

CSF herpes virus and autoantibody profiles in the evaluation of encephalitis

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Jenny J. Linnoila, MD,
PhD
Matthew J. Binnicker,
PhD
Masoud Majed, MD
Christopher J. Klein, MD
Andrew McKeon, MD

Correspondence to
Dr. McKeon:
mckeon.andrew@mayo.edu

ABSTRACT

Objective: To report the frequency of coexisting herpes viruses (herpes simplex virus 1 [HSV-1] or HSV-2, varicella zoster virus, Epstein-Barr virus [EBV], cytomegalovirus, or human herpes virus 6 [HHV-6]) and autoantibodies in patients with encephalitis (herpes or autoimmune) in clinical laboratory service.

Methods: Three groups were evaluated for herpes viruses and antibodies: group 1—patients whose CSF was positive for a herpes virus by real-time PCR over a period of 6 months; group 2—patients whose CSF was positive for an autoimmune encephalitis-associated antibody over 5 years (e.g., NMDA receptor [NMDA-R] antibody), and the same number of controls without autoimmune/infectious disease; and group 3—incidental autoimmune parainfectious encephalitis cases encountered over 1 year.

Results: In group 1, antibodies were detected in 27 of 100 herpes PCR-positive CSF specimens (CSFs), either unclassified neural or nonneural in all but one patient with NMDA-R antibody detected after EBV infection. Antibodies were also detected in 3 of 7 CSFs submitted for repeat PCR testing (unclassified, 2; AMPA receptor, 1). In group 2, herpes viruses were detected in 1 of 77 controls (HHV-6) and 4 of 77 patients with autoimmune encephalitis (EBV, 2; HHV-6, 2); autoantibodies targeted NMDA-R in 3/4 and GABA_B-R in 1/4. In group 3, NMDA-R antibody was detected in 7 patients post-HSV-1 encephalitis. Of the remaining 3 patients, 2 had unclassified neural antibodies detected, and one had GABA_B-R autoimmunity. Concomitant neoplasms were discovered in 2 patients each from groups 2 and 3.

Conclusions: Autoantibodies and herpes virus DNA frequently coexist in encephalitic CSF. Some patients develop parainfectious autoimmunity following viral CNS infection (usually HSV-1 encephalitis). The significance of detecting herpes nucleic acids in others remains unclear.

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GLOSSARY

AMPA-R = α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; **ANA** = antinuclear antibody; **AQP4** = aquaporin-4; **CASPR2** = contactin-associated protein-like 2; **CBA** = cell-based assay; **CMV** = cytomegalovirus; **CSFs** = CSF specimens; **DPPX** = dipeptidyl-peptidase-like protein-6; **EBV** = Epstein-Barr virus; **GABA_B-R** = γ -aminobutyric acid type B receptor; **GPC** = gastric parietal cell; **HHV-6** = human herpes virus 6; **HSV-1** = herpes simplex virus 1; **IFA** = immunofluorescence assay; **IgG** = immunoglobulin G; **LDT** = laboratory developed test; **LGI-1** = leucine-rich glioma-inactivated 1 protein; **mGluR** = metabotropic glutamate receptor; **NMDA-R** = NMDA receptor; **SMA** = smooth muscle antibody; **VZV** = varicella zoster virus.

Neurologic autoimmunity may be triggered by cancer (paraneoplastic), infection (parainfectious), or it may be cryptogenic.¹ Clinical disorders for which experimental models have been developed include anti-Yo autoimmunity, in women with cerebellar degeneration and breast or gynecologic adenocarcinoma, and ganglioside autoimmunity, in patients with Guillain-Barré syndrome arising after *Campylobacter* infection.^{2,3}

Ovarian teratoma is a frequent accompaniment of paraneoplastic autoimmune encephalitis targeting the GluN1 subunit of the NMDA receptor (NMDA-R).^{4,5} Recent studies revealed

From the Departments of Neurology (J.J.L., C.J.K., A.M.), and Laboratory Medicine and Pathology (M.J.B., M.M., C.J.K., A.M.), College of Medicine, Mayo Clinic, Rochester, MN.

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that NMDA-R antibodies frequently coexist in patients with herpes simplex virus 1 (HSV-1) encephalitis, and that some anti-NMDA-R encephalitis cases develop as a parainfectious autoimmune phenomenon, within weeks of HSV-1 encephalitis.^{6–11} Those studies confirmed suspicions that early relapse in HSV encephalitis, most notably in children, could be autoimmune.¹² Typically, a striking biphasic illness occurs, whereby initial remission from viral encephalitis is followed by relapse with autoimmune encephalitis within 1 to 7 weeks of the initial presentation.^{7–11}

Other viruses from the *Herpesviridae* family may trigger neurologic autoimmunity, and the spectrum of antibodies encountered may extend beyond the NMDA-R.^{7,13} In this study, we undertook a broad evaluation of CSF specimens (CSFs) clinically referred for either herpes or autoimmune testing in order to assess the following: (1) the frequency of neural and nonneural antibodies in CSFs from patients with herpes virus PCR positivity (identified over 6 months), (2) the frequency of herpes viruses in stored CSFs from patients with autoimmune encephalitis (identified over 5 years) and controls without inflammatory or infectious neurologic disorders, and (3) incidental cases of autoimmune encephalitis in which a parainfectious cause was considered (encountered in the Mayo Clinic Neuroimmunology Laboratory over 1 year).

