSF and serum proteins (mean value ± 2 standard deviations)*

Protein	CSF (mg/liter)	Serum (g/liter)	CSF/serum ratio	Index[a]
Total protein[b]	<493–667			
Albumin[b]	<262–394	38–49	<5.7–9.0	
IgG[c]	3.8–57.8	8.4–14.8		0.32–0.70
IgA[c]	0.15–6.7	0.5–3.44		0.12–0.62
IgM[c]	<2	0.1–2.5		≤1.0

[a] For explanation, see text.

[b] The normal values are age dependent and refer to a material of 56 individuals with functional neurological disorders (33); see text.

[c] Reference values; see text and reference 33.

The IgG level in CSF was calculated as the CSF-IgG index by the formula: (CSF-IgG/serum-IgG)/(CSF-albumin/serum-albumin) (33). This index takes into account the influence of the IgG level in serum as well as the occurrence of blood-brain barrier damage. The CSF-IgA index was calculated in a similar way. Only abnormally high index values, >0.70 for the CSF-IgG index and >0.62 for the CSF-IgA index, were taken into account. As the lower limit of IgM detection with the method used was 2 mg/liter, IgM was not demonstrable in normal unconcentrated CSF. Although a normal range for the CSF-IgM index has not been defined as accurately as for the other immunoglobulins, >0.7 should be abnormal, even when allowance is made for the age variation of albumin ratios. However, we regarded only values >1.0 as abnormal.

The kappa/lambda ratio (19) was determined. The normal range in our laboratory is 0.7 to 1.7 for CSF and 0.7 to 1.3 for serum.

Agarose gel electrophoresis was carried out as described previously (12). The occurrence of one or more homogeneous bands in the gamma globulin region in addition to those normally present was considered abnormal and evidence for the presence of oligoclonal immunoglobulins.

Viral serology. Antibodies to EBV viral capsid antigen were determined by indirect immunofluorescence (31, 32, 36), using P3H3-1 Burkitt lymphoma cells. Twofold dilutions of serum or CSF were applied for 30 min at 37°C for IgG staining or for 4 to 18 h at room temperature for IgM staining. After rinsing, fluorescein isothiocyanate-conjugated sheep anti-human IgG (National Bacteriological Laboratory, Stockholm, Sweden) or anti-human IgM (Dakopatts) was applied for 30 min. Antibodies to EBV nuclear antigen was determined by anticomplement immunofluorescence according to Reedman and Klein (28), using EBV-carrying but -nonproducing NC37 cells, human EBV antibody-negative serum as a source of complement, and goat anti-human β1C/β1A (Hyland Laboratories, Costa Mesa, Calif.). Antibodies to CMV were determined by indirect immunofluorescence using CMV strain Ad.169-infected human lung fibroblasts (30, 36).

To verify the presence of IgM antibodies, 5 to 30% sucrose velocity gradients were performed, and fractions containing maximum IgM and IgG, respectively, were used for both IgG and IgM immunofluorescence. The quantity of IgG and IgM within the gradient was determined with immunodiffusion plates (Tripartigen IgG and Tripartigen IgM, Behringwerke, Marburg-Lahn, West Germany). The heterophile antibody test was performed with undiluted sera or CSF and a sensitive Monospot assay with differential absorption (Johnson and Johnson, Malmö, Sweden). The presence of rheumatoid factor (RF) of IgM anti-IgG specificity was assayed by using latex beads coated with human IgG (Behringwerke) with serum dilutions of 1:

2 and 1:20 or undiluted CSF. This allowed a sensitivity comparable to that found in immunofluorescence, using RF as second antibody (our unpublished data). Using the latex beads, IgM anti-IgG can be absorbed without loss of antiviral IgM antibody (unpublished data).

To classify a viral infection as of recent origin, we required both a fourfold IgG antibody titer change and a titer of IgM antibodies ≥160. As evidence for a reactivated virus infection, two of the following three criteria were required: a fourfold titer change, IgG titers of ≥160, or IgM antibodies in a titer of 20 to 80. It is recognized that lower IgM titers may also signify primary or reactivated infections. However, CMV-IgM may persist for several months, and therefore these relatively strict criteria were used.

Antibodies to measles, rubella, and adeno- and polioviruses were determined to study the patient's general immune competence and to characterize possible blood-brain barrier damage. Measles virus hemagglutination-inhibiting and hemolysin-inhibiting antibody titers, rubella virus hemagglutination-inhibiting antibody titers, group-specific adenovirus penton hemagglutination enhancement titers, and, in some instances, neutralization enhancement tests with poliovirus type 1 were determined as described (25).

RESULTS

Clinical findings. Thirteen of the 24 patients were males; 11 were females. The patients were 20 to 74 years of age. Only five patients were less than 40 years of age. All had been in good health before the onset of GBS, except one patient who had rheumatoid arthritis. They had no history of blood transfusions or vaccinations in the 4 months before disease.

