

Viral Antibodies in Cerebrospinal Fluid of Multiple Sclerosis and Control Patients: Comparison Between Radioimmunoassay and Conventional Techniques

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Cerebrospinal fluid antibodies to measles, rubella, vaccinia, herpes simplex, and varicella-zoster viruses in four patient study groups (clinically definite multiple sclerosis [MS], early probable MS, optic neuritis, and control patients with other neurological diseases) were assayed by radioimmunoassay, complement fixation, hemagglutination-inhibition, or complement-enhanced plaque reduction methods. Antibodies were more frequently found and at higher dilutions by radioimmunoassay than by other techniques. Measles virus antibody, the most frequently found antibody, was present in the cerebrospinal fluid of 72% of MS patients and 5% of control patients. The differences between the numbers of MS patients and control patients with antibodies to other viruses were not as marked. Thus, 58% of MS patients versus 21% of control patients had antibody to rubella virus, 20 versus 3% had antibody to vaccinia virus, 50 versus 33% had antibody to herpes simplex virus, and 25 versus 8% had antibody to varicella virus. Sixty-seven percent of MS patients and 26% of control patients had antibodies to two or more viruses in their cerebrospinal fluid.

Serological studies of multiple sclerosis (MS) patients by conventional techniques have consistently shown increased levels of antibodies to viruses, particularly measles and rubella viruses, in both the serum and the cerebrospinal fluid (CSF) (20) of up to 57% of such patients. Because of the greater sensitivity of the radioimmunoassay (RIA) in the detection of antibodies (8, 9), as compared with conventional techniques, RIA was used in the present study for evaluation of antibodies in the CSF of well-characterized MS patients. CSF of patients with early probable MS, optic neuritis, and other neurological diseases was also studied. Since optic neuritis is often a prelude to MS, the results on this group, although it is small, were kept separate. Results obtained by RIA were compared with those obtained by conventional techniques.

MATERIALS AND METHODS

Patient selection and CSF analysis. Serum and CSF were obtained from patients who gave informed consent to be studied and were clinically characterized by neurologists, using the criteria of McDonald (18). Each patient was followed for 4 to 12 months after lumbar puncture, and the diagnosis was then reassessed. Control patients were chosen if they were

between the ages of 15 and 70, had an identified neurological disease other than MS (see Table 1), and were undergoing lumbar puncture. CSF cell counts and total protein levels were measured by standard techniques; immunoglobulin G (IgG) was measured by electrophoresis; and an assay for oligoclonal bands in concentrated (50×) CSF was done by agarose electrophoresis with commercial reagents as previously described (15). In addition, CSF albumin and IgG were also determined by radial immunodiffusion with commercial reagents (Hyland Laboratories, Inc., Costa Mesa, Calif.), and ratios were derived.

Conventional serological techniques. CSF antibodies to measles, rubella, herpes simplex, and varicella-zoster viruses were assayed by a standardized microcomplement fixation test (17), and antibodies to measles and rubella viruses were assayed by standardized micro-hemagglutination-inhibition tests (HI) (17, 28). Heparin-manganous chloride, used for removal of nonspecific serum inhibitors to the rubella viral hemagglutinin (28), could not be used with CSF. The CSF, therefore, were untreated except for absorption with chicken erythrocytes of those specimens showing agglutination in the control wells. Neutralizing antibody to vaccinia virus was assayed by a complement-enhanced plaque reduction (CPR) test modified from that of Takabayashi and McIntosh (24), using 80% plaque reduction as the end point. To determine the dose of virus for use in the test (40 to 60 plaque-forming units), viral stocks were titrated in the pres-

ence of 12 hemolytic units of guinea pig complement (1:16 dilution), the amount used in the test. After overnight incubation at 5°C of a mixture of CSF, virus, and guinea pig complement, 0.05 ml of the mixture was plated onto Vero cell monolayers in 16-mm-well plates, and the monolayers were overlaid with agarose. A second agarose overlayer with neutral red was added at 72 h, and the plaques were read at 96 h. The virus control (virus, guinea pig complement, and medium) was treated similarly.

CSF showing antibodies to rubella virus and/or vaccinia virus by HI and CPR, respectively, were checked for these antibodies by the indirect fluorescein-antibody technique (IFA), using fluorescein-labeled anti-human IgG and infected and noninfected cell cultures fixed to cover slips or slides as previously described (6). Appropriate controls were included (6).

