Post-transplant acute limbic encephalitis

Clinical features and relationship to HHV6

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ABSTRACT **Background:** Acute limbic encephalitis has been reported in the setting of treatment-related immunosuppression and attributed to human herpesvirus-6 (HHV6) infection. Clinical and laboratory features of the syndrome, however, have not been well characterized. **Methods:** We describe the clinical, EEG, MRI, and laboratory features of nine patients with acute limbic encephalitis after allogeneic hematopoietic stem cell transplantation (HSCT). To explore the relationship between HHV6 and this syndrome, we reviewed available CSF HHV6 PCR results from all HSCT patients seen at our center from March 17, 2003, through March 31, 2005. **Results:** Patients displayed a consistent and distinctive clinical syndrome featuring anterograde amnesia, the syndrome of inappropriate antidiuretic hormone secretion, mild CSF pleocytosis, and temporal EEG abnormalities, often reflecting clinical or subclinical seizures. MRI showed hyperintensities within the uncus, amygdala, entorhinal area, and hippocampus on T2, fluid-attenuated inversion recovery (FLAIR), and diffusion-weighted imaging (DWI) sequences. CSF-PCR assays for HHV6 were positive in six of nine patients on initial lumbar puncture. All patients were treated with foscarnet or ganciclovir. Cognitive recovery varied among long-term survivors. The one brain autopsy showed limbic gliosis and profound neuronal loss in amygdala and hippocampus. Among 27 HSCT patients with CSF tested for HHV6 over a 2-year period, positive results occurred only in patients with clinical limbic encephalitis. **Conclusions:** Patients undergoing allogeneic hematopoietic stem cell transplantation are at risk for post-transplant acute limbic encephalitis (PALE), a distinct neurologic syndrome. Treatment considerations should include aggressive seizure control and, possibly, antiviral therapy. PALE can be associated with the CSF presence of human herpesvirus-6, but the pathogenic role of the virus requires further exploration. **NEUROLOGY 2007;69:156-165**

Human herpesvirus-6 (HHV6) is an emerging and controversial pathogen in neurology. In adults, the virus has been associated with a broad range of common disorders, including multiple sclerosis,[1,2] temporal lobe epilepsy,[3,4] and encephalitis,[5,6] especially in immunocompromised hosts.[7] HHV6 infects nearly all children by 3 years of age,[8] and two variants, A and B, have been identified. Primary HHV6-B infection can be asymptomatic or cause exanthem subitum[9] but most often presents acutely with fever, fussiness, rash, diarrhea, or seizures.[10,11] Both HHV6 variants are neurotropic in vivo and reside latently throughout the adult brain,[12] complicating any attempt to link HHV6 to cerebral disease.

Encephalitis following allogeneic hematopoietic stem cell transplantation (HSCT) was first attributed to HHV6 in 1994.[13] The virus has since been held responsible for numerous cases, but the literature consists mainly of isolated reports spanning diverse clinical syndromes from cranial neuropathies to coma.[14,15] In an intriguing minority of patients, the limbic system has been the primary or exclusive target. To date, nine adult limbic-predominant cases have been reported in isolation,[15-22] along with a pediatric series of five patients[23] and four patients identified as part of a longitudinal study of plasma HHV6 levels after HSCT.[24] This limbic subgroup is of biologic interest, because it is unclear how HHV6 might induce such targeted limbic inflammation in some patients but a more diffuse or multifocal encephalitis in others.

