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VIRUS ANTIBODIES IN THE CEREBROSPINAL FLUID OF MULTIPLE SCLEROSIS PATIENTS DETECTED WITH ELISA TESTS

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SUMMARY

The enzyme-linked immunosorbent assay (ELISA) was used to determine levels of specific IgG antibodies against measles, rubella, vaccinia, corona (OC43) and mumps viruses in cerebrospinal fluid (CSF) and serum of 18 patients with clinically definite multiple sclerosis (MS), 8 patients with optic neuritis (ON), 27 patients with other neurological disease (OND), and 88 control subjects without central nervous system disease. Serum antibody levels were not significantly different between the four groups. Differences in the frequency and levels of CSF antibodies between the four groups were observed. Control patients had serum/CSF antibody ratios from 2.0 to 3.0 (log) with an average of 2.5 corresponding to a 320-fold difference between serum and CSF antibody levels. MS patients had ratios from 1.1 to 2.1 with an average of 1.6. The average was 2.0 for the ON patients. The average for the OND patients was similar to the controls. The altered serum/CSF ratios for several viruses within an individual patient was similar. These results suggest that nonspecific immunostimulation is responsible for the increased levels of CSF virus antibodies.

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INTRODUCTION

Increased antibody titers against several viruses have been found in cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients. The most constant finding has been elevated levels of measles antibodies but elevated rubella, herpes simplex, vaccinia and mumps antibodies also have been found (Haire 1977; Norrby 1978). The relative amount of CSF antibodies compared to serum antibody levels is higher in many MS patients than in most controls and has been associated with an intrathecal synthesis of antibodies (Frick and Sheid-Seydel 1958; Tourtellotte and Parker 1966; Norrby 1978). However, the low sensitivity of conventional serological techniques has limited the evaluation of CSF antibody levels in normal controls due to the low amounts of antiviral antibodies present. We have recently developed a sensitive enzyme immunoassay for virus antibodies (Leinikki and Pässilä 1976; Leinikki et al. 1978). By using this technique, we studied the occurrence and levels of CSF antibodies against unrelated viral antigens in MS and control groups.

MATERIALS AND METHODS

(1) Patient groups

Patients admitted to the outpatient Department of Neurology, University Central Hospital, Helsinki, Finland and in whom a lumbar puncture was performed at the admission were initially included. Careful clinical and laboratory investigations subsequently established the diagnosis and placed the patient either to clinically definite MS, optic neuritis (ON), other neurological disease (OND) or control group. The criteria for MS were those of Schumacher et al. (1965). The exclusion of neurological disease in the control group was based both on clinical examination following admission and on a further clinical follow-up. Altogether, 18 MS patients, 8 ON, and 27 OND patients were studied. The control group included 88 subjects with symptoms such as headache, vertigo, pain in the neck, hyperventilation without evidence of central nervous system (CNS) disease. CSFs were coded before study in the laboratory.

(2) ELISA for viral antibodies

Disposable polystyrene cuvettes and FP-9 photometer (Labsystems, Helsinki, Finland) were used as described earlier (Leinikki and Pässilä 1976). Measles (Edmonston strain) and rubella (Gilcrest strain) antigens (Leinikki et al. 1978) were prepared by purifying virions in sucrose gradients. Crude, commercially available vaccinia (Microbiological Associates, Bethesda, MD) and mumps (Enders strain) were used (Leinikki et al. 1979). Coronavirus antigen was prepared by growing strain OC43 in mouse brain and purified in sucrose gradient. Heavy chain-specific, alkaline phosphatase-conjugated anti-human IgG was prepared as described earlier (Leinikki and Pässilä 1976). Serum samples were diluted 1 : 50 and 1 : 500 while the CSFs were diluted 1 : 5. The amount of antibodies was calculated from a logarithmic scale where the dilution of the sample was compared to the dilution

of a reference serum giving the same optical absorbance. A zero-point was determined by analyzing negative sera. This method of quantitation was analogous to our previous "ED method" (Leinikki and Pässilä 1977) except that the scale was now positive: the higher the logarithmic value, the higher the antibody activity in the test sample.

(3) Other tests

Serum and CSF albumin and CSF IgG and total CSF protein were analyzed by automated fluoronephelometry (Technicon Autoanalyzer®). Blood–brain barrier was scored moderately impaired if the above mentioned values were from 2 to 6 standard deviations above the average of the control material and severely impaired if the deviation was more than 6 standard deviations.

