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Humoral and Cellular Immune Responses to Matrix Protein of Measles Virus in Subacute Sclerosing Panencephalitis

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The immune response to matrix (M) protein of measles virus was examined in patients with subacute sclerosing panencephalitis (SSPE) and controls. Antibodies specific for M and nucleocapsid (NC) proteins in 11 serum and 8 cerebrospinal fluid (CSF) samples from patients with SSPE were quantitated by enzyme-linked immunosorbent assay by using affinity-purified measles virus proteins. Geometric mean anti-NC antibody titers were higher in the serum (6.58 ± 0.98 [mean ± standard deviation]) and CSF (4.38 ± 0.74) of SSPE patients compared with controls. Anti-M antibodies were present in the serum and CSF of all SSPE samples tested but in titers lower than those of anti-NC antibodies. Geometric mean anti-M antibody titer was 3.35 ± 0.53 in sera from patients with SSPE compared with 3.05 ± 0.66 in sera from patients with other neurological diseases and 3.12 ± 0.74 in sera from healthy individuals. Geometric mean anti-M antibody titer was 2.59 ± 0.86 in the CSF of eight patients with SSPE compared with a mean <1.00 for patients with other neurological disease (controls). Intrathecal synthesis of anti-M or anti-NC antibodies was established in four patients with SSPE. The cellular immune responses to M, F, HA, and NC proteins were examined in four of the patients with SSPE by lymphoproliferation and were not significantly different from those in five healthy controls. The results demonstrate humoral and cellular immune responses to M protein in patients with SSPE and indicate that it is unlikely that a defect in the immune response to this virus component accounts for the disease process in the patients studied.

Subacute sclerosing panencephalitis (SSPE) is a persistent measles virus (MV) infection of the central nervous system. The course of the disease is usually progressive and fatal. The mechanism of MV persistence in this disease is not understood. It is not known whether the host immune system fails to clear the virus or whether the virus undergoes a postinfection mutation (22). A measles-like virus has been cocultivated from the brains of affected individuals (7, 17) and appears to be defective. The serum and cerobrospinal fluid (CSF) of some patients with SSPE have reduced or undetectable antibody to matrix (M) proteins (5, 6, 12, 27), suggesting a defect in the synthesis of or immune reactivity to this protein. However, antibodies to M protein have been reported by others (24). All five MV proteins including M protein have been demonstrated in acetone-fixed frozen brain tissue obtained from four patients with SSPE by using fluorescein-conjugated monoclonal antibodies (15). mRNAs for nucleocapsid (NC), phosphoprotein (P), and M protein have been demonstrated by Northern (RNA) blot analysis of the brains from four patients with SSPE, whereas those for hemagglutinin (HA) and fusion (F) proteins of MV were hardly detectable. In vitro translation of the M protein was defective in three of those four patients (2). Consequently, both the expression of M protein and its role in virus persistence in SSPE remain uncertain.

The M protein is essential for the assembly of MV (2), and reduced expression of this protein or production of a functionally defective protein could explain the persistence of MV in the brain, as well as the reduced antibodies to M protein. Alternatively, a defect in the immune response to M protein could account for the reduction or lack of antibodies to this protein. In this study, the immune response to M protein was examined in 11 patients with SSPE. Antibodies to M protein in serum and CSF were quantitated by enzyme-linked immunosorbent assay (ELISA), and the cellular immune response to this protein was examined in four patients.

MATERIALS AND METHODS

Patients. Eleven patients with SSPE (cases 1 to 11) were studied. The diagnosis was made on the basis of characteristic clinical findings, elevated CSF immunoglobulin G, and increased MV antibody titers in the serum and CSF. Paired serum and CSF samples were available for eight of those patients. Serum without CSF was obtained from three patients, and peripheral blood lymphocytes were obtained from four patients. Two patients with SSPE (cases 1 and 2) were on isoprinosine treatment when blood samples were obtained for this study.

Controls. Sera from five infants who had no history of natural measles or measles vaccination and were measles seronegative by immunoprecipitation methods were used as negative controls in the ELISA. Additional control sera for this assay were obtained from five healthy individuals aged 8 to 34 years and from two patients with acute measles (cases 12 and 13). Normal CSFs obtained from five healthy volunteers and from a patient with pseudotumor cerebri were used as controls. Paired serum and CSF samples obtained from five patients with other neurologic disease (OND) served as additional controls. Peripheral blood lymphocytes from five healthy individuals aged 25 to 40 years were used as controls in the lymphoproliferative studies.