METHODS Standard protocol approvals, registrations, and patient consents. The Mayo Clinic institutional review board (14-008716) approved this study. The figure demonstrates an outline of the patients.

Group 1: Autoantibody testing in CSFs with herpes PCR positivity. Residual CSFs from 100 patients evaluated in the Mayo Clinic Virology Laboratory between January 1, 2015, and June 30, 2015, that tested positive by real-time PCR for a herpes virus (HSV-1 and -2, varicella zoster virus [VZV], Epstein-Barr virus [EBV], cytomegalovirus [CMV], and human herpes virus 6 [HHV-6]), were prospectively collected and tested for immunoglobulin G (IgG) antibodies by indirect immunofluorescence assays (IFAs).

Patients in whom at least 500 μ L of residual CSF was available on completion of virology testing were included. CSFs were refrigerated at 4°C and tested in the Neuroimmunology Laboratory within 5 days. Ninety-two of the 100 CSFs were referred from practitioners outside of Mayo Clinic; 8 were internally referred. Serum was not available for testing. Of 10 CSFs subsequently submitted from the same patients for repeat virology testing within the 6-month timeframe, 7 had sufficient residual volume for neural antibody testing. Clinical histories were obtained for patients in whom well-characterized synaptic plasma

membrane protein-directed antibodies (such as NMDA-R) were detected and for those with antibody detected upon repeat testing.

The IgG-class antibody assays were all interpreted by experienced observers. The assays were indirect IFAs, which consisted of the following: (1) a tissue-based assay utilizing a composite substrate of mouse brain, kidney, and intestinal tissues to identify neural antibodies (either well-characterized, such as NMDA-R, or unclassified) and nonneural antibodies (such as antinuclear antibody [ANA], smooth muscle antibody [SMA] and gastric parietal cell [GPC] antibody) by established diagnostic criteria^{14,15}; and (2) cell-based assays (CBAs) for encephalitis-associated neural antibodies targeting cell surface synaptic antigens (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor [AMPA-R], aquaporin-4 [AQP4], contactin-associated protein-like 2 [CASPR2], dipeptidyl-peptidase-like protein-6 [DPPX], γ -aminobutyric acid type B receptor [GABA_B-R], metabotropic glutamate receptor 1 or 5 [mGluR1, mGluR5], leucine-rich glioma-inactivated 1 protein [LGI-1], or GluN1 subunit of the NMDA-R). For each CBA, HEK293 cells were transfected with the relevant complementary DNA and control cells were transfected with empty vector (EUROIMMUN, Luebeck, Germany).¹⁶

Five tests were performed in total on CSF at a dilution of 1:2 –1 tissue IFA, 3 composite CBAs, and 1 CBA to test for AQP4 antibody only. The composite CBAs contained chips expressing the following antigens: (1) NMDA-R, AMPA-R, and GABA_B-R; (2) LGI-1 and CASPR2; (3) mGluR1, mGluR5, and DPPX. Insufficient quantity existed to determine titers. Twenty of 100 CSFs had insufficient quantity available for complete antibody testing. One test was lacking in 13 specimens, 2 tests in 6, and 3 tests in 1; 5 total CSFs were lacking tissue IFA.

