Varicella-Zoster-Associated Encephalitis: Detection of Specific Antibody in Cerebrospinal Fluid

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Varicella-zoster (VZ) antibody measured by indirect immunofluorescence was used to identify patients with encephalitis due to VZ virus. In 15 of 16 (94%) patients with VZ infection and clinical evidence of central nervous system involvement, VZ antibody was present at a titer of 1:2 or greater in cerebrospinal fluid. In contrast, no VZ antibody was detected in the cerebrospinal fluid of seven patients with VZ infections without clinical signs of central nervous system involvement, nor was any detected in the cerebrospinal fluid of 25 patients with malignant or demyelinating disease or encephalitis due to other viral or toxic agents. All of these patients had detectable serum VZ antibody. Thus, in this study the presence of detectable VZ antibody in cerebrospinal fluid measured by indirect immunofluorescence seemed to correlate with clinical evidence of central nervous system involvement due to this virus.

The diagnosis of encephalitis caused by varicella-zoster (VZ) virus may be difficult to establish clinically. In certain forms of measles encephalitis (2, 9) and in encephalitis due to herpes simplex virus (5, 6), however, an increase of specific antibody in cerebrospinal fluid (CSF) has been observed and has been used for diagnosis of these conditions. An uncontrolled study of two patients suggested that this might also be true in VZ infections with encephalitis (7). We therefore examined the VZ antibody titers in CSF in patients with VZ infections and clinical encephalitis as well as in controls, to determine whether VZ antibody could be found in CSF and whether its detection could be helpful diagnostically.

MATERIALS AND METHODS

Tissue culture, virus, and VZ antibody assay. VZ virus was propagated in human embryonic lung fibroblasts as described previously (10). VZ antibody was measured by indirect membrane immunofluorescence (FAMA) with unfixed, infected tissue culture cells. This antibody assay is extremely sensitive and compares favorably with neutralization (4). A FAMA titer of 1:256 or greater is associated with VZ disease. This technique may be used to study immunoglobulin G (IgG) and IgM (1). We did not use complement fixation for testing because of its insensitivity and because, in our experience, CSF is anticomplementary in this assay.

Patients. Patients were seen by at least one of us or our associates. Patients with VZ infections with and without clinically diagnosed encephalitis and with meningitis and other kinds of encephalitis were studied. The patients with VZ encephalitis were identified

as VZ associated before knowledge of the results of CSF analysis for VZ antibodies. Patients with VZ infections and no evidence of VZ encephalitis had lumbar punctures performed for other clinically indicated reasons. Serum and CSF were obtained from most patients. These were stored at -20°C until testing. All specimens were coded and tested blindly. CSF was tested undiluted, at 1:2, and at doubling dilutions thereafter to endpoint. Serum was tested from 1:2 to endpoint. When there was sufficient CSF, we also looked for VZ IgM antibody.

RESULTS

There were 16 patients with varicella or zoster with clinical evidence of central nervous system (CNS) involvement (Table 1). Their symptoms included signs of encephalitis, such as lethargy, confusion, and obtundation or hallucinations or both. Other patients exhibited weakness, paralysis, and perturbations of sensation, such as numbness and paresthesias.

Samples of CSF were available from all patients with clinical CNS involvement. In 15 out of 16 patients, the VZ antibody titer in CSF measured by FAMA was equal to or greater than 1:2. In 6 of 12 patients VZ IgM antibody in CSF ranged between 1:2 and 1:4. In two patients, the VZ CSF titers were extremely high, 1:64 (no. 15) and 1:256 (no. 8). The one patient in whom the CSF VZ antibody titer was $<1:2$ had VZ virus isolated from the spinal fluid.

There appeared to be a temporal relationship between the presence of CNS symptoms and detection of VZ antibody in CSF. One patient (no. 7) had no CNS involvement initially and no