In 9 of the 24 patients, upper respiratory symptoms preceded the onset of neurological symptoms. Eight had respiratory symptoms within 14 days before onset, and one had upper respiratory distress 2 months before. One additional patient had gastroenteritis and another had urinary infection. One patient developed a myocarditis (no. 3) and another developed an HBs antigen-negative hepatitis (no. 4) during GBS.

Table 2 shows the course of GBS in terms of severity and the duration of neurological symptoms. The prognosis in all our cases was good. The mean observation time was 14 months. Twenty patients became healthy and four patients improved during the period of observation.

Mononuclear pleocytosis. In Table 3 the patients are grouped according to the presence or absence of mononuclear pleocytosis in CSF. Thirteen of the 24 patients had pleocytosis; in seven patients this exceeded 50 cells per mm^3.

Figure 1 gives the frequency of pleocytosis in specimens I through V, which were taken at different intervals after onset of neurological symptoms. About half of the patients displayed a pleocytosis in specimen IV, i.e., 2 to 4 months after onset, and three of these patients had more than 50 cells per mm^3 on this occasion. There was no apparent relation of pleocytosis to duration of symptoms (Tables 2 and 3).

TABLE 2. *Clinical findings in 24 patients with GBS*[a]

Pleocytosis	Patient no.	Age (yr)	Infections preceding neurology by up to 2 months	Severity of neurological symptoms	Durations of neurological symptoms (mo)
CSF	1	31	+	Moderate	10
	4	64	−	Severe	14
	5	63	−	Moderate	7
	10	29	−	Slight	3
	12	72	−	Moderate	8
	13	69	−	Moderate	4
	16	77	−	Moderate	>16
	17	74	−	Moderate	4
	20	65	−	Severe	5
	22	41	+	Slight	1
	23	71	−	Moderate	2
	24	62	+	Moderate	7
	25	20	+	Moderate	2
None	2	28	+	Severe	5
	3	59	+	Severe	12
	6	17	−	Moderate	3
	9	74	+	Moderate	12
	11	57	−	Severe	20
	14	60	+	Moderate	1
	18	45	−	Severe	>2
	19	64	+	Moderate	6
	21	51	+	Moderate	1
	26	57	+	Severe	8
	27	77	−	Moderate	6

[a] Patients are arranged according to presence or absence of pleocytosis.

Blood-brain barrier damage. All but two of the patients displayed evidence of blood-brain barrier damage as documented by elevated CSF/serum albumin ratios (Table 3). In the two cases where this ratio was normal, the first determinations were carried out 0.5 and 1.5 months, respectively, after onset of the disease, and one cannot rule out the possibility that blood-brain barrier damage occurred earlier than this.

Figure 1 shows the frequency of abnormal CSF/serum albumin ratios during the course of the disease. Persistent blood-brain barrier damage was documented in specimen V, i.e., 4 months or later after onset of GBS, in six cases

TABLE 3. *Pleocytosis, barrier damage, and immunoglobulins in CSF of GBS patients*[a]

Patient no.	Maximum no. of mononuclear cells per mm^3 in CSF	Duration of barrier damage (mo)	Oligoclonal immunoglobulin: bands on electrophoresis, presence and duration (mo)	Highest CSF/serum albumin ratio (ref. <5.7–9.0)	Highest index	
					CSF-IgG (ref. <0.70)	CSF-IgA (ref. <0.62)
1	26	>9	0	26.9	0.92	0.54
4	821	1	CSF and serum (2)	22.3	0.76	0.68
5	306	2	CSF (>13)	23.6	1.85	1.73
10	16	>5	0	34.0	0.63	0.45
12	201	2	CSF and serum (>6)	23.8	0.97	1.04
13	84	5	CSF (>6)	29.7	1.51	1.03
16	136	>3	3 in CSF, 1 in serum (>3)	45.9	1.22	0.62
17	618	>3	CSF	26.1	1.99	1.20
20	12	>2	CSF and serum (1.5)	15.9	0.80	0.41
22	11	>0.5	0	15.2	0.58	0.47
23	77	>1.2	CSF (>1)	34.7	0.74	0.35
24	8	3.5	0	13.0	0.60	0.42
25	11	>1	CSF and serum (0.5)	28.3	0.62	0.60
Frequency of abnormal values			9/13	13/13	9/13	5/13
2	4	3	0	83.3	0.78	ND[b]
3	5	1	0	19.4	0.68	0.49
6	5	28	0	25.0	0.81	0.60
9	2	<1.5	CSF (>7)	8.1 (normal)	1.18	0.63
11	5	4	0	54.1	0.86	0.66
14	2	>0.5	0	35.0	0.66	0.48
18	3	>2	0	9.3	0.87	0.35
19	3	1	0	26.0	0.69	0.43
21	2	<0.5	0	3.2 (normal)	0.75	0.41
26	2	>2.5	0	76.7	0.66	0.65
27	2	>0.5	0	16.2	0.54	0.48
Frequency of abnormal values			1/11	9/11	6/11	3/10

[a] Patients are grouped according to presence of pleocytosis.
[b] ND, Not done.