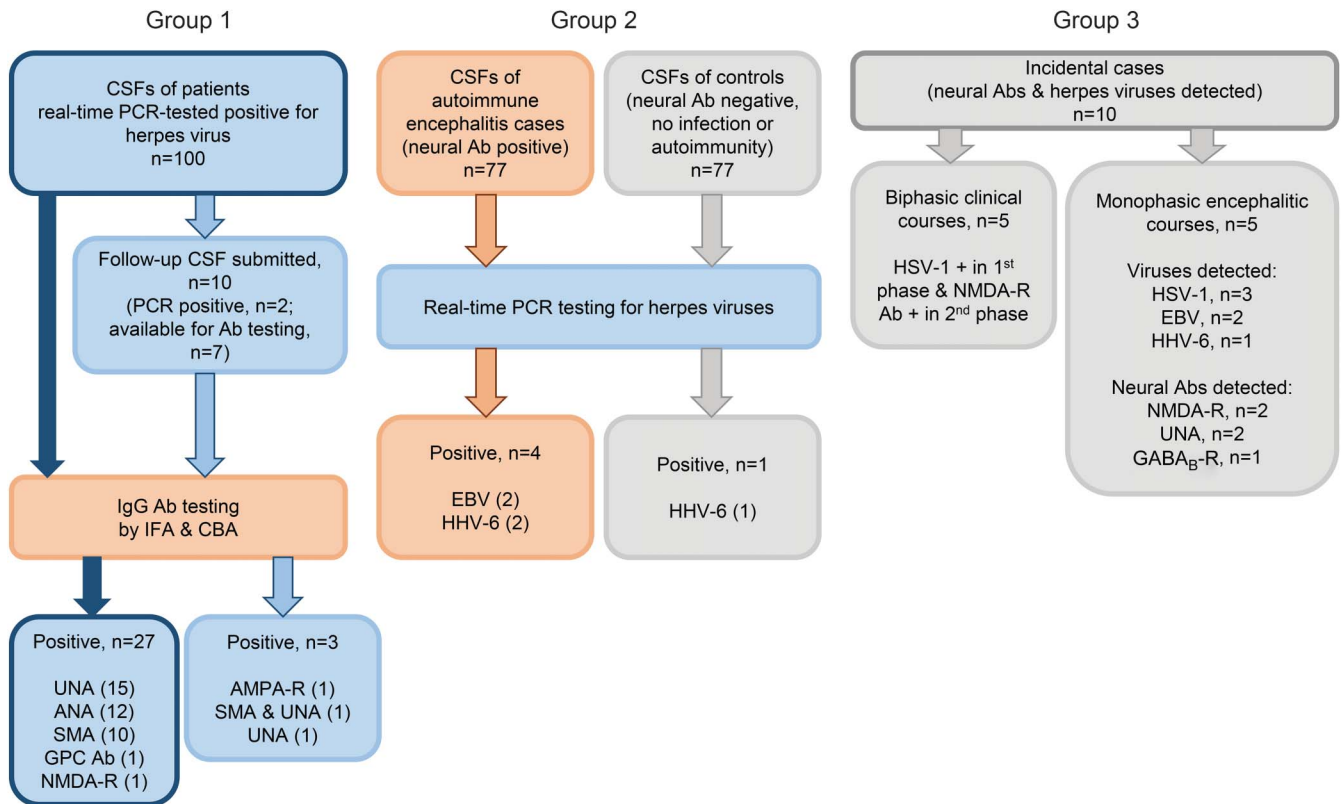
Group 2: Herpes virus testing in CSFs with synaptic antibody positivity and controls. Archived and frozen CSFs (2011–2015) from 77 patients with autoimmune encephalitis and known positivity for a neural antibody in one or more of the CBAs described above and 77 controls (antibody-negative CSFs from Mayo patients [2015] without known inflammatory or infectious CNS diseases) were tested retrospectively for herpes viruses (HSV-1/2, VZV, EBV, CMV, and HHV-6) by real-time PCR. The median patient age was 25 years (range, 2–85); 55 were female. Median control age was 49 years (range, 16–86); 49 were female. Control diagnoses were cognitive disorders ($n = 33$), epilepsy ($n = 15$), movement disorders ($n = 11$), no neurologic diagnosis ($n = 17$), or motor neuronopathy ($n = 1$).

For herpes virus real-time PCR testing, DNA from 200 μ L of raw CSF was extracted on the MagNA Pure LC 2.0 (Roche Diagnostics, Indianapolis, IN) using the Total Nucleic Acid extraction protocol. Subsequently, 5 μ L of extracted nucleic acid was added to a LightCycler capillary (Roche) containing 15 μ L of master mix for one of the following: CMV (laboratory developed test [LDT]), EBV (Roche analyte specific reagents), HHV-6 (LDT), HSV-1 and -2 (Roche analyte specific reagents), and VZV (LDT). Testing was then performed on a LightCycler 2.0 (Roche) as previously described.^{17–20}

Group 3: Incidental cases. Case histories of patients with findings suspicious for parainfectious autoimmune encephalitis incidentally encountered by J.L. in the course of clinical laboratory service between July 2014 and June 2015 were collated. Virology findings (from CSF only) and antibodies detected (from serum and CSF) are reported.

RESULTS Antibody testing results, virus PCR results, other CSF data, and clinical information are outlined in the figure and in tables 1–4.

Figure Groups 1-3, patients tested, and test results (viral PCR and antibodies)



Ab = antibody; AMPA-R = amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; ANA = antinuclear antibody; CBA = cell-based assay; CSFs = CSF specimens; EBV = Epstein-Barr virus; GABA_B-R = γ -aminobutyric acid type B receptor; GPC = gastric parietal cell; HHV-6 = human herpes virus 6; HSV = herpes simplex virus; IFA = immunofluorescence assay; IgG = immunoglobulin G; NMDA-R = NMDA receptor; PCR = polymerase chain reaction; SMA = smooth muscle antibody; UNA = uncharacterized neural antibody.

Group 1: Autoantibody testing of CSFs with herpes PCR positivity. One hundred CSFs that were positive by real-time PCR for a herpes virus (one virus per specimen) were encountered over the 6-month study period. Viruses detected included HSV-2 (n = 38), VZV (n = 30), HSV-1 (n = 18), EBV (n = 6), HHV-6 (n = 6), and CMV (n = 2).