Purification of MV structural proteins. Daudi cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, L-glutamine, penicillin-streptomycin, gentamycin, and HEPES (*N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer at 37°C in a 5% CO_2

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atmosphere. Cultures containing 3×10^9 Daudi cells were infected with the Edmonston strain of MV at a multiplicity of infection of 0.2 and harvested 3 days later. On day 2 postinfection, 5×10^7 cells were labeled with 0.5 mCi of [^{35}S]methionine in 95% methionine-free medium for 12 h. The cells were pelleted and solubilized in lysing buffer (pH 7.8) containing 0.01 M Tris, 0.075 M NaCl, 4.47% (wt/vol) KCl, 0.0005 M $MgCl_2$, 2% Triton X-100, 0.001 M phenylmethylsulfonyl fluoride, and 23.6 U of aprotinin. The solubilized membranes were separated from cellular debris and nucleic acid by ultracentrifugation (302,000 × *g*, 45 min).

MV structural proteins (HA, F, M, and NC) were purified from the cell lysate by affinity chromatography with monoclonal antibodies specific to each MV protein coupled to cyanogen bromide-activated Sepharose 4B, as described earlier (1, 3). The purity of the proteins was established by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and by immune reactivity with monoclonal antibodies specific for the purified MV protein.

Immunoprecipitation assay. MV Edmonston-infected Daudi cells labeled with [^{35}S]methionine were lysed (7.5×10^6 cells per ml) in radioimmunoprecipitation assay (RIPA) buffer (pH 7.4) containing 0.01 M Tris, 0.15 M NaCl, 1% deoxycholate, 1 % Triton X-100, 6.1% SDS, 0.002 M phenylmethylsulfonyl fluoride, and 100 k U of aprotinin per ml. A cytoplasmic extract was prepared by ultracentrifugation (302,000 × *g*, 45 min). Aliquots of the lysate containing 10^6 cpm were incubated with 0.1 ml of protein A-bearing *Staphylococcus aureus* in 0.3 ml of RIPA buffer with 0.5% myoglobin at 4°C for 1 h. The lysate was centrifuged in a Beckman Microfuge, and the supernatant was saved. Similarly, the serum and CSF samples were incubated with 0.1 ml of *S. aureus* in 0.3 ml of RIPA buffer–0.5% myoglobin for 1 h at 4°C and then washed twice with 1.5 ml of RIPA buffer–0.5% myoglobin centrifuged. The pellet was resuspended in the cleared lysate for 1 h at 4°C. The *S. aureus* immune complexes were then pelleted and washed three times with RIPA buffer–0.5% myoglobin and once with RIPA buffer. The immune complexes were dissociated in SDS under reducing conditions by boiling for 2 min and subsequently evaluated by SDS–9% polyacrylamide gel electrophoresis and autoradiography (3, 11).

ELISA. To quantitate antibodies to M and NC proteins, an ELISA was developed. Optimal concentrations of antigen and antisera were established in preliminary experiments. Sera were assayed at 10^{-1} to 10^{-5} dilutions made in phosphate-buffered saline containing 0.5% bovine serum albumin and 0.05% Tween 20. Assays were carried out in 96-well microdilution plates (Dynatech Laboratories, Inc., Alexandria, Va.). Each well was incubated with 2 μg of purified MV protein (M or NC) or bovine serum albumin dissolved in 100 μl of carbonate-bicarbonate coating buffer (pH 9.6) at 4°C for 16 h. The plates were washed with phosphate-buffered saline containing 0.05% Tween 20; specimens of serum or CSF were added and incubated with the purified MV protein or bovine serum albumin (100 μl per well) for 2 h at 25°C. The wells were washed and incubated with a 1:500 dilution of goat anti-human immunoglobulin G coupled to alkaline phosphatase for 1 h at 25°C. The color reaction was developed with 0.1% *p*-nitrophenol phosphate disodium in diethanolamine. Reactivity with M protein was quantitated after 90 min for serum and after 3 h for CSF. Reactivity with NC protein was quantitated after 90 min for both serum and CSF with a micro-ELISA automatic reader (MR 580; Dynatech). Optical density (OD) at 405 nm corresponding to reactivity with bovine serum albumin was subtracted from the OD corresponding to reactivity with the MV protein at each serum or CSF dilution.