(Fig. 1). Normalization of the blood-brain barrier was observed in 10 of the 22 patients who had demonstrable barrier damage, in one of them as late as 28 months after onset of disease. In the remaining 12 cases, no CSF specimens were available for additional follow-up. Determination of viral antibodies to measles virus, rubella virus, and adenovirus confirmed the existence of barrier damage and its duration. Neutralization tests with poliovirus type 1 in cases where measles virus or adenovirus antibody titers were too low for evaluation also agreed with albumin ratios.

In performing the reference viral antibody determinations it was noted that patients 1, 5, and 25 had high serum measles hemagglutination inhibition titers, ≥2,560. These titers persisted throughout the course of GBS.

CSF and serum immunoglobulins. An elevated CSF-immunoglobulin index may be used to indicate a synthesis within the CNS of the immunoglobulin in question, independently of the occurrence of blood-brain barrier damage (12). The CSF-IgG index was elevated in 15 of the 24 patients, and the CSF-IgA index was elevated in 8 patients (Table 3). We have considered CSF-IgM index values above 1.0 to be definitely abnormal. Values above 1.0 were found in six of the patients (Table 4).

Figure 2 shows the frequency of elevated CSF-IgG, CSF-IgA, and CSF-IgM index values during the course of disease. Elevated CSF-IgG index values were found in about half of the no. IV specimens. Elevated CSF-IgA index values were found at similar frequencies in specimens II through IV but not at all in the no. V specimens. The highest CSF-IgM index values were noted in specimens II through IV, i.e., 11 days to 4 months after onset of disease. All patients who had an elevated CSF-IgM index (Table 4) also had an elevated CSF/serum albumin ratio, indicating that CSF-IgM increases occurred to-

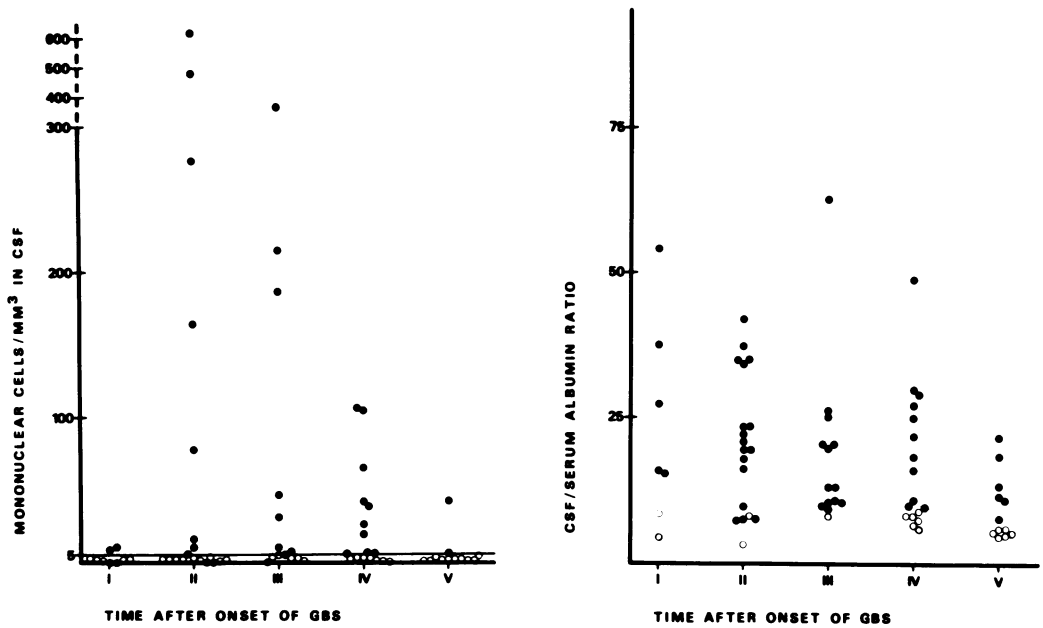

FIG. 1. *Temporal profiles of CSF pleocytosis and CSF/serum albumin ratio (normal value varies with age) during the course of GBS in 24 patients. I = ≤10 days; II = 11 days to 1 month; III = 1 to 2 months; IV = 2 to 4 months; V = >4 months after onset of disease. Symbols: (●) specimens with abnormal values; (○) specimens with normal values.*

TABLE 4. *IgM in serum and CSF of GBS patients*

Patient no.	Serum IgM highest value, g/liter (ref. 0.1-2.5)	CSF-IgM highest value, mg/liter (ref. <2)	CSF-IgM index (ref. <1.0)	Serum-IgM titers to:[a]		CSF-IgM titers to:[a]	
				CMV	EBV	CMV	EBV
1	2.8	29	0.62				
4	2.5	57	1.52		40		
5	1.5	44	2.90				
10	2.6	14	0.33	160	160		8
12	2.1	24	3.36		160		4
13	1.4	77	3.37				
16	2.1	100	1.76	10			
17	2.3	10	0.41				
20	5.7	10	0.17		20		
22	2.3	6	0.47				
23	2.7	11	0.15		20		
24	4.3	18	0.56	10	20		
25	5.6	37	1.04	160		2	
Frequency of increased values	6/13	13/13	6/13				
2	7.1	104	0.66	640		8	
3	2.9	19	0.46		80		
6	2.4	20	0.63				
9	1.8	<2	<0.20		160		2
11	1.5	39	0.52		10		
14	2.9	22	0.22		20		
18	2.5	<2	<0.14				
19	1.2	10	0.81	80			
21	2.5	<2	<0.25	10			
26	2.2	11	0.17				
27	2.2	<2	<0.11		20		
Frequency of increased values	3/11	7/11	0/11				

[a] Values not noted were negative (<10 for serum, <2 for CSF).