Antibodies detected in 27 of 100 CSFs by tissue-based IFA included uncharacterized neural antibodies (n = 15), ANA (n = 12), SMA (n = 10), and GPC antibody (n = 1) (table 1). Only one CSF, which was EBV-positive and drawn 1 month into a meningoencephalitic illness, was antibody positive by CBA (for NMDA-R antibody). This patient was also positive

Table 1 Group 1 patient demographics, viral species detected, and tissue IFA antibody findings in CSF (n = 100)

Herpes virus detected in CSF	No. positive for viral nucleic acid and tested for Abs (female sex)	Median age, y (range)	No. positive for Abs (% of tested); female sex	Autoantibodies detected (No.)
HSV-1	18 (9)	72 (0.6-89)	3 (16); 2	SMA (1), UNA (1), GPC Ab and UNA (1)
HSV-2	38 (28)	37 (0.1-70)	9 (23); 5	ANA (3), ANA and UNA (3), SMA (2), UNA (1)
VZV	30 (12)	79 (13-93)	9 (30); 5	ANA (3), UNA (3), SMA and UNA (2), SMA (1)
EBV	6 (2)	58 (34-85)	4 (67); 1	SMA, ANA, and NMDA-R (1), SMA and ANA (1), UNA (1), SMA and UNA (1)
CMV	2 (1)	41 (37-45)	1 (50); 0	SMA and UNA (1)
HHV-6	6 (1)	54 (1.5-67)	1 (17); 0	ANA and UNA (1)
Total	100 (53)	57 (0.1-93)	27 (27); 13	Total patients (27), UNA (15), ANA (12), SMA (10), NMDA-R (1), GPC Ab (1)

Abbreviations: Ab = antibody; ANA = antinuclear Ab; CMV = cytomegalovirus; EBV = Epstein-Barr virus; GPC = gastric parietal cell; HHV-6 = human herpes virus 6; HSV = herpes simplex virus; IFA = immunofluorescence assay; NMDA-R = NMDA receptor; SMA = smooth muscle Ab; UNA = unclassified neural Ab; VZV = varicella zoster virus.

for ANA and SMA by tissue-based IFA (tables 1 and 2) and had signs of systemic EBV infection as well as meningoencephalitis. CSF drawn 3 months after illness onset had neither antibodies nor EBV detected. The authors subsequently obtained an aliquot of CSF drawn 2 weeks into the illness from the referring provider. Viral testing was negative for EBV. Antibody testing revealed ANA alone.

Autoantibody testing of follow-up CSFs. CSFs from 10 of the 100 patients with prior herpes virus PCR positivity (including the patient with EBV infection described above, Table 2, row 1) were subsequently tested by real-time PCR within a median of 7 weeks after the initial evaluation (range, 1.3–21). Two were still positive for herpes viruses, one for CMV (1.3 weeks after the first CSF test), and one for HHV-6 (3.5 weeks later). Sufficient specimen volumes were available for antibody testing in 7 of the 10 CSFs (including the 2 with repeat herpes positivity). Antibodies were detected in 3 of 7 repeat CSFs (43%, table 2, rows 2–4). Of these patients, the first 2 described were considered to have a parainfectious autoimmune disorder by the treating physicians. The first patient with a biphasic encephalitic course had GPC antibody detected in the initial HSV-1

PCR-positive CSF, and AMPA-R antibody (but not GPC antibody) detected in CSF submitted 9 weeks later. The second patient with zoster myelitis who developed encephalopathy during recovery from myelopathy had SMA detected in the initial VZV PCR-positive CSF, and SMA and an unclassified neural antibody (but not VZV) detected in CSF submitted 3 weeks later. A third patient, with encephalopathy, recurrent lymphoma with spread to the CNS, and HHV-6 viremia, tested positive for both HHV-6 and an uncharacterized neural antibody in both CSFs submitted. The significance of the viral and antibody findings was uncertain in that patient.

Group 2: Herpes virus testing of CSFs with synaptic antibody positivity and controls. Among stored CSFs from 77 patients diagnosed with autoimmune encephalitis, the following antibodies were detected: NMDA-R (n = 56), GABA_B-R (n = 13), LGI-1 (n = 2), DPPX (n = 2), CASPR2 (n = 1), AMPA-R (n = 1), NMDA-R and AMPA-R (n = 1), and NMDA-R and GABA_B-R (n = 1). Four of 77 CSFs were positive by herpes virus real-time PCR: 2 for EBV (both patients had anti-NMDA-R encephalitis) and 2 for HHV-6 (one patient had anti-NMDA-R encephalitis, the other had

Table 2 Group 1 patients with serial CSF specimens in whom neural autoantibodies were detected (n = 4)