To quantitate antibodies to M protein in serum and CSF by ELISA, reference sera that lacked detectable antibodies to MV and normal CSFs were included in this assay. The reference sera did not precipitate any MV proteins at dilutions of 1:10 to 1:20 in the RIPA (Fig. 1A, lane 3). Mean ODs and standard deviations [SDs] were derived from the five MV-negative sera and the six normal CSF samples at several serial 10-fold dilutions. The mean ODs at serial dilutions for test serum or CSF were plotted on a semilogarithmic scale (OD on the linear scale and serial dilutions on the logarithmic scale). Antibody levels to M or NC protein were expressed as the maximal dilution (titer) which gave an OD greater than the mean OD + 3 SDs obtained with the MV antibody-negative samples at the same dilution as the test sample.

Estimation of intrathecal antibody synthesis. To determine whether anti-NC and anti-M antibodies were synthesized locally in the central nervous system, the approach of Johnson et al. (10) was employed. The anti-MV reactivities in the serum and CSF at a fixed immunoglobulin G concentration (1 μg/ml for NC and 10 μg/ml for M) were compared. A CSF-to-serum ratio greater than 1 was consistent with intrathecal synthesis.

Lymphocyte proliferation. Peripheral blood lymphocytes obtained by leukophoresis were purified and stored in liquid nitrogen vapor until used (9). The lymphoproliferative response (LPR) to MV and to purified NC, M, F, and HA proteins was measured in 96-well microdilution plates. Triplicate wells each containing 2×10^5 lymphocytes and appropriate concentrations of antigen were cultured in RPMI 1640 with 2% human AB serum. MV was used at a multiplicity of infection of 1. In pilot experiments, the optimal concentration of MV protein was found to be 1 μg per well. The response to MV and purified proteins was measured at 5 and 6 days, respectively. The cultures were then pulsed with 1 μCi per well of [^{3}H]thymidine for 4 h and harvested on a Mash II cell harvester. The results were expressed in counts per minute of [^{3}H]thymidine and as stimulation indices representing the ratios of [^{3}H]thymidine uptake in stimulated cells to that in unstimulated cells.

RESULTS

Detection of antibody to MV proteins by RIPA. Antibody reactivity to MV proteins was initially studied by RIPA. Sera from two individuals with acute measles were used as positive controls. These sera precipitated all five MV structural proteins (Fig. 1A and B, lanes 2). In contrast, serum from a negative control precipitated only a faint band with an approximate molecular size of 43 kilodaltons, which was believed to represent actin (Fig. 1A, lane 3). The RIPA of sera from the 11 patients with SSPE and controls are shown in Fig. 1A to C. Variations in the intensities of the bands corresponding to the five MV proteins were observed. A band with a molecular size of approximately 36 kDa was precipitated with a murine monoclonal antibody to M protein (Fig. 1A, lane 8). The intensity of this 36-kilodalton band in the sera of patients with SSPE was not appreciably different from that in control sera, and in the sera of both patients with SSPE and controls, the intensity of this band was much less than that in the sera from individuals with acute measles. Serum from one patient with SSPE (case 3) did not precipitate a band corresponding to M protein (Fig. 1C, lane 3).

RIPA bands obtained with the CSF from eight patients with SSPE also varied (Fig. 1D). The bands corresponding to