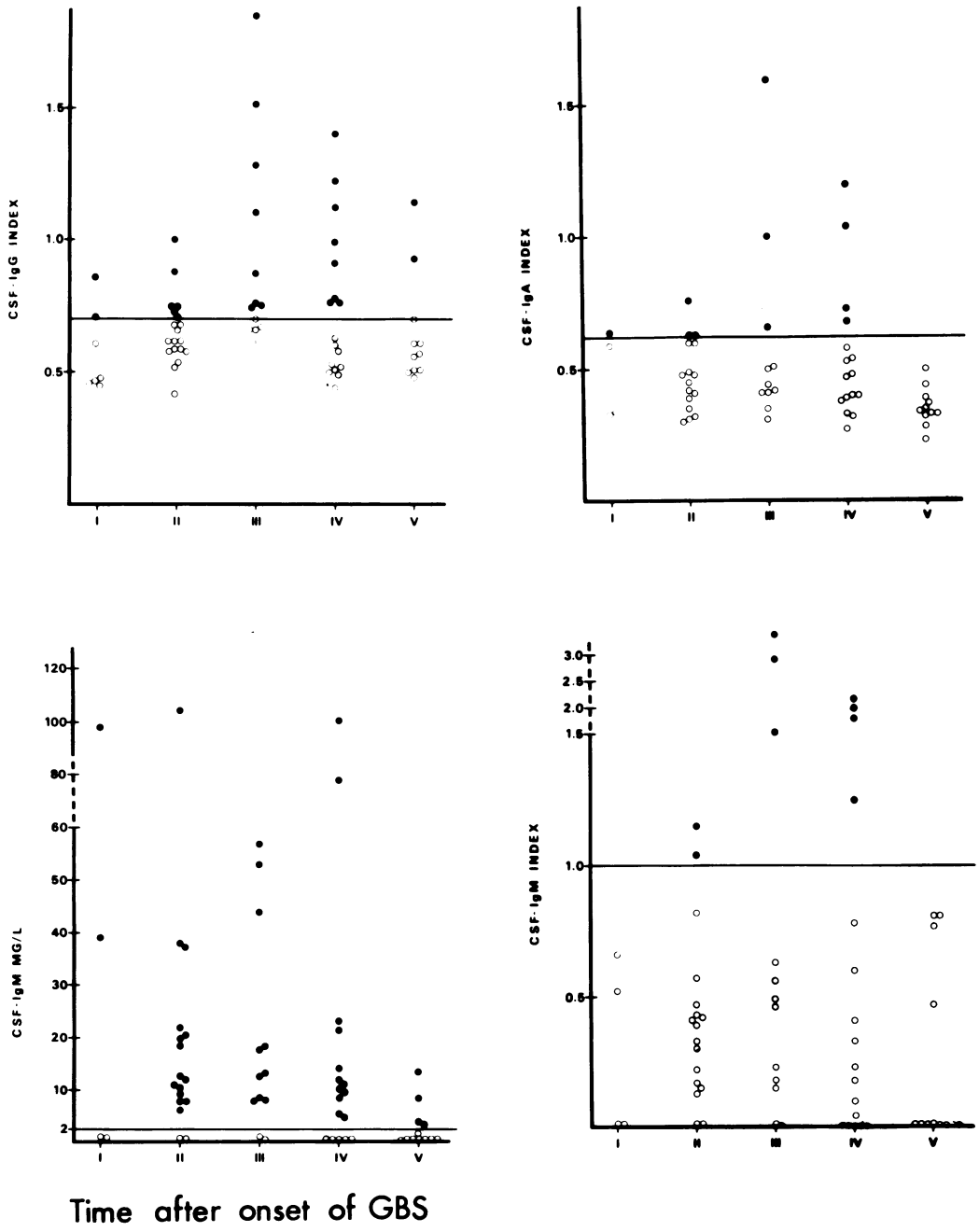

FIG. 2. *Temporal profiles of IgG, IgA, and IgM indexes and measurable CSF-IgM (≥2 mg/liter) in 24 patients during the course of GBS. Symbols:* (●) *specimens with abnormal values;* (○) *specimens with normal values (see Fig. 1).*

gether with blood-brain barrier damage in many cases (Fig. 1 and 2). The two patients with normal CSF/serum albumin ratios had no demonstrable CSF-IgM.