TABLE 1. *VZ infections with CNS involvement*

Patient no.	Titer (time) ^a		Cells ^b in CSF per mm ³		Diagnosis of VZ infection ^c	Symptoms ^d
	Serum	CSF	RBC	WBC (% lymphocytes)		
1	256 (5)	<1 (5)	0	3 (33%)	C, S	V; cerebral encephalitis; slow recovery
2	512 (10)	4 (10), IgM <2	0	8 (99%)	C, S	V; cerebral encephalitis; recovered
3	512 (9)	16 (9), IgM <2 ^e	0	59 (30%)	C, S	V; transverse myelitis 4 days after onset
4	256 (31) 256 (3 mo)	2 (30) ^e 4 (3 mo), IgM 1:2 ^e	NA/ 298	NA 10 (100%)	C, S	Z, cranial; facial paralysis persistent and progressive over a 3-mo period
5	NA	4 (6 wk), IgM <2	0	80 (84%)	C	Z, ophthalmic; ataxia, confusion 1 mo after onset
6	256 (4)	4 (11)	0	103 (90%)	S	No external skin lesions; Bell's palsy; confusion
7	32 (2 mo) 4 (8)	NA <1 (8), IgM <2	0	38 (20%)	C, S	DZ, ophthalmic; hemiplegic 3 mo after onset; recovered
	64 (3 mo)	16 (3 mo), IgM <2	0	16 (100%)		
8	NA	256 (2), IgM 1:2 ^e	0	100 (98%)	C	DZ, thoracic; confusion, lethargy, obtundation, underlying sarcoid; recovered
9	NA NA	8 (4), IgM $\leq 1:4^e$ 4 (10), IgM <2 ^e	0 0	261 (100%) 65 (95%)	C	DZ, ophthalmic; confused, obtunded; underlying obstructive pulmonary disease; recovered
10	NA	4 (7)	0	14 (100%)	C	DZ, sacral; slurred speech, underlying Marfan's syndrome
11	NA	4 (14)	0	33 (100%)	C, V	DZ, cervical; VZ virus isolated from CSF; obtunded; underlying lymphoma; expired
12	NA	2 (1), IgM <1:2	0	40 (85%)	C, V	DZ, thoracic; VZ virus isolated from CSF; hallucinations, altered mental state, lasting 1 day; renal transplant
13	16 (7) 64 (20)	NA 8 (21), IgM 1:2	0 0	560 478 (100%)	C, S	DZ, sacral; lethargic, stiff neck; eyes deviated to right, neurogenic bladder; underlying Hodgkin's disease; Ara-A; recovered
14	512 (6 wk)	4 (6 wk), IgM <1:2 ^e	0	0	C, S	DZ, thoracic; lethargic and confused 6 wk after onset; underlying carcinoma of lung; Ara-A; recovered
15	16 (4) NA	4 (4), IgM <1:2 64 (14), IgM 1:4	300	400 (NA)	C	DZ, thoracic; confused, semicomatose; underlying acute lymphatic leukemia; Ara-A; recovered
16	2,048 (5) NA	NA 4 (30), IgM 1:2	1,500	10 (NA)	C, S	DZ, cranial; paralysis 3 wk after onset of rash

^a Reciprocal of titer determined by FAMA at time (days or as indicated) after onset of rash.^b RBC, Erythrocytes; WBC, leukocytes.^c C, Clinical; S, serological; V, virus isolation.^d V, Varicella; Z, zoster; DZ, disseminated zoster; Ara-A, adenine arabinoside.^e Measles hemagglutination inhibition titer, <2; hemagglutination inhibition titer in serum, $\geq 1:32$.^f NA, Not available.^g Measles hemagglutination inhibition titer in CSF, <2.

VZ antibody in CSF. Approximately 3 months after the onset of rash, however, he developed hemiplegia, and VZ antibody was detected in his CSF. Several other patients had CNS symptoms which became prominent 1 to 3 months after the onset of zoster (no. 4, 5, 14, and 16), and all had detectable VZ antibody in CSF. The phenomenon of delayed onset of CNS symptoms after onset of zoster has been described previously (8).

Of the 11 patients with CNS symptoms associated acutely with the onset of infection, the mean day of examination of CSF for VZ antibody was day 8. There was no correlation between the day after onset of rash and VZ antibody in CSF. Seven control patients with varicella or zoster and no clinical evidence of CNS involvement (Table 2) had no detectable VZ antibody in CSF (<1:2). The mean day after onset of rash on which CSF was tested for VZ antibody for this group was day 7.

Serum antibody titers were available at the same time as the lumbar puncture for 9 patients with VZ infections and CNS involvement. The geometric mean titer (GMT) of VZ serum antibody in patients with varicella was 1:406 (range, 1:256 to 1:512), and with zoster it was 1:114 (range, 1:16 to 1:512). The GMT of five patients with VZ infections and no CNS symptoms was 1:8 in the patient with varicella and 1:80 in the patient with zoster. The VZ GMT of CSF antibody from the 13 patients with CNS symptoms was 1:8 (including the negative patient). The VZ GMT of CSF antibody from eight VZ patients without CNS symptoms was <1:2.