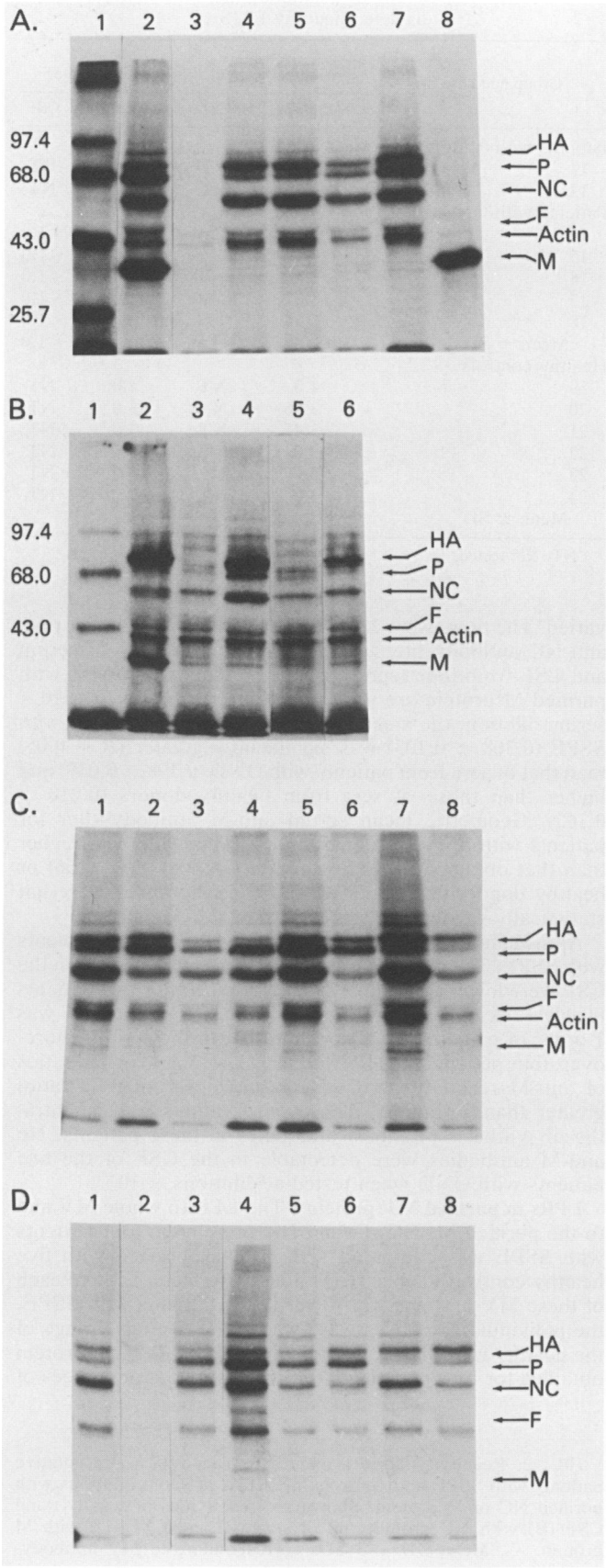

FIG. 1. Immunoprecipitation of [^{35}S]methionine-labeled MV proteins from MV Edmonston-infected Daudi cell lysates by serum or CSF. All sera were tested at a 1:20 dilution except for case 13, and all CSF samples were tested at a 1:5 dilution. Autoradiographs were exposed for 1 week except for that shown in panel A, lane 2, which was exposed for 5 days. (A) Results are shown for molecular weight standards (lane 1), serum from a patient with acute convalescent measles (case 13) (1:40 dilution) (lane 2), MV-negative serum (lane 3), serum from a patient with OND (case 16) (lane 4), sera from SSPE cases 10, 9, and 11 (lanes 5 to 7, respectively), and mouse monoclonal antibody to M protein (1:100 dilution) (lane 8). (B) Results are shown for molecular weight standards (lane 1) and sera from a patient with acute convalescent measles (case 12) (lane 2), a normal control (lane 3), SSPE case 10 (lane 4), a patient with OND (case 14) (lane 5), and a normal control (case 24) (lane 6). Numbers at the left indicate molecular sizes in kilodaltons. (C and D) Results are shown for sera (C) and CSF (D) from eight patients with SSPE (cases 1 to 8 for lanes 1 to 8, respectively). The positions of the relevant proteins are indicated.

TABLE 1. Serum and CSF $\log_{10}$ antibody titers for patients with SSPE as determined by ELISA

Case no.	Anti-M antibody titer		Anti-NC antibody titer	
	Serum	CSF	Serum	CSF
1	2.50	1.95	6.68	5.00
2	2.93	3.00	6.69	3.00
3	4.00	3.00	7.11	5.00
4	3.00	4.00	6.82	4.00
5	3.78	2.60	6.68	4.00
6	2.83	1.18	4.00	4.00
7	3.30	2.00	6.90	5.00
8	3.90	3.00	6.00	5.00
9	3.00	NT[a]	6.83	NT
10	3.60	NT	6.48	NT
11	4.00	NT	8.00	NT
Mean ± SD	3.35 ± 0.53	2.59 ± 0.86	6.58 ± 0.98	4.38 ± 0.74

[a] NT, Not tested.