Seven patients had concomitantly raised CSF-IgG and CSF-IgA indexes. In one patient the CSF-IgM index only was raised (case no. 25), in two cases a raised CSF-IgM index preceded a raised CSF-IgG index by 3 days (no. 4 and 12), and in three cases which were investigated some-

what later, after onset of symptoms, raised CSF-IgM and CSF-IgG indexes were present simultaneously.

Abnormally high serum levels of IgG, IgA, and IgM were registered at some time during the course of disease in ten, four, and nine patients, respectively. Six of the nine patients with high serum IgM had concomitant serum IgG elevation.

The subgrouping of the patients according to presence of pleocytosis (Table 3) did not reveal a significantly higher frequency of elevated CSF-IgG or CSF-IgA index values in those with elevated CSF cell counts, nor did the mean index values differ ($P = 0.1$ to 0.2, t test). However, all six patients with a raised CSF-IgM index (Table 4) had pleocytosis ($P < 0.01$, χ^2 test), and the mean values of the two groups also differed ($P < 0.1$, t test).

Figure 3 illustrates the variations in the CSF/serum IgM ratio in relation to time in four patients. The first one (no. 11) shows a close relationship of CSF/serum albumin ratios to CSF/serum IgM ratios. The IgM in CSF therefore probably derives from serum. Deviations between the temporal profiles of the CSF/serum IgM ratio and the CSF/serum albumin ratio were seen in three cases. Patient 6 had a severe blood-brain barrier damage for more than 2 years, although his neurological symptoms lasted only 3 months. His CSF/serum IgM ratio was initially high but returned to normal despite long-lasting barrier damage. This indicates local production of IgM within the CNS or, perhaps, persistence. Patients 4 and 5 showed an early increase of the CSF/serum IgM ratio despite normalizing CSF/serum albumin ratios. A local IgM synthesis within the CNS may be postulated in these two patients. None of the three latter patients was evaluated to have a primary CMV or EBV infection (see below).

Oligoclonal immunoglobulins in CSF and serum. Ten of the patients had oligoclonal immunoglobulin in CSF demonstrable by agarose gel electrophoresis (Table 3). The frequency was significantly higher ($P < 0.01$, χ^2 test) in the group with pleocytosis. Five of the patients had oligoclonal immunoglobulin bands in CSF only, indicating that the oligoclonal immunoglobulin was synthesized within the CNS. All five patients had abnormally high CSF-IgG index values; four had abnormally high CSF-IgA index values and two had abnormally high CSF-IgM index values as well. Only one of the five patients with selective oligoclonal CSF immunoglobulin displayed abnormally high serum immunoglobulin levels (IgG). Five patients had oligoclonal immunoglobulin bands in CSF as well as in serum, and in four of them the numbers of visible bands were identical in the two body fluids. The remaining patient had three bands visible on CSF electrophoresis and one band on serum electrophoresis, again indicating synthesis within the CNS. All these five patients displayed abnormally high serum IgG values; three of them had elevated serum IgM and one had elevated serum IgA. Disappearance of oligoclonal immunoglobulin was confirmed in four of the ten patients during the period of observation. The bands in CSF of the remaining six patients persisted for at least 3 months after onset of disease, and in one case it persisted for as long as 13 months.

Additional evidence for the presence of oligoclonal immunoglobulin was obtained from determinations of the kappa/lambda ratio. This ratio was abnormally low in CSF of six patients (patients 9, 12, 13, 16, 17, and 20), all of whom displayed oligoclonal bands on electrophoresis. Three of these patients had normal kappa/lambda ratios of serum and normal serum electrophoresis patterns, indicating that the oligoclonal immunoglobulin of the lambda type was synthesized within the CNS. Three patients had abnormally low kappa/lambda ratios in serum also, and they belonged to the group of patients who displayed oligoclonal immunoglobulin in CSF as well as in serum. In the latter three patients the oligoclonal immunoglobulin of the lambda type is most probably synthesized outside the CNS. The remaining 18 patients had normal kappa/lambda ratios of both CSF and serum.

Virological studies. Serum antibodies to EBV. These studies are summarized in Tables 4 and 5. All patients but one had a demonstrable titer to EBV nuclear or capsid antigen in the first blood sample. The remaining patient (no. 12) had a seroconversion to both EBV nuclear antigen and EBV viral capsid antigen. Of the 24 patients, three (no. 9, 10, and 12) had high EBV-IgM and at least fourfold titer increases to indicate a primary infection (Table 5). One of these patients (no. 10) had an IgM titer of 160 to both EBV and CMV and rising IgG titers to EBV but a high IgG titer to CMV. This patient may have had two primary infections, which is not uncommon (36), but was evaluated to have had a recent EBV infection, due to his changing EBV-IgG titers. Eleven of the patients had fourfold changes in EBV antibody titers or high IgG antibodies but IgM titers below 160, thus perhaps indicating an activation of a latent infection. None had heterophile antibodies at the time of the first investigation.