Serum and CSF specimens were obtained

from a number of other control patients. All had VZ serum antibody titers of 1:4 or greater. These included six patients with malignant or demyelinating diseases and 19 patients with bacterial meningitis or encephalitis caused by other viral or toxic agents. Four patients had herpes simplex virus encephalitis proven by brain biopsy. The GMT for VZ antibody in their serum was 1:21 (range, 1:8 to 1:64). In their CSF, it was <1:2.

DISCUSSION

Of the patients with CNS symptoms and VZ infections, 94% had VZ antibody detectable in their CSF by the FAMA technique. In contrast, none of the control patients studied had detectable VZ antibody in CSF by this method. The control patients had conditions which might conceivably cause them to have VZ antibody in CSF, such as zoster with concomitantly high serum VZ antibody titers, and inflammatory diseases, such as viral and bacterial meningitis and other types of encephalitis, which would be expected to compromise the blood-brain barrier. Although the CSF of a few patients contained erythrocytes it seems unlikely that the small amounts of blood in CSF could account for the VZ antibody in CSF. Several control patients also had CSF which was bloody; nevertheless, they had no detectable VZ antibody in their CSF. In addition, of six VZ antibody-positive CSF specimens also tested for measles antibody by hemagglutination inhibition, none had detectable measles antibody hemagglutination inhibition (assays performed by Saul Krugman; see Table 1).

The serum GMT of VZ patients with CNS involvement was somewhat higher than that of

TABLE 2. Simultaneous CSF and serum FAMA antibody titers in patients with and without VZ infections

Type of infection	No.	Mean serum titer			Mean CSF titer		
		Serum Ab ^a	Range	No. tested	CSF Ab ^a	Range	No. tested
VZ infection with CNS symptoms							
Varicella	3	406	256-512	3	4	<1-16	3
Zoster	13 ^c	114	16-512	6	8	2-256	13
VZ infection without CNS symptoms							
Varicella	1	8	8	1	<2		1
Zoster ^c	6	80	64-128	3	<2		6
Other CNS diseases ^b	25	21	8-128	25	<2		25

^a Reciprocal; Ab, antibody.

^b Malignancy, demyelinating disease, encephalitis, meningitis.

^c Two localized, four disseminated.

VZ patients without CNS symptoms. Serum antibody titers overlapped considerably, however. For example, three patients who had serum VZ titers of 1:64 or less and CNS symptoms also had detectable VZ antibody in CSF. In contrast, three patients with serum titers of 1:64 or greater and no CNS symptoms did not have detectable VZ antibody in CSF. Thus, the level of VZ antibody in serum alone probably cannot account for its presence in the CSF. Whereas we found a correlation between the heights of serum and CSF antibody titers for all the patients (including controls), we found no correlation in the group with VZ infections and CNS symptoms.

Detection of specific antibody in CSF has been used to identify encephalitis caused by measles virus and herpes simplex virus (2, 5, 6, 9). To interpret the results of testing for antibody in CSF to diagnose herpes simplex virus encephalitis, it has been necessary to determine the ratio between antibody in serum and that in CSF. A serum/CSF antibody ratio of 20 or less has been found to be diagnostic of biopsy-proven herpes simplex virus encephalitis (6). In addition, it is not unusual for apparently normal persons to have low levels of detectable antibody to herpes simplex virus in their CSF (7). It appears, however, that for diagnosis of VZ-associated encephalitis by FAMA measurement of specific CSF antibody, a serum/CSF ratio may not be needed. In our study, persons without clinical VZ encephalitis did not have detectable VZ antibody in their CSF by the FAMA assay. This is in contrast to the results of others, who have utilized different techniques to measure VZ antibody in CSF (3).

Although the pathogenesis of VZ-associated encephalitis is not completely defined, the observation that VZ antibody is normally absent from CSF whereas herpes simplex virus antibody may normally be present suggests basic differences in the usual state of these viruses in the CNS. One may surmise that, normally, VZ virus is not present in the CNS. When VZ encephalitis occurs, it may be due to multiplication of virus in the CNS, thus resulting in the production of local antibody. This concept is sup-

ported by our finding specific IgM in 6 out of 12 patients' CSF and by isolation of VZ virus from the CSF of two of our patients. In addition, inclusion bodies and viral particles in the autopsied brain of persons dying of VZ encephalitis have been observed (7). These observations do not exclude the other possibility that VZ encephalitis may be due to an immunological reaction to the virus. For diagnosis of VZ encephalitis, however, our data suggest that measurement of VZ CSF antibody by FAMA may be helpful.

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