RESULTS

The number of patients in each group who had antibody in the serum was very similar. Seventeen out of 18 MS patients had detectable measles antibodies and all 18 had rubella antibodies. All 88 patients in the control group had measles and rubella antibodies. The frequency of other viral antibodies was similar with each virus tested in all groups (Table 1). Vaccina virus antibody levels were generally very low, reflecting the infrequent vaccination of the Finnish population during the last ten years.

Viral antibodies were detected in the CSF samples of all groups, although the levels and the frequencies were lower in the control and OND groups than in MS and ON. This was also reflected in differences in the serum/CSF antibody ratios. The average serum/CSF antibody ratio, calculated for each individual patient for all viral antibodies, varied from 3.0 to 2.0 in control patients while in MS patients the range was from 2.1 to 1.1 (Fig. 1). ON patients had a ratio range between MS and control patients while OND patients showed a wider variation from 3.0 to 1.6. A lower than average index (2.0) in OND and control patients seemed to be

TABLE 1

MEAN VALUES (±SD) (log scale) OF SERUM ANTIBODY LEVELS AND NUMBERS OF SEROPOSITIVES (N) IN DIFFERENT PATIENT GROUPS

Virus	Patient group						Controls	(N)
	MS	(N)	ON	(N)	OND	(N)		
Measles	1.9 ± 0.6	(17)	1.7 ± 0.2	(8)	1.8 ± 0.5	(27)	1.7 ± 0.5	(88)
Rubella	1.6 ± 0.4	(18)	1.6 ± 0.5	(6)	1.9 ± 0.6	(26)	1.7 ± 0.5	(88)
Vaccinia	0.6 ± 0.7	(12)	0.5 ± 0.2	(7)	0.7 ± 0.4	(25)	0.7 ± 0.3	(73)
Corona	0.6 ± 0.3	(7)	0.6 ± 0.2	(5)	0.7 ± 0.4	(21)	0.8 ± 0.4	(78)
Mumps	0.9 ± 0.3	(14)	1.3 ± 0.3	(8)	1.0 ± 0.3	(23)	1.1 ± 0.5	(86)

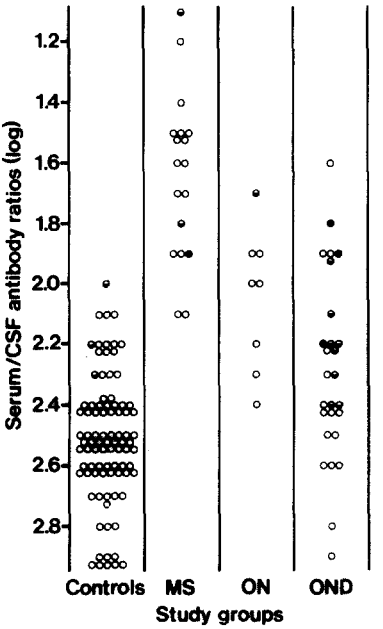

Fig. 1. Distribution of mean serum/CSF virus (measles, rubella, vaccinia, corona OC43 and mumps) antibody ratios (log) related to blood–brain barrier function in different patient groups. Note that the ratios are highest in control subjects and lowest in MS patients without any relation to blood–brain barrier. O = intact blood–brain barrier; ◒ = moderately impaired blood–brain barrier; ● = severely impaired blood–brain barrier.

associated with an impaired blood–brain barrier, but this was not the case in MS and ON patients (Fig. 1).

The average serum/CSF ratios of different viral antibodies in the study groups are presented in Table 2. The control group showed relatively little variation from one virus antibody to another with an average for all antibodies of 2.5. This corresponds to a 320-fold difference between serum and CSF antibodies. In MS the average ratios varied from 1.3 for vaccinia to 1.8 for mumps virus antibodies with an overall average of 1.6. The ratios in ON were between those of MS patients and controls; only the vaccinia virus antibody ratio was less than 2.0 and this was derived from two patients. The average ratios in OND patients were slightly lower than in controls.

The serum/CSF ratios were analyzed statistically by a multiple regression analysis and compared with other data of serum and CSF proteins including CSF total protein, CSF albumin and serum/CSF albumin ratio. No significant correlation was found in MS or ON groups indicating that the altered serum/CSF antibody ratio was not due to altered blood–brain barrier.

The serum/CSF antibody ratio, in general, was very similar for different viruses in individual patients. This was equally true for control patients with normal ratios as well as for MS patients with abnormal ratios. A few examples are given in Table 3.