Serum antibodies to CMV. Sixteen of the

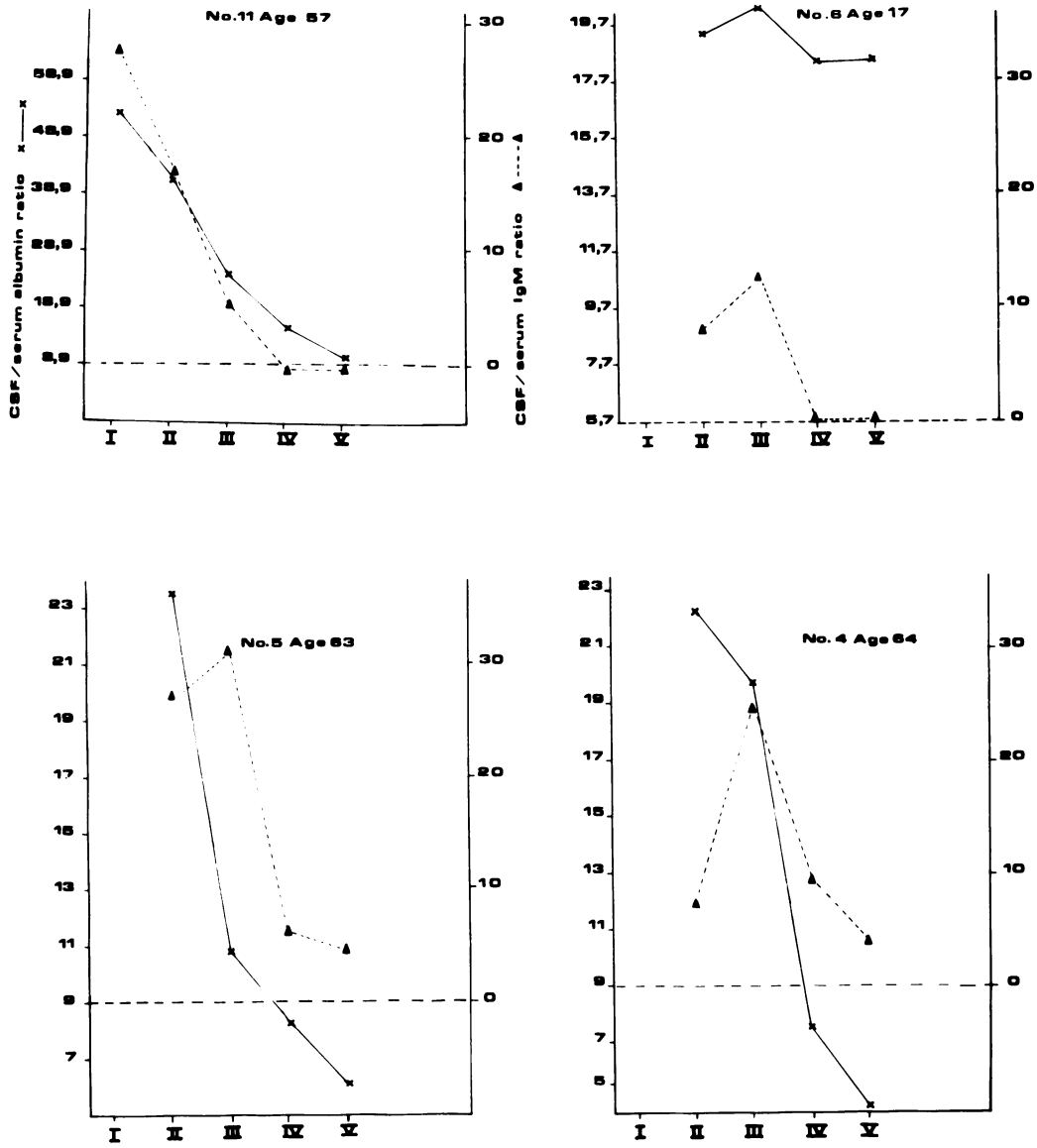

FIG. 3. *Time-dependent variations in the CSF/serum ratios of albumin (×) and IgM (▲) of four patients with GBS. Time after onset of disease is indicated as in Fig. 1. The dashed horizontal line represents the upper normal value for the CSF/serum albumin ratios.*

24 patients were initially seropositive for CMV. In two of them, high CMV-IgM and fourfold IgG titer increases indicated a primary infection (no. 2 and 25). Two patients had serological data indicating a reactivated CMV infection. Eight patients (not shown in Table 5) had no serological signs of reactivated CMV or EBV.