Supplemental data at
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Table 1   Baseline patient characteristics

Patient	Age, y	Sex	Hematologic malignancy	Day 0*	Conditioning regimen	Donor relationship†	Stem cell source	GVHD prophylaxis	Engraftment	Acute GVHD	GVHD treatment
1	56	M	MDS	10/2001	Myeloablative cyclophosphamide and TBI	MRD	PBSC	CD8 T-cell depletion, tacrolimus	Day 12	Day 33, Grade III, GI, liver	Steroids, cyclosporine, tacrolimus, mycophenolate
2	53	M	CLL	11/2001	Non-myeloablative fludarabine and busulfan	MUD	PBSC	Prednisone, cyclosporine, mycophenolate	No neutropenia	Day 25, Grade IV, GI, liver, skin	Steroids, cyclosporine, tacrolimus, mycophenolate, daclizumab, infliximab
3	60	M	CML	1/2002	Non-myeloablative fludarabine and busulfan	MUD	PBSC	Prednisone, cyclosporine, mycophenolate	Day 7	Day 91, Grade II, skin	Steroids, mycophenolate, sirolimus, daclizumab
4	36	M	CML	3/2002	Myeloablative cyclophosphamide and TBI	MUD	PBSC	Tacrolimus, sirolimus	Day 20	Day 15, Grade IV, skin, GI	Steroids, tacrolimus, sirolimus, mycophenolate, denileukin diftitox
5	32	M	HD	12/2003	Myeloablative cyclophosphamide and TBI	MUD	PBSC	Tacrolimus, sirolimus, mycophenolate	Day 14	Day 85, Grade IV, liver	Steroids, tacrolimus
6	41	M	CLL	2/2004	Myeloablative cyclophosphamide and TBI	MUD	PBSC	Tacrolimus, sirolimus	Day 14	Day 13, Grade I, skin	Steroids, tacrolimus, sirolimus
7	59	M	CLL	10/2004	Non-myeloablative antithymocyte globulin fludarabine, melphalan	MUD	CORD	Cyclosporine, mycophenolate	Day 22	Day 22, Grade II, skin	Steroids, cyclosporine, mycophenolate
8	22	M	AML	1/2005	Myeloablative cyclophosphamide and TBI	MMUD	PBSC	Tacrolimus, sirolimus, methotrexate	Day 12	Day 13, Grade III, GI, liver	Steroids, tacrolimus, sirolimus, mycophenolate
9	38	M	AML	2/2005	Myeloablative cyclophosphamide and TBI	MMUD	PBSC	Tacrolimus, sirolimus, methotrexate	Day 12	Day 56, Grade IV, liver, GI	Steroids, mycophenolate

*Exact date omitted to protect patient anonymity.
†Patients were considered HLA-matched if all 6/6 HLA-A, -B, and -DR were identical.
GVHD = graft vs host disease; MDS = myelodysplastic syndrome; TBI = total body irradiation; MRD = matched-related donor; PBSC = adult peripheral blood stem cells; GI = gastrointestinal; CLL = chronic lymphocytic leukemia; MUD = matched-unrelated donor; CML = chronic myelogenious leukemia; HD = Hodgkin's disease; CORD = umbilical cord blood stem cells; AML = acute myelogenous leukemia; MMUD = mismatched-unrelated donor.

Here we describe nine patients with acute limbic encephalitis after HSCT, including six with PCR evidence of HHV6 DNA in the CSF. Although a subset of these patients has been described from a radiological perspective,[25] this is the first adult series of post-transplant acute limbic encephalitis (PALE) based on clinical features. By characterizing this emerging syndrome, we seek to improve recognition of the illness and promote tractable studies of its pathogenesis.

METHODS Patients. Nine patients with acute limbic encephalitis were seen on the Brigham & Women's Hospital/ Dana-Farber Cancer Care Hematopoietic Stem Cell Transplant Service between October 2001 and March 2005. Neurologic consultations, performed for each patient by one of the authors (W.W.S., K.U., T.M.H., or E.B.B.), were requested for unexplained amnesia, confusion, or a first-ever seizure. MR images were obtained from a Signa 1.5 T unit (GE Systems, Milwaukee, WI) using T1-weighted (before and after IV contrast), T2-weighted, fluid attenuation inversion recovery (FLAIR), diffusion-weighted (DWI), and apparent diffusion coefficient (ADC) sequences, though not all sequences were available for

all patients. CSF assays for HHV6 DNA were performed at different reference laboratories during the study period. Samples from Patients 1 through 6 underwent non-quantitative PCR amplification with primers common to both HHV6 variants (A and B), precluding specification of the detected variant (Focus Technologies, Cypress, CA). For Patients 5 and 6, confirmatory testing was submitted for HHV6 DNA measurements using quantitative PCR (ViraCor Laboratories, Lee's Summit, MO).[26] Patients 6 through 9 had additional quantification of viral loads using a PCR assay with a quantitative range between 1,000 and 1,000,000 copies/mL that distinguishes between variants A and B (Associated Regional & University Pathologists [ARUP], Inc., Salt Lake City, UT).[27]