M protein were fainter in the CSF than in the serum, except for one patient (case 4; Fig. 1D, lane 4). The findings indicated the importance of using a quantitative assay to compare antibodies to MV proteins. Consequently, an ELISA was employed.

Detection of antibodies to NC and M proteins by ELISA. Antibodies to NC protein in the serum of 11 patients with SSPE were measured by ELISA. ODs obtained for the patients with SSPE were significantly higher than those obtained for either patients with OND or healthy donors ($P < 0.01$) and comparable with those obtained for patients with acute convalescent measles (Fig. 2A). Patients with SSPE had a geometric mean serum anti-NC antibody titer of 6.58 ± 0.98 (mean ± standard deviation) (Table 1), significantly greater ($P < 0.001$) than those obtained for patients with OND (2.8 ± 0.84) or healthy donors (3.33 ± 1.03) (Table 2).

CSF anti-NC antibody titers were elevated in eight patients with SSPE, with a geometric mean of 4.38 ± 0.74 (Fig. 2B). Anti-NC antibodies were undetectable in the CSF of patients with OND when tested at dilutions $\geq 10^{-1}$ (Table 2). Four patients with SSPE (cases 1, 4, 6, and 8) had ratios of anti-NC reactivity in CSF to that in serum (OD ratio) greater than 1 (Fig. 3). This ratio is consistent with intrathecal synthesis of anti-NC antibodies.

Antibodies to M protein were detected in the serum and CSF of all patients with SSPE by ELISA, but the quantities