Analysis of serum and CSF IgM antibodies with viral specificity. Figure 4 illustrates the serum and CSF antibody titers and CSF/serum albumin ratios in relation to time after onset of disease in two patients evaluated to have primary EBV or CMV infection. Measurable CSF-IgM of EBV specificity was seen in

TABLE 5. *Serological data for EBV and CMV of 24 GBS patients*[a]

Patient no.	IF antibody titers to:				
	EBV(IgM)	EBNA	EBV-VCA (IgG)	CMV(IgM)	CMV(IgG)
Primary EBV infection					
9	160, <10	20, 160	40, 10	<5, <5	<10, 10
10[b]	160, 10	80, 320	80, 40	160, 10	160, 160
12	160, <10	<10, 40	<10, 40	<5, <5	10, 10
Primary CMV infection					
2	<5, <5	<5, <5	160, 80	320, 640	320, 1,280
25	<5, <10	80, 80	80, 160	160, 40	80, 320
Reactivated EBV infection					
1	5, 5	20, 80	20, 80	<5, <5	<5, <5
3	80, 10	20, 40	160, 40	<5, <5	40, 80
4	40, 20	80, 80	160, 320	<5, <5	<10, <10
11	10, 10	20, 80	160, 160	<10, <10	<10, <10
13	<5, 5	10, 80	40, 320	<5, <5	160, 80
14	20	10	320	<5	10
19[b]	<5, <5	320, 320	160, 40	80, 40	80, 10
20	20, <5	<10, <10	5, 40	<10, <10	<10, <10
23	20, <5	20, 160	80, 320	5, <10	10, 10
24	20, <5	<5, 10	80, 80	10, <5	10, 10
27	40	80	320	<10	80

[a] The highest and lowest values of serum titers during follow-up are given. For serological criteria for evaluating titers as representing a primary or a reactivated infection, see text. IF, Immunofluorescence; EBNA, EBV nuclear antigen; EBV-VCA, EBV viral capsid antigen.

[b] These patients were also evaluated as having a reactivated CMV infection.

all of the three cases with high serum EBV-IgM and measurable CSF-IgM to CMV in the two cases with high serum CMV-IgM. Of the five patients with measurable virus-specific CSF-IgM (Table 4), four had barrier damage at the time that detectable viral IgM occurred. The fifth patient (no. 9) had no barrier damage, but the IgM titer was too low (= 2) to allow any conclusions about local specific antiviral IgM synthesis.

In determining IgM antibodies to viral antigens, there is a risk of false positive reactions due to the RF IgM anti-IgG. The possible presence of RF was determined in all sera. Two patients had RF (no. 20 and 27). In case 20, the EBV-IgM titers fell, despite increasing EBV-IgG antibody titers. In case 27, only EBV-IgM and not CMV-IgM antibody titers were found despite the presence of CMV-IgG antibody. It is thus not probable that our IgM titers were caused by RF or that RF interfered with our interpretations of the obtained antibody titers.

CSF-IgG antibodies with viral specificity. IgG viral antibodies to CMV or EBV in titers of 2 to 16 were measured in the CSF of 10 patients (data not shown). Eight patients had blood-brain barrier damage simultaneously, whereas two patients had normal barrier parameters. In one patient CSF-IgG to EBV was demonstrated 3.5 months after a normalization of the blood-brain barrier (case 4, CSF anti-EBV viral capsid antigen titer 8). In the other patient it occurred without any previous barrier damage (case 9, CSF anti-EBV nuclear antigen titer 4). In the remaining 14 patients, EBV-IgG or CMV-IgG were not demonstrable by immunofluorescence using unconcentrated CSF.

DISCUSSION

According to the classical description, GBS is characterized by a progressive, self-limiting symmetric paralysis, which in about one-third of the cases is preceded by infection or vaccination. In the present study, the diagnostic criteria for GBS as proposed by Masucci and Kurtzke (22) were used. No patients with neuropathy secondary to known recent immunization were included.

A raised CSF total protein combined with a normal cell count or only a slight mononuclear pleocytosis, the so-called albumino-cytological dissociation, is also claimed to be characteristic of the disease. However, our sequential CSF studies revealed that mononuclear pleocytosis may occur in more than half of patients with GBS. A similarly high frequency of patients (55%) displayed pleocytosis in CSF 2 to 4 months after onset of neurological symptoms, and in a few patients more than 50 cells/mm^3 were found from 11 days up to 4 months. This prolonged mononuclear pleocytosis has not been described previously in GBS, probably because it is unu-