For all nine patients, relevant clinical data were collected, including age, sex, underlying hematologic malignancy, date of HSCT, conditioning regimen, source of stem cells, relationship and HLA matching of the donor and recipient, GVHD prophylaxis and treatment medications, and time and type of GVHD manifestations. Engraftment was defined as an absolute neutrophil count greater than 500 cells/µL for 2 consecutive days. GVHD was classified according to the consensus scale.[28] Information related to the clinical encephalitis was recorded, including day of onset after HSCT, presenting symptoms and findings on neurologic examination, EEG and MRI results, antiviral and anticonvulsive treatments used, concomitant medical and laboratory conditions, and clinical outcomes. Follow-up ended on June 30, 2005, for surviving patients.

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Table 2   Post-transplant acute limbic encephalitis (PALE) after hematopoietic stem cell transplantation

Patient	PALE symptom onset*	Symptoms	1st EEG	1st MRI: foci of T2/FLAIR/DWI hyperintensity	Antiviral treatment	Anticonvulsive treatment	Concomitant conditions	Outcome
1	Day 25	Confusion, amnesia	Day 40: Diffuse theta slowing	Day 27: R>L uncus/amygdala, R=L hippocampus	Acyclovir ×10 d →valganciclovir, IVIg TIW	None	Hyponatremia (nadir 126 meq/L, Day 25)	Alive Day 1351; Good cognitive recovery
2	Day 29	Amnesia, episodic staring with oral automatisms	Day 75: PLEDS, L anterior temporal	Day 31: R>L uncus/amygdala, hippocampus	Acyclovir→ganciclovir × 12 d →foscarnet × 18 d	Phenytoin→ levetiracetam	Hyponatremia (nadir 130 meq/L, Day 35)	Died Day 144 from severe GI GVHD and bacteremia; poor cognitive recovery; no autopsy
3	Day 55	Amnesia, episodic confusion with complex motor behavior	Day 60: Bitemporal slowing with sharp waves	Day 60: R>L uncus, R=L hippocampus	Acyclovir→valganciclovir × 21 d	Valproate & levetiracetam	Hyponatremia (nadir 118 meq/L, Day 60); posterior leukoencephalopathy (cyclosporine)	Alive Day 1254; poor cognitive recovery with persistent episodic memory impairment
4	Day 61	Confusion, amnesia, visual hallucinations	Day 62: Excessive beta activity, diffuse theta activity	Day 62: R=L uncus, hippocampus	Valganciclovir × 10 d	Gabapentin (ongoing for neuropathy)	Hyponatremia (nadir 129 meq/L, Day 68); S aureus bacteremia, DAH	Died Day 157 from refractory GI GVHD and enterococcal sepsis; good cognitive recovery; autopsy (see text)
