

Viral Antibody in the Cerebrospinal Fluid of Patients with Acute Central Nervous System Infections

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Cerebrospinal fluid (CSF) and sera from 129 patients of a study population of 139 were tested for antibody to herpes simplex, measles, and mumps viruses. Herpes simplex virus antibody was found in three of five patients with laboratory-confirmed herpes simplex infection and in eight patients without serological or virological evidence of current infection with this or other common neurotropic viruses. Eleven of the 139 patients were studied for antibody to lymphocytic choriomeningitis (LCM) virus. Eight of these had laboratory-confirmed LCM infection, and antibody was detected in the CSF of five of them. In one of these five, complement-fixing antibody appeared earlier in the CSF than in the blood. Assay of LCM virus antibody in the CSF may thus indicate infection with LCM virus more rapidly than serological and virological studies. The diagnostic and the possible prognostic significance of herpes simplex virus antibody in CSF remains to be ascertained.

Measles antibody has been found in the cerebrospinal fluid (CSF) of patients with chronic or degenerative conditions such as multiple sclerosis (5, 21, 24) and subacute sclerosing panencephalitis (9, 10, 18). On occasion, antibodies to other viral agents, including mumps, herpes simplex, rubella, and varicella, have also been observed in the CSF (5, 6, 18, 21, 25). Herpes simplex antibody has been found in the CSF of patients with encephalitis and primary herpes simplex infections and in patients with evidence of preexisting herpes simplex infection (6, 19, 20). Presence of antibody in CSF may reflect a lesion in the blood-brain barrier. Evidence is accumulating, however, which suggests that there is also local production of these antibodies (6, 9, 10, 19, 20, 21, 24), possibly induced by an antigenic stimulus from an activation of latent virus (18, 21), from viral components formed in an incomplete cycle of viral replication (5, 21), or from components of latent virus released from cells destroyed by other agents (24). Although the presence of viral antibody in the CSF does not by itself confirm a viral etiology, herpes simplex antibody findings have been accepted as supportive evidence in patients with encephalitis (19, 20).

Any of the mechanisms that could cause the appearance of viral antibody in the CSF of patients with chronic central nervous system (CNS) diseases could presumably be invoked in acute CNS illness. In the present study, evidence for viral antibody was found in the CSF of patients with aseptic meningitis, meningoen-

cephalitis, or encephalitis. The data indicate that in some cases a local production of antibody has occurred and that in primary infections the findings may be evidence of viral etiology.

MATERIALS AND METHODS

The study population was 139 patients with clinical diagnoses of aseptic meningitis (42 patients), meningoencephalitis (17), encephalitis (51), or other CNS infections (18), or with suspected CNS infections (11). Samples from all patients were submitted to the virus diagnostic services for isolation attempts and antibody assays for neurotropic viruses. In 32 patients, evidence of a viral etiology was found: 16 by virus isolation from CSF, from brain obtained at biopsy, and/or seroconversion; eight by virus isolation from stool and/or throat swabs; and eight by unusual antibody findings. The viral agents implicated were lymphocytic choriomeningitis (LCM), eight patients), enteroviruses (eight), herpes simplex (five), mumps (five), influenza B(two), and influenza A, measles, varicella, and California encephalitis (one each).

For comparison of antibody levels in CSF and serum, both samples were tested simultaneously in the same assay. Antibodies against herpes simplex (strain MacIntyre), measles (strain Edmonston), mumps (strain Enders), and LCM (strain WE) viruses were assayed by the indirect immunofluorescence test. Virus-infected cells (human embryonic lung cells for measles and herpes simplex viruses, BHK-21 cells for mumps and LCM viruses) were the antigens. The staining procedure followed a previously published protocol (8). If antibody was detected in the CSF, additional tests were performed depending on the available amounts of the samples.

Evidence for local antibody production in the CNS was determined by a procedure based on the observations of Clarke et al. (7) and Connolly et al. (10). If antibody was detected in the CSF, a ratio was calculated for the serum:CSF titers of the individual patient. This ratio was compared with the ratio of the patient's antibody against a reference virus, such as polio 1 (strain Mahoney), 2 (strain YSK), or 3 (strain Leon), or Coxsackie B2 (strain Ohio 1), 3 (strain Nancy), or 4 (strain JVB), as determined by a micro-neutralization test in LLCMK₂ cells. Test doses of virus were 32 to 320 mean tissue culture infective doses. Virus and serum or CSF dilutions were incubated for 1 h at 22 C. A difference was considered to be significant if the ratio was at least fourfold lower than the corresponding ratio for the reference antibody (21). Low serum:CSF antibody ratios (≤ 4) were considered as additional presumptive evidence for local antibody production. Some samples of special interest were tested for total albumin, immunoglobulin (Ig) G, and IgM by radial immunodiffusion (15). For assay of IgM-associated antibody, samples were fractionated by sucrose gradient centrifugation (3, 4), and the fractions were tested for virus immunofluorescent antibody and for IgG and IgM by radial immunodiffusion.