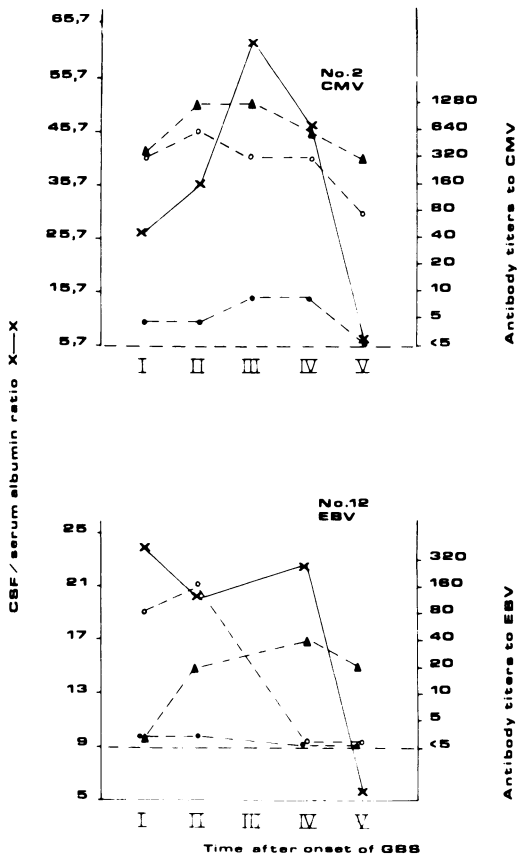

FIG. 4. *Temporal profiles of the CSF/serum albumin ratios and antibody titers of two patients evaluated as primary CMV and EBV infections, respectively. Symbols:* (×) *CSF/serum albumin ratio;* (○) *IgM virus-specific antibody in serum;* (●) *IgM virus-specific antibody in CSF;* (△) *IgG antibody in serum (for CMV);* (▲) *anticomplement immunofluorescence antibody titer to EBV nuclear antigen.*

sual to repeat lumbar punctures. It also appears later than the pleocytosis in, e.g., acute viral meningitis. The patients with pleocytosis displayed no clinical differences from those without detectable pleocytosis.

Oligoclonal immunoglobulin has been demonstrated before in the CSF and serum of GBS patients (13) and has been claimed to be due to immunoglobulin synthesis outside the CNS (14). In the present study, agarose gel electrophoresis revealed oligoclonal immunoglobulin bands simultaneously in CSF and serum of five patients. Five additional patients displayed oligoclonal immunoglobulin bands in CSF only, indicating that in at least these five cases immunoglobulin was synthesized within the CNS. The finding of abnormally low kappa/lambda ratios means that oligoclonal immunoglobulin synthesized by GBS patients either outside the CNS or intrathecally is mainly of the lambda light-chain type. No abnormally high kappa/lambda ratios were found. This contrasts to the findings in multiple sclerosis, where the immunoglobulin synthesized within the CNS is mostly of the kappa light-chain type (15), and is similar to acute aseptic meningitis, where the frequencies of lambda seem to dominate (A. Fryden and H. Link, Arch. Neurol., in press).

In subacute sclerosing panencephalitis, oligoclonal immunoglobulin bands have been shown to consist of measles virus antibodies (16, 34). In multiple sclerosis patients virus-specific antibodies of restricted heterogeneity can be found in CSF (24). It may therefore be suggested that the oligoclonal immunoglobulin demonstrable in GBS also contains specific antibodies. We were not able to relate the occurrence of oligoclonal immunoglobulin in GBS to any of the specific antibodies we were measuring (antibodies to EBV nuclear antigen, EBV viral capsid antigen, or CMV late antigens). On the contrary, the low CSF titers of IgG or IgM antibodies to CMV and EBV that we found indicate that these antibodies are not the main constituent of oligoclonal CSF-immunoglobulin bands visible on electrophoresis. Investigations are in progress to elucidate the antibody character of oligoclonal immunoglobulin in GBS.

One of our questions was whether the pleocytosis was a result of active CNS viral infection and whether, in that case, synthesis of immunoglobulin or of specific viral antibodies could be demonstrated. Viral diseases of various kinds, especially caused by CMV or EBV, have been associated with the development of a GBS syndrome. These two viruses occasionally are also associated with CNS disease of types other than GBS (4, 6, 27). After a primary infection, herpesviruses seem to remain latent in the host and are often reactivated in diseases such as malignancy, immunosuppression, graft versus host rejection, transplantation, and sarcoidosis (35). High and persistent IgM differentiates patients with primary CMV from those with reactivated disease (29, 31), and the same is probably true for EBV (23). We used more conservative criteria than previously used (7, 37) and evaluated only 5 of the 24 cases as having a recent CMV or EBV infection at the time of GBS. Thus, we suggest that although some cases have a primary infection, many more displayed reactivation of previous infections. Eleven patients were evaluated to have a reactivated EBV and two patients, a reactivated CMV. It must be remembered in this context that fewer of the patients had anamnestic CMV titers compared to EBV titers.

Primary and reactivated infections with EBV or CMV were equally common in patients with and without pleocytosis.

Three patients had high serum measles hemagglutination-inhibiting antibodies, which remained unchanged during the follow-up. In subacute sclerosing panencephalitis this is a common finding (16), but we have at present no explanation for these high titers in GBS patients since they all recovered.

Whether the low titers of antiviral IgM found in CSF are due to local synthesis or consist of serum IgM retained after previous leakage has not yet been established. Since IgM in serum has a very short half-life, we find it probable that intrathecal synthesis occurred in at least three of our cases.

In mycoplasma, early appearing IgM antibodies react with the I-antigen of normal B and T lymphocytes (1). This autoimmune reactivity, which is common in mycoplasma disease, might induce GBS in certain patients (8). We found that GBS patients with pleocytosis had a high CSF-IgM index more often than GBS patients without pleocytosis. Perhaps, therefore, antiviral IgM antibody synthesized within CSF, or IgM-containing immune complexes, can have pathogenetic importance for development of the clinical syndrome, which is strongly suspected to be caused by abnormal immune reactivity.

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