5	Day 31	Generalized tonic-clonic seizures, confusion, amnesia, emotional outbursts	Day 31: Diffuse theta and delta slowing, nonspecific sharps	Day 31: R>L uncus/amygdala, hippocampus	Acyclovir × 1 d→ganciclovir × 12 d →foscarnet × 31 d→repeat foscarnet × 11 d	Phenytoin→ levetiracetam	Hyponatremia (nadir 128 meq/L, Day 34); VOD of the liver, ARF	Died Day 108 from liver GVHD, VOD, ARF; modest cognitive recovery; autopsy excluded brain
6	Day 25	Generalized tonic-clonic seizures, confusion, amnesia, emotional outbursts (aggressive, hypersexual)	Day 27: L anterior temporal spikes, intermittent runs of L>R temporal theta; R frontotemporal PLEDS 4 d later	Day 26: L>R uncus, hippocampus	Acyclovir × 1 d →foscarnet × 28 d; IVIg	Phenytoin→ levetiracetam	Hyponatremia (nadir 126 meq/L, Day 31)	Alive Day 663; modest cognitive recovery, persistent episodic memory impairment
7	Day 32	Confusion, amnesia, anomia; depression and withdrawal after antiepileptic treatment	Day 35: R frontotemporal PLEDS	Day 32: normal; Day 37: R=L amygdala, hippocampus	Acyclovir→ganciclovir × 5 d→ foscarnet × 28 d	Levetiracetam	Neurocardiogenic syncope; no hyponatremia, (nadir 135 meq/L, Day 41); hypernatremia to 155, peak Day 66)	Alive Day 251; modest cognitive recovery, persistent episodic memory impairment
8	Day 14	Agitation and aggression followed by lethargy and hypercarbic respiratory failure; after extubation on Day 22, amnesia and confusion	Day 26: R>L theta and delta activity; periodic right temporal sharp waves	MRI not done due to indwelling bullet and TEN; Day 23: CT normal	Foscarnet × 4 d→ganciclovir × 6 d→foscarnet × 8 d	Levetiracetam	Hyponatremia (nadir 130 meq/L, Day 15); aspiration pneumonia, TEN vs skin GVHD	Died Day 42 from MRD-Acinetobacter baumannii bacteremia and septic shock, persistent TEN; recovery not assessable; no autopsy
9	Day 18	Confusion, fever, resolving to amnesia; one emotional (hypersexual) outburst	Day 28: Poor organization, theta and delta slowing; Day 68: Diffuse theta and delta activity, one run of asymmetric beta followed by sharp alpha and 4–5 Hz R temporal theta	Day 18: R=L uncus/amygdala, hippocampus	Foscarnet × 28 d	Levetiracetam	Hyponatremia (nadir 126 meq/L, Day 18); incident VOD, ARF, and aspiration pneumonia	Died Day 114 from persistent liver GVHD and VOD, ARF; modest cognitive recovery; no autopsy