Age, y/sex/race/ interval between CSF tests, wk	Available CSF findings		Clinical details	Diagnosis
	1st CSF	2nd CSF		
46/F/C/9	WCC, 600 (50% PMNs); protein, 228; EBV; ANA ^a , SMA ^a , NMDA-R Ab ^{a,b}	Negative	Presented with fatigue, headache, dysphagia, diplopia (bilateral V1th CN palsies), mild hallucinations, and lymphadenopathy. MRI demonstrated leptomeningeal enhancement. Initial CSF, not obtained by authors, demonstrated 1,100 WBCs (50:50, PMNs: lymphocytes); viral studies were negative. Subsequent worsening coincided with 1st CSF evaluated by authors. Patient had gradual clinical improvement and resolution of scan findings, partly attributable to steroid therapy. Died suddenly in her sleep 2 mo later	Likely systemic EBV and EBV meningoencephalitis, followed by anti-NMDA-R encephalitis
62/F/NA/9	HSV-1; GPC Ab ^a	AMPA-R Ab ^{a,c}	Severe encephalitis, requiring tracheostomy and percutaneous feeding tube. Mild improvement with antiviral treatment. Re-presented 2.5 mo later in coma after cardiac arrest. Care withdrawn; patient died	HSV-1 encephalitis followed by AMPA-R encephalitis
43/M/C/3	WCC, 486 (60% lymphs); protein, 429; VZV; SMA ^a	WCC, 183 (87% lymphs); protein, 270; SMA ^a , UNA ^a	Diagnosed with zoster myelitis in setting of immunosuppression for neurosarcoidosis. Immunosuppression was stopped to treat zoster. He developed encephalopathy, which resolved on restarting immunosuppression	VZV myelitis followed by autoimmune encephalopathy
63/M/H/4	WCC, 7 (87% lymphs; 9% atypical); protein, 99; HHV-6; UNA ^a	WCC, 34 (84% lymphs; 13% atypical); protein, 140; HHV-6; UNA ^a	History of HTLV-associated T cell lymphoma. Remitted after chemotherapy and SCT. Developed GVHD. Presented with month of encephalopathy. Found to have CNS lymphoma. MRI demonstrated diffuse leptomeningeal enhancement. Did not respond to chemotherapy. Had concomitant worsening of HHV-6 viremia. Discharged to hospice	CNS lymphoma, HHV-6 viremia; possible autoimmune, HHV-6, or paraneoplastic encephalitis

Abbreviations: Ab = antibody; AMPA-R = amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; ANA = antinuclear Ab; C = Caucasian; CN = cranial nerve; EBV = Epstein-Barr virus; GPC = gastric parietal cell; GVHD = graft vs host disease; H = Hispanic; HHV-6 = human herpes virus 6; HSV = herpes simplex virus; HTLV = human T lymphotropic virus; lymphs = lymphocytes; NA = not available; NMDA-R = NMDA receptor; PMN = polymorphonuclear white cell; SCT = stem cell transplant; SMA = smooth muscle Ab; UNA = unclassified neural Ab; VZV = varicella zoster virus; WBC = white blood cell; WCC = white cell count.

Normal CSF values: protein, ≤35 mg/dL; WCC, ≤5/μL.

^aDetected by tissue-based immunofluorescence assay.

^bDetected by cell-based immunofluorescence assay.

^cHSV-1 not retested.

Table 3 Group 2 patients with diagnoses of autoimmune encephalitis in whom herpes viruses were detected (n = 4)

Age, y/sex/race	Available CSF findings ^a		Serum antibody specificity	Available clinical details	Diagnosis
	WCC, protein, Ab specificity	Herpes virus detected			
13/F/AA	Elevated WCC; NMDA-R ^b	EBV	Serum NA	Presented with typical anti-NMDA-R encephalitis requiring ICU hospitalization. No teratoma found	Anti-NMDA-R encephalitis
34/F/AA	WCC, 115 (94% lymphs); protein, 86; NMDA-R ^c	EBV	Serum NA	Presented with typical anti-NMDA-R encephalitis requiring ICU hospitalization. Bilateral teratomas found. Poor response to steroids and IVIg. Died of multiple pulmonary emboli after 1 mo	Anti-NMDA-R encephalitis
15/F/NA	NMDA-R ^c	HHV-6	Serum NA	Presented with encephalopathy, emotional lability, seizures, dysarthria, and focal weakness of upper extremity. Globus pallidus T2 hyperintensities on MRI. Had concurrent HHV-6 viremia	Anti-NMDA-R encephalitis
67/M/NA	GABA _B -R ^c	HHV-6	VGCC-N ^d	Presented with limbic encephalitis. Small cell carcinoma of lung found	GABA _B -R encephalitis

Abbreviations: AA = African American; Ab = antibody; EBV = Epstein-Barr virus; GABA_B-R = γ -aminobutyric acid type B receptor; HHV-6 = human herpes virus 6; ICU = intensive care unit; IVIg = IV immunoglobulin; lymphs = lymphocytes; NA = not available; NMDA-R = NMDA receptor; VGCC-N = N-type voltage-gated calcium channel; WCC = white cell count.

CSF normal values: protein, ≤ 35 mg/dL; WCC, $\leq 5/\mu\text{L}$.

^aAll detected in the same CSF specimen.

^bDetected by tissue-based immunofluorescence assay only.

^cDetected by tissue-based and cell-based immunofluorescence assays.

^dDetected by immunoprecipitation assay.

GABA_B-R encephalitis [table 3]). None had a history of infectious encephalitis recorded. Two of the 4 patients also had neoplasia (bilateral ovarian teratomas and small cell lung carcinoma) detected and were diagnosed with a paraneoplastic (rather than parainfectious) encephalitis. Among 77 control CSFs (antibody negative, from patients without inflammatory or infectious CNS disease), one tested positive for HHV-6. The patient was a 21-year-old woman with chronic intractable cryptogenic focal epilepsy, without clinical, radiologic, or CSF evidence of CNS infection or autoimmunity.