TABLE 2

MEAN (± SD) OF SERUM/CSF VIRUS ANTIBODY RATIOS (log scale) IN VARIOUS PATIENT GROUPS

Virus	Patient group						Controls	(N)
	MS	(N)[a]	ON	(N)	OND	(N)		
Measles	1.6 ± 0.2	(17)	2.0 ± 0.3	(8)	2.2 ± 0.3	(27)	2.5 ± 0.2	(78)
Rubella	1.6 ± 0.3	(18)	2.1 ± 0.3	(6)	2.4 ± 0.2	(26)	2.5 ± 0.2	(74)
Vaccinia	1.3 ± 0.7	(4)	1.5 ± 0.2	(2)	2.2 ± 0.3	(6)	2.3 ± 0.2	(8)
Corona	1.5 ± 0.1	(4)	—	(0)	2.1 ± 0.2	(9)	2.3 ± 0.2	(8)
Mumps	1.8 ± 0.1	(9)	2.1 ± 0.2	(6)	2.3 ± 0.1	(7)	2.5 ± 0.3	(31)
All viruses	1.6 ± 0.3	(18)	2.1 ± 0.2	(8)	2.3 ± 0.3	(27)	2.5 ± 0.2	(87)

[a] Number of patients with detectable CSF antibody levels.

TABLES 3

EXAMPLES OF SERUM/CSF VIRUS ANTIBODY RATIOS (log scale) IN DIFFERENT PATIENT GROUPS AS MEASURES BY ELISA AGAINST DIFFERENT VIRAL ANTIGENS

Clinical groups	Case No.	Serum/CSF-antibody ratio				
		Viral antigen				
		Measles	Rubella	Vaccinia	Corona	Mumps
MS						
	1	1.5	1.4	1.5	1.4	1.5
	2	1.3	1.6	—[a]	1.4	2.1
	3	1.6	1.4	—	1.4	—
	4	1.3	1.5	1.4	—	—
	5	2.0	1.9	—	—	1.7
Controls						
	1	2.9	2.8	—	—	—
	2	2.6	2.7	—	—	—
	3	2.3	2.1	—	—	—
	4	2.3	2.1	—	—	—
	5	2.5	2.6	—	—	—
ON						
	1	2.2	2.6	2.1	—	—
	2	2.3	2.3	—	—	2.6
	3	1.7	1.8	1.7	—	—
	4	1.4	—	1.3	—	1.8
	5	2.3	2.3	—	—	2.3
OND						
	1	1.9	2.0	1.9	1.8	—
	2	2.3	2.1	—	—	2.2
	3	2.4	2.4	—	—	—
	4	2.3	2.4	2.7	2.4	2.4
	5	2.0	2.4	2.7	2.1	—

[a] Serum and/or CSF antibody levels undetectable.

DISCUSSION

The sensitivity of the ELISA test permits the detection of CSF antibodies when the amount is too low to be detected by conventional serological methods. The serum/CSF ratio for total IgG is about 320 (2.5 in log scale) in normal, healthy individuals. Alteration of this ratio may be associated with *in situ* formation of antibody in the CNS or impairment of the blood–brain barrier. The increased sensitivity of the present ELISA technique made it possible to detect viral antibodies in CSF in a majority of the control subjects. In fact, all individuals who had serum antibodies against measles and/or rubella had detectable levels in their CSF. Corona, vaccinia, and mumps virus antibodies when occurring in low concentrations in the serum could not be detected in the CSF.

The simultaneous elevation of multiple antibody levels in the CSF of MS patients was clearly demonstrated in the present study. The role and evolution of viral antibodies in the CSF of MS patients is not understood. The diversity of the viral agents that have been implicated in MS makes it improbable that any of these agents or some cross-reacting, unknown agent would cause the simultaneous multiple antibody stimulus observed in this study. The best explanation is that the viral antibodies produced within the CNS of MS patients are related to the immunopathophysiology of the disease rather than a specific etiology. Altered serum/CSF virus antibody ratios have also been occasionally detected among patients with other neurological diseases (Norrby 1978; Iivanainen et al. 1981). In diseases such as subacute sclerosing panencephalitis (SSPE) the serum/CSF antibody ratio is exclusively altered for measles virus antibodies (P. Leinikki, D. McFarlin, I. Shekarchi and J. Sever, unpublished observation). It would be of interest to study multiple virus antibody ratios in acute inflammatory diseases such as mumps meningitis, uncomplicated measles, encephalitis, neurosyphilis and toxoplasmosis.

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