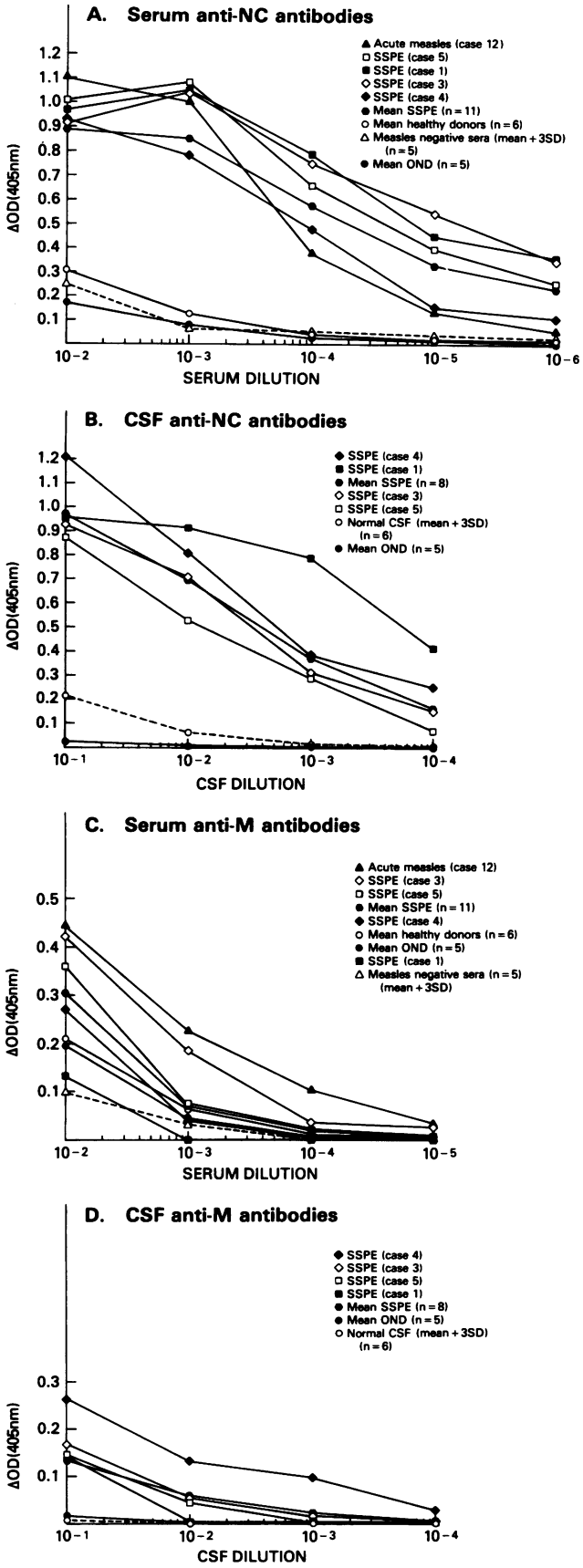

FIG. 2. Reactivity of sera and CSF from four representative patients with SSPE and controls in ELISA at serial dilutions with purified NC or M protein. Shown are reactivities of sera (A) and CSF (B) with NC protein and of sera (C) and CSF (D) with M protein. ---, Mean OD + 3 SDs corresponding to MV antibody-negative samples.

TABLE 2. Serum $\log_{10}$ antibody titers for controls as determined by ELISA

Group and case no.	Anti-M antibody titer		Anti-NC antibody titer	
	Serum	CSF	Serum	CSF
Patients with acute measles				
12	5.0	NT[a]	6.0	NT
13	5.0	NT	8.0	NT
Patients with OND				
14	3.70	<1.0	4.0	<1.0
15	3.48	<1.0	3.0	<1.0
16	2.30	<1.0	2.0	<1.0
17	3.41	<1.0	2.0	<1.0
18	2.38	<1.0	3.0	<1.0
Mean ± SD	3.05 ± 0.66	<1.0	2.80 ± 0.84	<1.0
Healthy controls				
19	4.0	NT	4.0	NT
20	3.66	NT	3.0	NT
21	3.45	NT	3.0	NT
22	2.0	NT	5.0	NT
23	2.6	NT	3.0	NT
24	3.0	NT	2.0	NT
Mean ± SD	3.12 ± 0.74		3.33 ± 1.03	

[a] NT, Not tested.

varied. The titers were 2 to 3 orders of magnitude lower than anti-NC antibody titers (Table 1). The reactivity of serum and CSF from four representative patients with SSPE with purified M protein are shown in Fig. 2C and D. At a 10^{-2} serum dilution, the mean OD of the sera from patients with SSPE (0.308 ± 0.103) was significantly greater ($P < 0.05$) than that of sera from patients with OND (0.198 ± 0.087) and higher than those of sera from healthy donors (0.210 ± 0.162). Geometric mean serum anti-M antibody titer for patients with SSPE was 3.35 ± 0.53. This value was higher than that obtained for patients with OND (3.05 ± 0.66) or healthy donors (3.12 ± 0.74), but the differences were not statistically significant (Tables 1 and 2).

Anti-M antibody was detected in the CSF of eight patients with SSPE. The mean titer was 2.59 ± 0.86. The levels in the CSF were lower than those in serum, except for two patients (Table 1, cases 2 and 4). In case 4, the titer in the CSF was 1 order of magnitude greater than that in the serum. Moreover, four patients with SSPE (cases 1, 4, 5, and 6) had ratios of anti-M reactivity in CSF to that in serum (OD ratio) greater than 1 (Fig. 3). These observations suggest intrathecal synthesis of anti-M antibodies in these patients. No anti-M antibodies were detectable in the CSF of the five patients with OND when tested at dilutions $\geq 10^{-1}$.

LPRs to purified MV proteins. The LPR to whole MV and to the purified M, NC, F, and HA proteins in four patients with SSPE was compared with results obtained with five healthy controls (Table 3). Although the mean LPR to each of these MV proteins was lower in the patients with SSPE, the individual responses were within the response range of the healthy individuals. Specifically, the LPRs to M protein obtained for four patients with SSPE (stimulation indices of