In all assay procedures, equal numbers of patients' specimens and control specimens were assayed in the same run to equalize differences between runs. Doubling dilutions of an initial 1:2 dilution of CSF were assayed.

Radioimmunoassay. The assay for viral antibodies by solid-phase RIA was performed as described by Forghani et al. (8). Briefly, infected and noninfected cell monolayers (BHK-21 cells for rubella virus and human fetal diploid lung cells for the other viruses), grown in 1-dram (ca. 1.18-g) vials and fixed with acetone, were incubated for 3 h at 37°C with CSF at a 1:50 dilution. The selection of a 1:50 dilution of CSF as a suitable screening dilution was based on previous published (8, 9) and unpublished studies in which serial dilutions of sera and CSF were tested for specific antibody and nonspecific binding in the RIA system. After washing with phosphate-buffered saline, pH 7.2, the cell monolayers were reacted for 30 min at 37°C with ¹²⁵I-labeled goat anti-human IgG labeled by the chloramine-T method (14) (specific activity, 0.5 μCi/μg of protein). The anti-human IgG was purchased from Antibodies, Inc., Davis, Calif. Its specificity was verified by immunoelectrophoresis. After thorough washing with phosphate-buffered saline, the contents of the vials were counted to 3% error in a Beckman 300 gamma ray spectrometer. A binding ratio of 2.1 or greater, determined by the ratio of counts bound by infected cultures to those bound by noninfected cultures, was considered indicative of the presence of specific antibody in the tested specimen. A binding ratio of 2.1 was used because of the demonstrated reliability of this value in the serodiagnosis of hepatitis B by the Abbott Laboratories RIA method.

RESULTS

Patient data. The entire sample included 94 patients. Twenty-five clinically definite MS patients (mean age, 43 years), 21 patients with early probable MS (mean age, 35 years), 7 patients with primary optic neuritis as their only neurological symptom, and 41 control patients with other neurological diseases (mean age, 41 years) were studied. The clinical diagnosis in each case was made by a neurologist and confirmed 4 to 12 months after lumbar puncture.

The diagnoses of patients in the control group are listed in Table 1. Sexes were approximately equally distributed in each group.

CSF. CSF total protein, IgG, and cell measurements are noted in Fig. 1. In a few cases, CSF data were incomplete, as some CSF quantities were inadequate for all tests. Nineteen of 25 clinically definite MS patients (76%) and 17 of 21 early probable MS patients (81%) had 2 to 8 oligoclonal IgG bands in their CSF. The CSF of 5 MS patients and 3 early probable MS patients displaying bands had normal IgG levels. Of the 4 (of 41) control patients (10%) with CSF oligoclonal IgG bands, 2 had central nervous system (CNS) syphilis, one had a proven CNS vasculitis, and one had epilepsy of undetermined origin. The range of CSF IgG/albumin ratios for the MS group was 0.11 to 0.49, with a mean of 0.265; for the early probable MS group, it was 0.11 to 0.53, with a mean of 0.259; and for the control group, it was 0.08 to 0.36, with a mean of 0.148. Using a *t* test for unequal variances, the ratios for both the clinically definite MS and the probable MS groups were significantly different from the control group, with a value of *P* < 0.001.

Antibody assays. Results of the antibody assays are shown in Table 2. Based on RIA data, 43% of all patients had CSF antibody to herpesvirus, and 35% had antibodies to measles and rubella viruses. More patients with clinically definite MS had antibodies to the various viruses than did patients in the control group. Antibody to measles virus was found infrequently in the control group (5%) but was the most frequently found antibody in the MS group (72%).

Usually, a higher percentage of patients had CSF antibodies by RIA than by standard tech-

TABLE 1. Diagnoses of control patients^a

Diagnosis	No. of patients
CNS degeneration	7
Epilepsy	7
Peripheral neuropathy	4
Cerebellar ataxia	3
Brain tumor	3
Lumbar disk disease	3
Functional disease	1
CNS syphilis	2
Stroke	2
CNS vasculitis	2
Jakob-Creutzfeldt disease	2
Pseudotumor cerebri	2
Carcinomatous meningitis	2
Labyrinthitis	1

^a In addition to the 39 CSF studied for viral antibodies, the CSF of 4 more patients was analyzed for oligoclonal bands.