*Days refer to interval from hematopoietic stem cell transplantation.

IVIg = IV immune globulins; TIW = three times weekly; PLEDS = periodic lateralized epileptiform discharges; GI = gastrointestinal; GVHD = graft vs host disease; DAH = diffuse alveolar hemorrhage; VOD = veno-occlusive disease; ARF = acute renal failure; TEN = toxic epidermal necrolysis; MRD = multi-drug resistant.

Clinical database search. The first PALE cases to be recognized at our center were encountered in 2001. Thereafter, HHV6 PCR testing was routinely included when HSCT patients underwent lumbar puncture for an acute neurologic change. To evaluate the specificity of a positive HHV6 CSF PCR assay for PALE and to minimize the possibility of inception bias, we searched the Partners Healthcare System Research Patient Data Repository for all CSF HHV6 PCR determinations performed for HSCT recipients, beginning when the test became available to all clinicians in March 2003 and ending on March 31, 2005. Medical records from all tested patients were examined for clinical and radiographic (MRI) evidence of limbic encephalitis. Indications for CSF sampling and final diagnoses rendered for each indication were recorded. When testing limbic encephalitis patients and patients identified through the database search,

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the reference laboratories were unaware of the clinical syndromes that prompted HHV6 testing.[26,27]
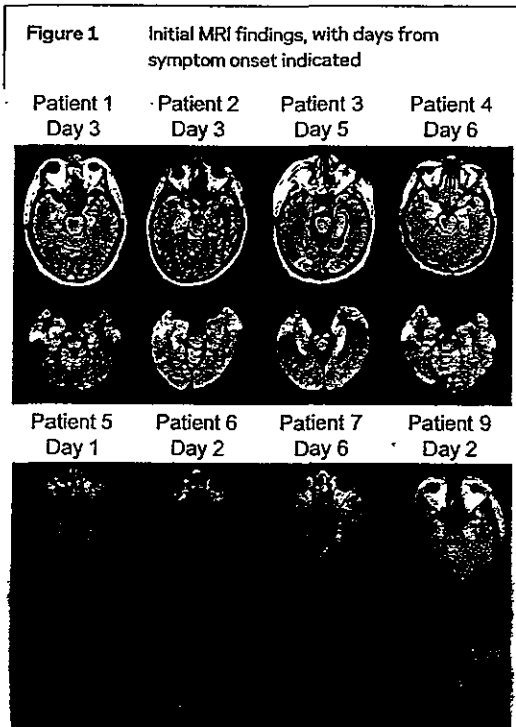
The study was approved by the institutional review boards for the Partners Healthcare System and the University of California, San Francisco.

RESULTS Clinical features. We encountered nine HSCT patients with an acute limbic encephalitis syndrome between October 2001 and March 2005. During this period, 584 patients underwent allogeneic HSCT, suggesting a cumulative PALE incidence of 1.5%. Table 1 summarizes the baseline clinical characteristics of the patients in the series. All were men with a median age of 41 years (range 22 to 60 years) who underwent HSCT for a range of hematologic malignancies (table 1). Donors were unrelated to the recipient for all but Patient 1, who underwent a T-cell depleted transplant from a HLA-matched sibling donor. Six patients underwent myeloablative conditioning, three non-myeloablative. All developed GVHD. Severity and organ involvement varied. GVHD diagnosis and treatment preceded onset of limbic encephalitis in five and followed it in four patients. Prophylactic and treatment GVHD immunosuppressive regimens also varied (table 1). Engraftment preceded neurologic symptom onset in all patients.

Table 2 summarizes the clinical characteristics of the limbic encephalitis syndrome. Neurologic symptoms began a median of 29 days after HSCT (range 14 to 61 days). Herpes simplex and herpes zoster virus prophylaxis with oral or IV acyclovir was ongoing in all patients, and cytomegalovirus (CMV)

surveillance was performed one to two times per week by use of a whole-blood CMV hybrid capture assay. Clinical features common to all patients included acute onset over 1 to 3 days, dense anterograde amnesia, and patchy retrograde amnesia for up to 4 years. All patients lost memory of their underlying HSCT-requiring condition. Patient 1 failed to recognize an examiner returning from a 3-minute telephone call. Patient 3 was surprised to find a tunneled central venous catheter in his upper chest wall when prompted by his wife to clean it. Patient 4 had no memory of the September 11, 2001, tragedy when queried in April 2002. Language, praxis, and visuoconstructive skills were spared on routine bedside testing. A confusional state was present at symptom onset in Patients 1 and 4 through 9 but gave way to amnesia within the first several days. Fever was present only in Patients 5 and 6 and could be attributed to ongoing GVHD or concomitant infection. Clinical seizures occurred without question in Patients 2, 3, 5, and 6, were suspected in Patients 7 through 9, and typically did not respond to the first medication given (table 2). Patients 2 and 3 had complex partial seizures that were ultimately controlled with levetiracetam, valproic acid, or both. In Patients 5 and 6, a generalized tonic-clonic seizure marked symptom onset. Periods of intense agitation followed antiepileptic treatment in both cases. Patient 6 cursed at family members (including a small child) and struck a nurse. Patient 7 was started on levetiracetam after his EEG and became emotionally withdrawn for several days thereafter. Hyponatremia due to inappropriate production of antidiuretic hormone (SIADH) heralded or began with symptom onset in all but Patient 7. No other consistent general laboratory abnormality was seen.