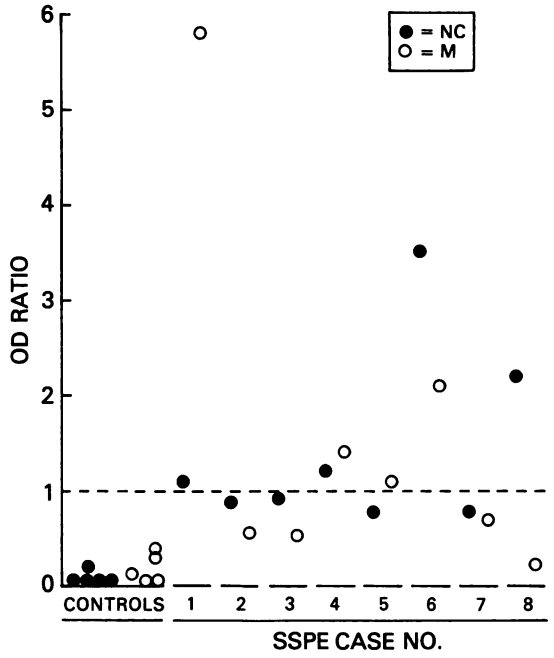

FIG. 3. Ratios of anti-NC or anti-M reactivity of CSF to that in serum for patients with SSPE and patients with OND (controls) measured at 3 h at a constant immunoglobulin G concentration. A ratio greater than 1 suggests intrathecal antibody synthesis.

13.9, 5.7, 3.0 and 2.9) were within the response range of the controls (stimulation indices of 2.5 to 20.9). Because two of the patients with SSPE (cases 1 and 2) were taking isoprinosine when the LPR was tested, the in vitro effect of this drug on the LPR to MV and to M protein was examined in four healthy controls and in one patient with SSPE (case 9) who was not on treatment. In pilot experiments, the optimal concentration of 100 μg of isoprinosine per ml was established. At this dose, there was a 30% enhancement in the LPR to MV in one of the four controls, but no effect on the LPR to MV or to M protein in the patient with SSPE or the other three controls was observed.

DISCUSSION

Previous studies (5, 6, 12, 27) using RIPA have described relatively low amounts of antibodies to the M protein in sera and CSF of patients with SSPE, in contrast to the high levels of antibodies to other MV proteins. For example, sera from only 4 of 28 patients with SSPE immunoprecipitated the M protein of MV from MV-infected cell lysates. In contrast, sera from patients with atypical measles precipitated all MV proteins at dilutions as high as 10^{-4} (6). It was suggested that defective synthesis or the lack of immune recognition of M protein may be the underlying cause for the lack of antibodies to this protein. However, in other reports, some reactivity of sera from patients with SSPE with M protein has been described (19, 24). In these previous studies, the methods used to evaluate antibodies to MV proteins were not quantitative. Because of this, an ELISA was developed for the quantitation of antibodies specific for MV proteins. With this approach, antibodies to M protein were detected in 11 serum and 8 CSF samples from the patients with SSPE. The log of anti-M antibody titers varied from 2.5 to 4.00 in the serum and from 1.18 to 4.00 in the CSF. The serum titers for the patients with SSPE were higher than those for controls but less than those for two patients with acute convalescent measles. Furthermore, for four of the patients with SSPE, there was evidence for intrathecal synthesis of anti-M antibodies.

In general, there was a correlation between the antibody titers measured by ELISA and the intensity of the corresponding band detected by RIPA. However, this relationship did not exist in specimens with low amounts of antibody. Analysis by SDS-polyacrylamide gel electrophoresis of such specimens gave faint bands that did not correlate with antibody titers detected by ELISA. For example, SSPE case 9 had an anti-M antibody titer of 3.0 but failed to show a band corresponding to M protein on SDS-polyacrylamide gels (Fig. 1A, lane 6). Another example is the serum from SSPE case 3 that had an anti-NC antibody titer greater than that for SSPE case 4 (Tables 1 and 2), yet the corresponding band in the specimen from case 3 was fainter than that obtained from case 4 (Fig. 1C, lanes 3 and 4). The discrepancy between the intensity of a band and the ELISA titer in some specimens may be related to the antibody/antigen ratio