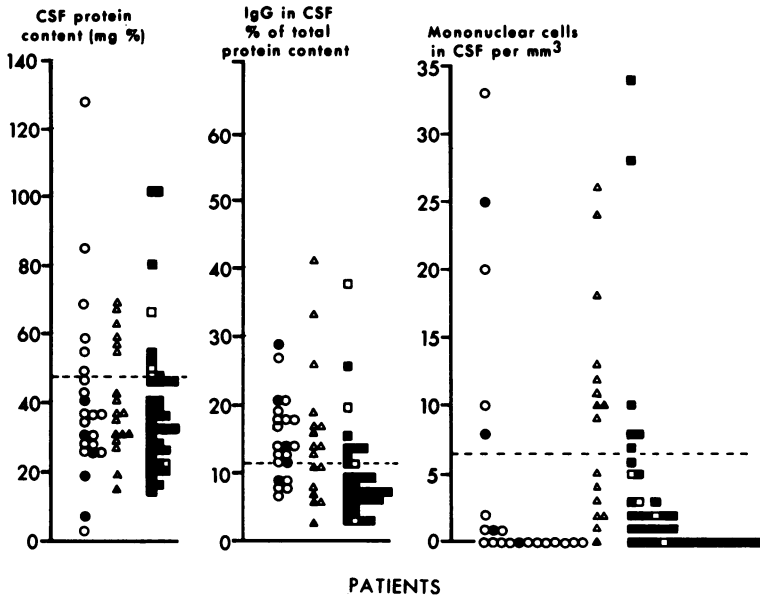


FIG. 1. Total protein concentration, IgG percentage of total protein, and cell counts of CSF in MS patients and control patients. (●, ○) MS patients; (■, □) early probable MS patients; (▲, △) control patients. Open symbols denote CSF with oligoclonal IgG bands, and closed symbols denote CSF without bands, as determined by agarose electrophoresis of concentrated CSF. Dotted lines denote accepted normal values for each assay. CSF data are incomplete in some cases, as some CSF quantities were inadequate for all tests.

TABLE 2. Comparison of RIA with other techniques for detection of viral antibodies in the CSF^a

Patient group (no.)	Patients with antibodies (%) to:									
	Measles		Rubella		Vaccinia		Herpes simplex		Varicella	
	RIA	HI	RIA	HI	RIA	CPR	RIA	CF	RIA	CF
MS (25)	72	32	58	44	20	32	50	0	25	4
Early probable MS (21)	52	19	38	15	9	5	43	0	28	5
Optic neuritis (7)	28	14	29	29	0	0	85	0	14	0
Controls (39)	5	5	21	13	3	16	33	0	8	3
All patients	35	16	35	23	9	17	43	0	18	3

^a CSF were screened at a 1:50 dilution for RIA and titrated for other tests at a starting dilution of 1:2.

niques. For example, by RIA and HI, respectively, 72 versus 32% of MS patients had antibody to measles virus, and 58 versus 44% had antibody to rubella virus. By complement fixation, antibody to herpes simplex virus was not detected, whereas, by RIA, this antibody was frequently observed. However, antibody to vaccinia virus was detected more frequently by CPR than by RIA.

Multiple antibodies were present more frequently in the CSF of MS and early probable MS patients than in the control patients and in a higher percentage of individuals by RIA than by a combination of the other techniques (Tables 3 and 4). A correlation was made between

results from standard techniques and those of RIA and IFA of all CSF (entire study group) showing antibody to measles, rubella, or vaccinia virus by standard techniques (Table 5). It should be noted that standard analyses and IFA started with a 1:2 dilution of the CSF, whereas RIA used a 1:50 dilution. The best correlation occurred between HI and RIA assays for antibody to measles virus. Of 14 CSF with antibody by HI, 13 also had antibody by RIA. The least correlation occurred between CPR and the two other assays for antibody to vaccinia virus. The lack of correlation occurred primarily with CPR titers of 1:2. Complement fixation was the least sensitive test for detection of antibody.

TABLE 3. *Patients with viral antibodies in CSF by RIA*

Patient group (no.)	Positive		Patients with antibody in CSF (%) to:				% with multiple anti-bodies
	No.	%	One virus	Multiple viruses			
				2	3	4	
MS (24)	21	88	21 ^a	25	25	17	67
Early probable MS (21)	15	71	19 ^b	19	19	14	52
Optic neuritis (7)	7	100	57 ^c	29	14	0	43
Controls (39)	29	74	49 ^d	21	5	0	26

^a Herpes simplex (2), measles (2), varicella (1).