Clinical course varied. In most instances, suspicion for HSV encephalitis prompted acyclovir treatment within 1 to 2 days of symptom onset. Therapy changed when HSV PCR testing was negative or concern for HHV6 rose (table 2). Cognitive recovery ranged from excellent to none. Upon retesting, anterograde amnesia had improved considerably in Patients 1 and 4, little in Patient 3, and modestly in Patients 6, 7, and 9. Patient 2 remained densely amnesic prior to his death from GVHD and sepsis. Patient 5 made modest gains in anterograde memory before developing GVHD, veno-occlusive disease of the liver, and ultimately death from pneumonia. Patient 6 was examined in the neurology clinic 15 months after his illness. Digit span backward was six. On a verbal learning test, he was able to recall 7/7 words after the first entrainment trial. After a 60-second distraction, he recalled 5/7, at 3 minutes 5/7, and at 10 minutes he was able to recall only 3/7

Figure 1	Initial MRI findings, with days from symptom onset indicated

Patient 1 Day 3	Patient 2 Day 3	Patient 3 Day 5	Patient 4 Day 6

Patient 5 Day 1	Patient 6 Day 2	Patient 7 Day 6	Patient 9 Day 2

Axial fluid attenuation inversion recovery (FLAIR) (top) and diffusion-weighted (bottom) sequences demonstrate limbic system involvement, including the uncus, amygdala, and anterior hippocampus in all patients. For Patient 6, only coronal FLAIR images from day 2 were available. An axial FLAIR image from day 6, with findings similar to those on day 2, is shown. The left side of each image corresponds to the right side of the brain

Coronal FLAIR MR images from Patient 1 demonstrate the focality of the encephalitis, which can be traced bilaterally from the uncus through the hippocampal body and into the fornix. The amygdala is partly affected and the parahippocampal gyrus is conspicuously spared. The left side of each image corresponds to the right side of the brain.

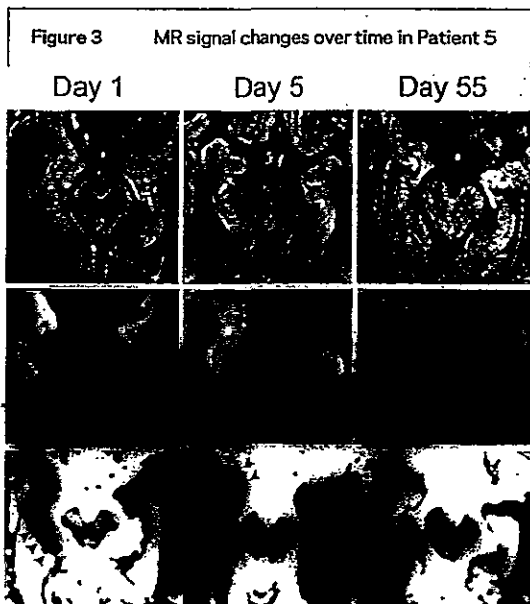**Figure 2**   Coronal fluid attenuation inversion recovery (FLAIR) MR images from Patient 1
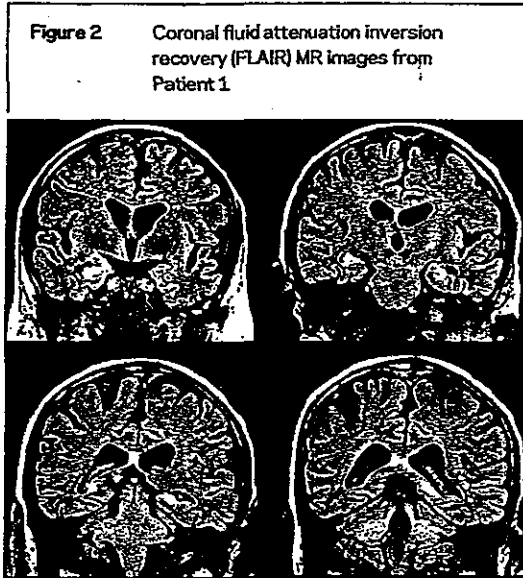
words. He requires a global positioning system device to drive without getting lost. Patient 7 performed similarly when examined 4 months after his illness during an unrelated hospital admission. Patient 8 developed toxic epidermal necrolysis due either to trimethoprim-sulfamethoxazole or severe GVHD. He required intubation, mechanical ventilation, and heavy sedation that precluded cognitive follow-up. He later died from septic shock caused by multidrug-resistant *Acinetobacter baumannii*. Absent or early treated seizures were associated with a better cognitive outcome. Patients 2 and 3 recovered least and both had refractory complex partial or clinically "silent" electrographic seizures early in their course.