Group 3: Incidental cases. In the course of clinical consultative correspondence with outside physicians over 12 months, 10 patients were encountered with CSF positivity for both herpes PCR and neural antibodies concurrently or sequentially (table 4). Eight patients had HSV-1 alone detected (3 were children), one had both EBV and HHV-6 detected, and one had EBV alone.

Five patients had biphasic clinical courses and were recognized to have a postinfectious autoimmune disorder. All had HSV-1 detected during the first phase and NMDA-R antibody detected during the second phase. Three were older than 60 years, and 2 were younger than 2 years; all were female. Initial encephalitic illnesses were followed by improvement attributed to antiviral therapy. Relapse of encephalitic symptoms occurred a median of 4 weeks later (range, 1–6). One pediatric patient developed a hyperkinetic movement disorder during the second phase. Immunotherapy data were available for 3 patients, 2 of whom improved.

Five patients had monophasic encephalitic courses. In one child, HSV-1 infection was initially suspected and was confirmed by PCR. Further evaluation was prompted by lack of improvement and development of orofacial dyskinesias despite prompt treatment with antiviral therapy. Anti-NMDA-R encephalitis was suspected and confirmed by CSF testing. A second patient had a clinical course typical for severe anti-NMDA-R encephalitis from the outset, and had both NMDA-R antibody and EBV detected in the same CSF specimen. The significance of the virology finding was unknown. Two further patients had both HSV-1 and neoplasia detected during evaluations for encephalitis. One of those 2 patients had GABA_B-R encephalitis and small cell lung carcinoma, and was concluded to have paraneoplastic encephalitis. The other had an unclassified neural antibody and renal cell carcinoma detected, but the cause of encephalitis (infectious, parainfectious, or paraneoplastic) was uncertain. The final patient had encephalopathy, which progressed to a minimally responsive state over the course of 1 year. Despite extensive evaluations, a cause was not determined. Both EBV and HHV-6 were detected in the initial CSF. Repeat CSF testing over the year persistently demonstrated an elevated white cell count, HHV-6 PCR positivity, and an unclassified neural antibody. There were no neurologic improvements after multiple antiviral treatments and immunotherapies.

DISCUSSION In this study based in the Mayo Clinic Virology and Neuroimmunology clinical laboratories, autoantibodies and herpes nucleic acids were commonly

Table 4 Group 3 (incidental cases) in whom parainfectious autoimmunity was considered (n = 10)

Age, y/ sex/race	Available CSF findings			Serum Abs detected (wk after positive herpes CSF)	Clinical data	Diagnosis	Treatment, response
	WCC, protein, OCBs	Herpes viruses detected	CSF Abs (wk after positive herpes CSF)				
1/F/NA	NA	HSV-1 ^{a,b}	NMDA-R Ab, ^{c,d} ANA (4) ^{d,e}	SMA (4)	Biphasic neurologic course; relapse with chorea occurred after 4 wk	Anti-NMDA-R encephalitis, post-HSV-1 encephalitis	NA
1/F/AA	1st: WCC, 21 (40% PMNs); 2nd: WCC, 3 (90% lymphs); protein, 24	HSV-1 ^{a,b}	NMDA-R Ab (11) ^{d,f}	NMDA-R (13) ^f	Severe biphasic neurologic course	Anti-NMDA-R encephalitis, post-HSV-1 encephalitis	No improvement after IVMP and IVIg. Persistent seizures and developmental delay
61/F/NA	NA	HSV-1 ^a	UNA, ^{a,d,f} NMDA-R Ab (6) ^{c,d}	Negative (0)	Biphasic clinical course. Relapse occurred after 6 wk	Anti-NMDA-R encephalitis, post-HSV-1 encephalitis	NA
63/F/C	1st: WCC, 14; protein, 60; 2nd: WCC, 79 (76% lymphs); protein 79; OCBs, 15	HSV-1 ^{a,b}	NMDA-R Ab, ^{c,d} ANA, ^{d,e} SMA (4) ^{d,e}	Negative (4)	Biphasic neurologic course. Relapse after 3 wk	Anti-NMDA-R encephalitis, post-HSV-1 encephalitis	1st event: improved with antiviral therapy; 2nd event: improved with IVMP × 5, still severe cognitive dysfunction
67/F/C	1st: WCC, 730 (95% lymphs); 2nd: WCC, 10 (90% lymphs)	HSV-1 ^{a,b}	NMDA-R Ab, ^{c,d} ANA, ^{d,e} SMA (6) ^{d,e}	Negative (6)	Biphasic neurologic course. Relapse after 6 wk	Anti-NMDA-R encephalitis, post-HSV-1 encephalitis	1st event: improved with antiviral therapy; 2nd event: normalized with IVIg, rituximab, and cyclophosphamide after long recovery
4/M/H	1st: WCC, 18 (91% lymphs); 2nd: WCC, 3 (80% lymphs); 1 wk after initial CSF)	HSV-1 ^{a,b}	NMDA-R Ab, ^{d,f} ANA (3.5) ^{d,e}	Negative (3.5)	Presented in status epilepticus	Anti-NMDA-R encephalitis, post-HSV-1 encephalitis	Some response to antivirals. No improvement after IVMP and IVIg. Remained nonambulatory and minimally interactive
21/M/H	WCC, 60 (90% lymphs)	EBV ^a	NMDA-R Ab (0) ^{a,c}	Negative (0)	Severe course of progressive decline typical of NMDA-R encephalitis requiring ICU admission	Anti-NMDA-R encephalitis	Limited response to IVMP, PLEX, IVIg, rituximab, and cyclophosphamide
57/F/C	1st: WCC, 59 (78% lymphs); protein, 46; 2nd: WCC, 2; protein, 74	HSV-1 ^{a,b}	UNA, ^{d,e} SMA (12) ^{d,e}	Negative (16)	Presented with encephalopathy, diagnosed with HSE. Renal cell carcinoma found	Paraneoplastic encephalitis	Some response to antivirals and oral prednisone but still had severe residual amnesic disorder
65/M/NA	1st: WCC, 27; protein, 200; 2nd: WCC, 10; 3rd: WCC, 10; protein, 54	EBV, ^{a,b} HHV-6 ^{a,g}	UNA (12) ^{d,e,g}	Striational, AChRbi, AChRmo, UNA (0)	Progressive encephalopathy, became minimally responsive over 1 y. No cancer	Encephalitis of unknown cause	Minimal response to antivirals, steroids, and IVIg
71/F/NA	WCC, 47	HSV-1 ^a	GABA _B -R Ab (0) ^{a,c}	VGCC-N and VGCC-P/Q (0) ^h	Progressive cognitive decline and aphasia. Identified HSV-1 and GABA _B in CSF; small cell carcinoma of lung found	GABA _B -R encephalitis	Some improvement with IVIg