RESULTS

Sera and CSF from 129 of the 139 patients were tested for antibody to herpes simplex, measles, and mumps viruses. These agents were chosen for their neuropathogenicity. In addition, herpes simplex and mumps viruses are major pathogens in CNS infections in New York State (11-13), and herpes simplex and measles virus may be able to remain latent in nerve cells of the peripheral or CNS tissue (1, 2, 17, 23). The incidence of serum antibodies to these viruses generally increased with age but decreased for mumps in individuals 51 years or older (Table 1). The level of antibody, expressed as the geometric mean of the titer for each age

group, was highest for measles in school children and for herpes simplex and mumps in young adults.

In 11 patients, herpes simplex antibody was detected in the CSF collected 3 to 150 days after onset of symptoms (Table 2).

The CSF of 11 of the 139 patients were studied for LCM virus antibody in separate studies during a statewide outbreak of pet hamster-associated LCM (14) and during a local outbreak at a university research facility. Antibody was detected in five of these patients in the CSF collected 4 to 24 days after onset of symptoms (Tables 2 and 3).

A total of 16 patients thus had CSF antibody to either herpes simplex or LCM virus. For 11 of these patients the serum:CSF antibody ratio could be compared with the ratio of a reference antibody. Fourfold or greater difference in the ratios suggested a local antibody production to herpes simplex virus in four patients and to LCM virus in four others (Table 2). For three patients with LCM antibody, enough material was available for us to determine the immune globulin class of the CSF and serum antibody. Evidence for both IgG- and IgM-associated LCM serum antibody was obtained in one patient (Table 3). IgM and IgG were demonstrated in this patient's CSF, but where the LCM antibody class could be determined, the activity was associated only with IgG. The LCM antibody in the other two patients was also IgG-associated.

DISCUSSION

Virus antibody occurs frequently in the CSF of patients with clinical manifestations of acute CNS infections where there is laboratory evidence of herpes simplex or LCM virus etiology.

TABLE 1. Serum antibody determined by the indirect immunofluorescence method in 129 patients with CNS illness

Age group (years)	No. studied	Patients with serum antibody ≥ 4 to:								
		Herpes simplex			Measles			Mumps		
		No.	%	GM ^a	No.	%	GM ^a	No.	%	GM ^a
≤ 0.5	3	2		64	0			0		
$>0.5-5$	23	9	39	11	5	22	5	5	22	14
6-18	34	15	44	17	11	32	20	11	32	14
19-35	32	23	72	43	19	59	17	11	34	18
36-50	21	16	76	25	14	67	17	7	33	10
51-65	16	14	88	39	11	69	12	3	19	5
Total	129	79			60			37		

^a GM, Geometric mean titer.

TABLE 2. Findings on patients with herpes simplex or LCM virus antibody in CSF

Virus and patient (age and sex) ^a	Diagnosis	Antibody titer			Reference antibody		Other diagnostic findings ^a
		Days after onset	CSF	Serum: CSF ^b	Virus ^a	Serum: CSF ^b	
Herpes simplex							
1(NB, M)	Meningitis	5	4	32	ND		-
2(7, M)	Encephalitis	3	4	8	Coxsackie B2	8	Herpes simplex ^c
3(18, F)	Meningitis	NG	4	16	ND		-
4(21, F)	Encephalitis	4	128	4	Measles ^d	≥16 ^e	-
5(24, M)	Meningitis	10	16	32	Coxsackie B2	≥64	Herpes simplex ^c
6(24, M)	Meningitis, herpes genitalis	12	32	2	Coxsackie B1	128 ^e	-
7(25, F)	Encephalitis	150	16	4	Polio 3	32 ^e	-
8(29, F)	Meningitis	16	8	4	ND		-
9(38, F)	Meningitis	4	8	8	Polio 2	64 ^e	-
10(42, F)	Meningitis	5	4	4	ND		-
11(85, F)	Encephalitis	33	16	32	Coxsackie B4	16	Herpes simplex ^c
LCM							
12(22, M)	Meningitis	10	128	1	Coxsackie B3	16 ^e	LCM ^c
13(42, F)	Meningoencephalitis	14	8	4	Herpes simplex ^d	≥64 ^e	LCM ^f
14(43, M)	Meningoencephalitis	4	16	2	Coxsackie B4	≥64 ^e	LCM ^{c, g}
15(NG, F)	Meningitis	16	8	8	Coxsackie B4	≥128 ^e	LCM ^f
16(NG, M)	Meningitis?	17	8	8	ND		LCM ^f

^a NB, Newborn; M, male; F, female; NG, not given; -, no diagnostic findings by virus isolation and serology; ND, not done; LCM, lymphocytic choriomeningitis.