^b Herpes simplex (2), measles (1), rubella (1).

^c Herpes simplex (3), measles (1).

^d Herpes simplex (16), rubella (2), varicella (1).

TABLE 4. *Patients with viral antibodies in CSF by standard techniques*

Patient group (no.)	Positive		Patients with antibody in CSF (%) to:				% with multiple anti-bodies
	No.	%	One virus	Multiple viruses			
				2	3	4	
MS (24)	15	62	25 ^a	29	8	0	37
Early probable MS (19)	5	26	10.5 ^b	10.5	5	0	16
Optic neuritis (7)	3	43	43 ^c	0	0	0	0
Controls (39)	9	23	15 ^d	5	3	0	8

^a Vaccinia (2), rubella (4).

^b Measles (1), rubella (1).

^c Measles (1), rubella (2).

^d Vaccinia (3), rubella (2), varicella (1).

TABLE 5. *Correlation between antibody titers in the CSF by HI or CPR and presence of antibody by RIA and IFA^a*

Measles virus		Rubella virus			Vaccinia virus		
HI titer ^b	RIA + ^c	HI titer ^b	RIA + ^c	IFA + ^c	CPR titer ^b	RIA + ^c	IFA + ^c
1:2 (6)	6	1:2 (16)	11	10	1:2 (11)	2	1
1:4 (3)	2	1:4 (2)	1	1	1:4 (2)	2	1
1:8 (2)	2	1:8 (2)	2	2	1:8 (2)	1	2
1:16 (3)	3	1:16 (1)	1	1	1:16		

^a Starting dilution by CPR, IFA, and HI was 1:2; CSF were screened by RIA at a 1:50 dilution.

^b Numbers in parentheses are numbers of patients with indicated antibody titer in the CSF.

^c Number of same patients as in *b* that have antibody by the indicated test.

DISCUSSION

Oligoclonal IgG bands are known to occur in up to 85% of MS patients but rarely in controls, except for those with chronic CNS infections or CNS immunological disorders (22). In the present study, three of four control patients with oligoclonal IgG bands had proven diagnoses of CNS infection or possible immunological disease. Bands were present in approximately 75% of MS and probable MS patients. Several CSF with bands had normal IgG levels. CSF IgG/albumin ratios indicated that the MS group was distinct from the control population, suggesting IgG synthesis within the CNS, a finding also noted by others (10).

The greater sensitivity of solid-phase RIA for detection of viral antibodies in the CSF, as compared with the more conventional techniques used in the present study and by others, is readily apparent. Using several serological methods to detect antibodies to measles antigen, Norrby et al. (20) found evidence of CSF antibody, thought to have been produced in the CNS, in 57% of 150 MS patients. Haire et al. (13), using IFA, reported the prevalence of measles virus-specific IgG in CSF, concentrated five-fold, to be 80.6% in MS patients and 34.5% in patients with other neurological diseases. Their figure of 80.6% prevalence in concentrated CSF samples from MS patients agrees well with ours of 72% with a 1:50 dilution of CSF (Table 2).

The prevalence of such antibody in the CSF of our control patients, however, was considerably lower, approximately 5% by both RIA at 1:50 dilution and HI at 1:2 dilution, but agrees with a previous report of 6.7% by Haire et al. (11).

Two other RIA antibody studies on the CSF of MS patients (1, 7) have been published. In the report of Cunningham-Rundles et al. (7), the test was done with ¹²⁵I-labeled soluble components of the measles virion and 20-fold-concentrated CSF. Ten of 10 patients with MS and a similar number with other neurological diseases had antibody in the CSF to the six known proteins of measles virus. The relative sensitivity of the two RIA tests cannot be compared, since, in that study, labeled antigen and 20-fold-concentrated CSF were used, whereas the present study used an infected-cell sheet as antigen, labeled anti-IgG, and a 1:50 dilution of CSF. In the report of Arstila et al. (1), three of four MS patients tested had CSF antibody titers to measles virus that were equal to or greater than 1:128. A binding ratio of a positive to a negative serum of 3 was taken as the end point for the serum assays in this study. However, the method for calculating binding ratios for CSF antibody was not indicated. In the present study, a ratio of 2.1 was considered positive for the presence of antibody. Counts bound by patients' CSF with noninfected cells, rather than those bound by a negative CSF to infected cells, were used as the denominator in determining binding ratios. Since individual CSF may differ in their reaction with nonviral cellular antigens, nonspecific binding can be evaluated for each specimen in this way.