Abbreviations: AA = African American; Ab = antibody; AChRbi = acetylcholine receptor binding Ab; AChRmo = acetylcholine receptor modulating Ab; ANA = antinuclear Ab; C = Caucasian; EBV = Epstein-Barr virus; GABA_B-R = γ -aminobutyric acid type B receptor; H = Hispanic; HHV-6 = human herpes virus 6; HSV = herpes simplex virus; HSE = herpes simplex encephalitis; ICU = intensive care unit; IVIg = IV immunoglobulin; IVMP = IV methylprednisolone; lymphs = lymphocytes; NA = not available; NMDA-R = NMDA receptor; OCB = oligoclonal band (CSF exclusive); PLEX = plasma exchange; PMN = polymorphous neutrophil; SMA = smooth muscle Ab; UNA = unclassified neural Ab; VGCC-N = N-type voltage-gated calcium channel; VGCC-P/Q = P/Q-type voltage-gated calcium channel; WCC = white cell count.

CSF normal values: protein, ≤ 35 mg/dL; WCC, $\leq 5/\mu\text{L}$; CSF-exclusive OCBs, < 4 .

^a Detected in first CSF tested.

^b Virus testing documented and negative in 2nd CSF tested.

^c Ab detected by tissue-based immunofluorescence assay and cell-based assay.

^d Ab detected in 2nd CSF tested.

^e Detected by tissue-based immunofluorescence assay.

^f Ab detected by cell-based assay only.

^g Detected in all CSF specimens tested.

^h Detected by immunoprecipitation assay.

codetected in CSFs submitted for herpes and autoimmune encephalitis evaluation. The species of herpes viruses (HSV-1/2, VZV, EBV, HHV-6, and CMV) and antibodies detected (most commonly unclassified neural or nonneural IgGs) were diverse. For some patients in whom longitudinal clinical data and specimens were available (mostly anecdotal), clinical and CSF testing results indicated sequential clinical events (remitting viral encephalitis, followed by autoimmune encephalitis). Some patients had the now well-described parainfectious phenomenon of anti-NMDA-R encephalitis after HSV-1 encephalitis. Consistent with previous reports, many (half) of those patients were children and neoplasms were not found.⁷⁻¹¹ For others, testing results were also suggestive of sequential viral and parainfectious autoimmune disorders, although distinct clinical phases could not be discerned. In other patients, the clinical significance of the detected herpes viruses and antibodies was uncertain. Latent neurotropic virus may be reactivated or shed as a consequence of CNS inflammation, and autoimmune responses to disparate neural antigens during viral infection may occur.

The herpes virus PCR-positive cohort (group 1), which consisted of patients with CSFs submitted on a clinical basis for herpes virus testing, and evaluated on a research basis for autoantibodies, provided a snapshot of other parainfectious autoimmune phenomena arising during CNS herpes infection. Consistent with a previous report, uncharacterized neural antibodies were commonly detected in CSF.⁷ In addition, we also detected a high frequency of nonneural autoantibodies, either organ-specific (such as SMA and GPC antibody) or non-organ-specific (ANA). A similar profile of nonneural antibodies is frequently detected in patients with paraneoplastic autoimmunity.²¹ We did not have clinical data or sera available to determine whether the detected non-organ-specific autoimmunity was attributable to coexisting systemic autoimmune disease or advanced age, rather than occurring in the context of CNS infection.²² Nonneural autoantibodies detected in CSF may also indicate nonspecific immune activation during infection, rather than heralding impending neural-specific autoimmunity. Repeat CSF data were limited to those patients who had CSF resubmitted on a clinical basis.