TABLE 3. LPRs of patients with SSPE and controls to MV and its purified proteins

Group	Radioactive cpm[a] (stimulation index)				
	MV	M	NC	F	HA
Healthy donors (n = 5)					
Range	3,787–20,413 (7.5–19.7)	3,480–16,155 (2.5–20.9)	3,679–21,086 (2.4–30.7)	3,868–21,461 (3.4–31.3)	3,207–11,988 (2.8–17.5)
Mean ± SD	9,660 ± 7,223 (13.0 ± 5.0)	8,451 ± 6,253 (8.6 ± 7.6)	8,175 ± 7,355 (10.1 ± 12)	8,990 ± 7,317 (10.1 ± 11.9)	6,368 ± 3,352 (7.0 ± 6.2)
SSPE cases					
1	4,214 ± 964 (21.5)	8,847 ± 1,795 (13.9)	3,844 ± 454 (6.0)	4,067 ± 1,062 (6.4)	2,731 ± 871 (4.3)
2	21,122 ± 6,456 (13.1)	7,321 ± 1,373 (5.7)	4,813 ± 820 (4.3)	7,902 ± 796 (7.0)	8,144 ± 666 (7.2)
9	26,586 ± 4,505 (21.8)	968 ± 75 (3.0)	7,152 ± 1,013 (4.1)	2,816 ± 124 (1.6)	3,913 ± 139 (2.2)
10	4,321 ± 1,293 (4.1)	4,531 ± 570 (2.9)	5,652 ± 1,328 (3.6)	5,919 ± 1,071 (3.7)	4,456 ± 999 (2.8)
Mean ± SD	14,061 ± 11,526 (15.1 ± 8.0)	5,417 ± 3,463 (6.4 ± 5.2)	5,365 ± 1,402 (4.5 ± 1)	5,176 ± 2,226 (4.7 ± 2.5)	4,811 ± 2,336 (4.1 ± 2.2)

[a] Values are means ± SDs. The background counts per minute ranged from 196 to 1,617.

in the RIPA. This discrepancy emphasizes the importance of using a quantitative assay for the comparison of antibodies to MV proteins, particularly at levels that are low or undetectable by RIPA.

The relatively low amount of antibody to M protein is not specific for patients with SSPE. Sera from some healthy individuals, patients with OND, and patients with conditions associated with increased MV antibody titers, such as chronic active hepatitis (16), contain less antibody to M protein than to NC. The mechanisms responsible for the lower amount of antibody to M protein than to NC are not known. NC protein may be produced in larger amounts than M protein and thus provides a more potent immunologic stimulus. Another possibility is that the responses to NC and M proteins are different. To assess this hypothesis, the LPRs to HA, F, and NC proteins were compared. The responses of patients with SSPE to MV and its purified structural proteins were comparable with those of healthy individuals. Further, significant differences in the response to M protein compared with HA, F, or NC protein were not observed. It is therefore unlikely that a diminished cellular immune response to M protein accounts for the relative reduction in anti-M antibodies in patients with SSPE.

It is possible that the level of anti-M antibodies fluctuates during the course of SSPE, and this fluctuation can account for the differences in the results reported from various laboratories. Multiple samples were not available to allow us to examine this possibility, but sera from patients at different stages of the disease were studied and no correlation between disease status and level of antibody to M protein was observed.

Previous studies of the LPR to MV in patients with SSPE have reported conflicting results (4, 8, 14, 20, 23, 26; E. B. Matthew, M. Krasny, D. A. Fucillo, and J. L. Sever, Arch. Neurol. 32:497–498, 1975). Some investigators described low or undetectable responses (8, 23, 26), and in an occasional patient, a high response was found (20). This is consistent with the LPR to MV in normal patients and in disorders other than SSPE (13). These variations in results may be related to differences in methodology, antigen preparations, and concentrations of MV used. To minimize such variables, MV proteins of known purity and in optimal concentration were used in this study. It is noteworthy that two of the patients with SSPE (cases 1 and 2) were on isoprinosine treatment at the time peripheral blood lymphocytes were obtained. Isoprinosine has been shown by in vitro studies to restore concanavalin A-induced lymphocyte proliferation in aging humans (25) and to enhance phytohemagglutinin and Epstein-Barr virus-induced lymphoproliferation in healthy donors (21). In view of these findings, the possibility that the LPR to MV and its proteins in the two patients with SSPE was enhanced by isoprinosine was considered. The results do not support a role for isoprinosine in the LPR to MV proteins. However, because LPR studies were not performed prior to isoprinosine treatment, the effect of this agent on the LPR of these two patients with SSPE was not conclusively established.

The progressive nature of MV infection in SSPE in spite of the presence of high levels of MV antibodies in the CSF and serum suggests that the disease is not due to a defect in the humoral immune response. The presence of both antibody and LPR to M protein indicates that this protein is being produced, a finding consistent with the observation of Norrby et al. (15). These findings do not exclude the possibility that M-protein production or function or both are altered in a manner that affects viral mutation but not the immune response.

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