^b CSF and sera were collected within 24 h of each other with the following exceptions: CSF of patients 9 and 12 were collected 4 and 5 days, respectively, before the blood. CSF of patient 8 was collected 11 days after the blood.

^c Antibody conversion.

^d Immunofluorescent antibody. All others are neutralizing antibodies.

^e Reference antibody ≥fourfold higher than antibody ratio.

^f Unusual antibody and/or CF titer ≥64.

^g Virus isolation.

TABLE 3. Serological findings in sera and CSF of patient 12 (See Table 2)^a

Days after onset	Serum					CSF				
	LCM antibody		Albumin (mg/dl)	IgG (mg/dl)	IgM (mg/dl)	LCM antibody		Albumin (mg/dl)	IgG (mg/dl)	IgM (mg/dl)
	IF	CF				IF	CF			
6	<4	<4	5,700	900	56					
9						64 ^b	<4	420	66	26
10	128 ^c	<4	3,530	632	83	128 ^d	8	570	90	28
15						16 ^d	8	ND	Positive <90	Positive <28
19	128 ^c	8	3,300	1,000	125					
24						8 ^d	<4	ND	ND	ND
31	≥128 ^b	8	4,400	925	101					
33	128 ^b	8	4,800	975	118					

^a LCM, Lymphocytic choriomeningitis; IF, indirect immunofluorescence test; CF, complement fixation test; ND, not done.

^b LCM antibody activity in IgG but not in IgM fraction.

^c LCM antibody activity in IgG and IgM fractions.

^d Insufficient amounts for differentiation of IgG- and IgM-associated LCM antibody.

The data presented here suggest that some of the LCM antibody in the CSF is produced in the CNS. This assumption is supported by the relatively low (≤4) serum:CSF LCM antibody ratio in three patients, the high ratio for the refer-

ence antibody in four patients, and the appearance of complement-fixing (CF) antibody in the CSF of one patient (Table 3) before it could be detected in the serum. That the appearance of CF antibody in the CSF may precede the serum

antibody has also been observed in measles and respiratory syncytial virus infections (6) and has been interpreted as evidence of local antibody production. In our patient, however, the albumin and globulin levels in the CSF also indicated a minor lesion of the blood-brain barrier.

LCM virus is a rodent agent, and its spread in man is an unusual event. It can invade the CNS and has been isolated from the CSF (14). The production of LCM CSF antibody is most likely stimulated by the invasion of the virus into the CNS in a primary infection. Therefore these findings are of significance and can be considered evidence for the etiology of the disease.

The findings of herpes simplex virus immunofluorescent antibody in the CSF of three patients with herpes encephalitis or meningitis are analogous to the finding by MacCallum et al. (20) of CF and neutralizing herpes simplex antibody and by Lerner et al. (19) of passive hemagglutinating herpes antibody in patients with herpetic encephalitis. However, the remaining eight patients with herpes simplex virus antibody had no evidence of a current infection with this or any other common virus. An explanation for the appearance of herpes simplex virus antibody activity in the CSF is therefore more difficult. Herpes simplex virus is carried asymptotically, possibly in nerve cells (1, 2), and involves the CNS in primary infections as well as in reinfections (16, 22). The virus is widespread, as indicated by the incidence of its antibody (Table 1). Activation of latent herpesvirus that results in production of complete or incomplete virus, or leaking of "dormant" herpesvirus or its components from cells damaged by other factors, may induce local antibody formation. This would be analogous to the events that are considered to cause the stimulation of measles antibody production in the CNS of patients with multiple sclerosis or subacute sclerosing panencephalitis.

Five of the 11 patients with herpesvirus CSF antibody had relatively low serum:CSF ratios (≤ 4). In three of these patients and in one additional person, with a ratio of 8, the reference antibody ratios were significantly higher. Thus, in approximately half of the cases, the data are compatible with the assumption of some local antibody production. In three other patients, however, herpes simplex virus antibody ratios of 32 suggest a leaking of high-level serum antibody through lesions of the blood-brain barrier. The finding of herpes simplex antibody in the CSF does not necessarily implicate this virus in the etiology of the clinical

disease. It may reflect instead the nonspecific results of lesions caused by other factors. For this reason, the diagnostic significance of such findings is uncertain.

Of the 11 patients with CSF herpes simplex antibody, none died, whereas in our experience from 1972 to 1974 10 of 34 patients with herpes encephalitis confirmed by isolation and/or serum conversion expired. These observations suggest a protective effect. However, CSF was submitted from only one patient with fatal, laboratory-confirmed herpes simplex encephalitis, and thus the incidence of herpes simplex virus antibody in the CSF of such patients could not be determined. Therefore, at this time, the prognostic significance of herpes antibody in CSF cannot be ascertained and requires further study.

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