Our findings confirm that parainfectious autoimmunity may occur in the context of EBV and VZV, as reported previously.^{10,13,23} In 4 of 5 cases (from groups 1-3) where the clinical histories were known, patients with EBV-positive CSF also had NMDA-R antibodies detected. With this study, we additionally demonstrated AMPA-R autoimmunity post-HSV-1 encephalitis and neurally directed antibodies in the setting of HHV-6 infection. Together, these results

demonstrate a spectrum of coexisting herpes viruses and antibodies extending beyond HSV-1 and NMDA-R antibodies.

The rate of resubmission of CSF (10%) from group 1 is consistent with a sizable number of patients with herpes encephalitis having prolonged or complicated courses, prompting clinicians to re-evaluate. A recent prospective study of herpes simplex encephalitis cases found that 25% of patients had immune-mediated relapsing neurologic sequelae.¹² It was also found in the same study that adults who did not have hyperkinetic movements and altered consciousness often had delayed diagnoses when compared with children who had these symptoms. Those findings and our data suggest that postviral autoimmune encephalitis remains underrecognized in clinical practice, and that opportunities for neurologic improvement with immunotherapy may be missed.¹²

Our autoimmune encephalitis cohort (group 2) demonstrated herpes virus PCR positivity (HHV-6 and EBV) in 4 of 77 patients (5%), although the timing of CSFs submission may have affected sensitivity. CSFs may have been submitted for antibody testing weeks to months after symptom onset, by which time herpes PCR testing might have proven negative.

The significance of herpes virus PCR positivity was uncertain in 3 of 4 patients with autoimmune encephalitis (group 2) and 4 of 10 incidental cases (group 3). Of those 7 patients, a paraneoplastic cause (rather than parainfectious) seemed most likely in 3. This uncertainty seemed particularly true for those patients in whom HHV-6 was detected, and was exemplified further by one control patient with cryptogenic intractable chronic focal epilepsy who was HHV-6-positive (and neural autoantibody-negative). Of 4 patients in groups 1, 2, or 3 who had HHV-6-positive CSF and histories known, one had HHV-6 viremia and likely CNS infection, one had encephalopathy of unknown cause, and 2 had malignancies (small cell carcinoma and T cell lymphoma) prompting consideration of a paraneoplastic rather than parainfectious encephalitis. Post hoc, we reviewed the available Mayo Clinic histories of 2 additional antibody-negative patients from group 1 with HHV-6 CSF positivity. In contrast to our antibody-positive patients, both were immunocompromised and had unambiguous clinical and radiologic features supportive of HHV-6 encephalitis. One had myelodysplastic syndrome and the other had diffuse systemic (excluding CNS) large B cell lymphoma.

It is possible that CSF herpes PCR positivity may occur in some patients because of shedding of latent neurotropic viral DNA in the course of paraneoplastic encephalitis. Reactivation of infection in that context cannot be excluded either (and we did not undertake confirmatory viral cultures from archived CSFs).²⁴

PCR positivity for HSV-1, HHV-6, and EBV in CSF, occurring without additional evidence supporting CNS infection, has also been reported among immunosuppressed patients and those with neurodegenerative disorders or stroke.^{25,26}

Although serum was not available for groups 1 and 2, antibody data were available for both serum and CSF among our incidental cases. In 6 of those 10 cases, antibodies were detected in CSF alone, supporting the concept of a CNS-predominant immune response (antiviral and autoimmune). An animal model study of herpes simplex encephalitis, which did not include testing for neural autoantibodies, demonstrated a sustained inflammatory response after recovery from herpes infection.²⁷ Ours and previous data support the possibility of autoimmunity contributing to this persistent CNS inflammation.⁷ Before the availability of antiviral therapy, corticosteroids (anti-inflammatory) had been reported to be beneficial for reducing edema in HSV encephalitis.^{28,29} Use of corticosteroids combined with antiviral therapy was associated with improved outcome in HSV encephalitis in a retrospective study, but controlled clinical trials are lacking in this area.^{30,31}

Detection of a herpes virus (usually HSV-1) and diverse autoantibodies in the CSF may signify a parainfectious autoimmune response in many patients. That response may initially be nonneural but, in some cases, matures into an attack directed at specific neural receptors (such as NMDA-R), which may manifest as a relapsing biphasic illness. The frequent coexistence of autoimmune markers in patients with CNS herpes infection raises several mechanistic and therapeutic questions, which may be answered by animal model studies and clinical trials.³² Caution regarding the interpretation of herpes virus PCR, unclassified, and nonneural antibody results in CSF is advised where other clinical or supportive features of CNS infection or autoimmunity are absent.

AUTHOR CONTRIBUTIONS

J.L.: study design, data collection and analysis, and drafting and critical revision of manuscript. M.B.: data collection and analysis, and critical revision of manuscript. M.M.: data collection and critical revision of manuscript. C.K.: study concept and design, and critical revision of manuscript. A.M.: study concept and design, data analysis, and drafting and critical revision of manuscript.

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