Kempe et al. (16) first reported antibody to vaccinia virus in the CSF of MS patients by CPR. In a series of 187 MS patients, 30% had antibody, whereas, of 75 patients with other neurological disease, 4% had such antibody. Thompson et al. (25) found a similar antibody prevalence: 30% of MS patients and 4.3% of patients with other neurological diseases had antibody. In the present series, 32% of MS patients and 16% of patients with other neurological disorders had antibody by CPR. Unlike the high titers (as great as 1:256) reported by Kempe et al. (16), the CPR titers in this report were low and, at the 1:2 dilution, not always confirmed by IFA or RIA (Table 5). If CPR titers of 1:2 are disregarded, 16% of clinically definite MS patients and no patients in the other three groups showed CSF antibody to vaccinia virus. By RIA, 5 of 25 MS patients (20%) and very few in the other groups had antibody to vaccinia virus (Table 2). Brown et al. (4), in a study of MS patients from France, found no CSF anti-

body to vaccinia virus.

Salmi et al. (23) detected CSF antibody to rubella virus in a higher proportion of MS patients (20%) than control patients (2%). In the present study, 58% of MS patients and 21% of control patients had antibody by RIA, and 44 and 13%, respectively, had antibody by HI. Better correlation, although not perfect, occurred between RIA and IFA results than between either of these test results and those of HI (Table 5).

The finding of antibody to herpes simplex in a significant number of CSF of all groups was of interest (Table 2). There is good evidence that herpes simplex virus commonly causes a latent nervous tissue infection in humans, for Baringer (2) has recovered virus from the sensory ganglia of over 40% of unselected autopsied adults. Norrby et al. (20), using a passive hemagglutination test, found an 11% prevalence of antibody to herpesvirus in their MS groups, and Haire et al. (12), using IFA, found 38%. The induction of nonspecific Fc receptors by herpes simplex reported by Westmoreland and Watkins (30) appears not to play a role in our system or in that of Haire et al., for, if reactivity were due to a reaction of IgG with induced Fc receptors, all specimens should be positive, as should also be our labeled human anti-IgG, the IgG fraction of goat antiserum. Furthermore, the presence of antibody to herpes simplex virus was not dependent upon the concentration of IgG in the CSF, i.e., low levels of IgG were not confined to those CSF that were negative for such antibody.

Whereas only 18% of the study group had CSF antibodies to varicella by RIA, the MS patients, nevertheless, had the largest number (25%).

As techniques become more sensitive, or when CSF is concentrated, more diverse viral antibodies are detected in MS CSF (11, 20). The presence of multiple antibodies reported in this study and by others could be explained either by local synthesis of antibody within the CNS or by leakage of serum antibodies into the CSF. For complete evaluation of CSF antibody synthesis, serum/CSF ratios should be determined (5) and, preferably, compared to antibody ratios to other antigens in the same material. A low ratio, such as that seen with antibody to measles virus in subacute sclerosing panencephalitis, suggests antibody synthesis within the CNS. Another, indirect method to test for leakage of serum antibody into the CNS is the evaluation of IgG/albumin ratios of CSF or of serum and CSF (16). In our series, only CSF IgG/albumin ratios were determined, but such methods did distinguish the MS groups from the control group,

suggesting that the CSF IgG was locally produced, within the CNS.

The presence of multiple CSF antibodies reported in this study adds complexity to the viral theory of the etiology of MS. Although the consistent observation (3) that measles antibodies appear most commonly in MS CSF and are seldom noted in controls was confirmed, it is difficult to consider "a candidate MS virus" when these studies suggest possible infection with several agents in the same patient. The presence of antibodies to multiple viral agents (especially measles and rubella viruses) in the sera of patients with "autoimmune" diseases (26) or chronic active hepatitis (27) has been explained as a nonspecific immune enhancement phenomenon. If this were true in MS, even synthesis of antibody within the CNS may not be directly related to viral antigens within the brain or to the cause of the disease. Study of CSF from various other patient groups with evidence of immune enhancement may help in determining if the CSF findings noted in the present study are, in fact, unique to MS.

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