MRI findings. All but Patient 8 underwent MRI of the brain. Without exception, focal medial temporal

Figure 3   MR signal changes over time in Patient 5

Day 1   Day 5   Day 55

Limbic fluid attenuation inversion recovery hyperintensities (top row) were prominent in the early subacute phase, even as diffusion-weighted imaging (DWI) signal (middle row) waned. Apparent diffusion coefficient maps (bottom row) showed restricted diffusion within DWI-bright regions (arrowheads). Prominent hippocampal atrophy was demonstrated 55 days from symptom onset (right column). The left side of each image corresponds to the right side of the brain.

abnormalities were identified (table 1, figure 1). This radiographic signature involved circumscribed, bilateral, non-enhancing, T2/FLAIR/DWI hyperintensities within the uncus, amygdala, and hippocampal body, typically extending to the entorhinal cortex and subiculum. Parahippocampal gyrus was conspicuously spared. The posterior extent of the lesions varied but reached as far as the intersecting crura of the fornix (figure 2). Some asymmetry was common. Initial MR imaging in Patient 3 also revealed a posterior leukoencephalopathy that resolved after discontinuation of cyclosporine. ADC maps were available for four patients and suggested true restricted diffusion in Patient 5 (figure 3) but T2 "shine through" effects in the others (Patients 6, 7, and 9).[25] Serial imaging of Patients 1, 5 through 7, and 9 revealed a consistent radiographic progression (figure 3). Within affected areas, increased DWI signal appeared acutely, but over time increasing T2/FLAIR signal emerged as diffusion characteristics returned to normal. Four patients were rescanned at least 6 weeks from symptom onset. In each case, hippocampal volume was notably reduced (see figure 3); whether this finding represents atrophic change or resolution of edema is uncertain since patients were not imaged prior to developing limbic encephalitis.

EEG findings. EEG was performed for all patients due to observed or suspected seizures. Focal abnormalities were seen over the temporal or frontotemporal leads in seven of nine patients, usually involving both sides. All seven had epileptiform activity-including two with electrographic seizures, three with periodic lateralized epileptiform discharges (PLEDS), one with both seizures and PLEDS, and one with sporadic interictal discharges. The remaining two patients had diffuse slowing, in one case recorded on the same day as a convulsive seizure. A representative EEG tracing from Patient 6 illustrates the epileptogenic nature of PALE (figure E-1 on the *Neurology* Web site at www.neurology.org).

CSF and HHV6 PCR results. Findings at lumbar puncture are shown in table 3. No specimen was xanthochromic. Mild CSF pleocytosis with lymphocyte predominance was present in all but Patient 4 (median 5 leukocytes/mm^3, range 1 to 41). Protein elevations were typically mild (median 48 mg/dL, range 19 to 189). Patients 1, 2, and 5 through 9 tested positive for HHV6 by PCR on at least one CSF sample. Traumatic blood contaminated the HHV6-positive specimen from Patient 2, raising the possibility that HHV6 DNA from blood was detected. Further, this specimen was obtained 3

Patient	PALE onset	LP timing	Protein mg/dL	Glucose mg/dL	WBC cells/mm³ (% lymphs)	RBC cells/mm³	CSF HHV6 PCR copies/mL	HHV6 variant
1	Day 25	Day 26	45	72	4 (93)	0	Positive	ND
		Day 40	58	80	2 (82)	2	Negative	
		Day 158	91	46	14 (90)	0	Negative	
2	Day 29	Day 30	97	56	12 (93)	0	Negative	
		Day 51	189	71	41 (98)	...178	Negative	
		Day 125	19	78	1 (88)	3,444*	Positive	ND
3	Day 55	Day 56	53	69	5 (91)	0	Negative	
		Day 58	33	73	7 (82)	3	Negative	
		Day 71	90	62	7 (92)	0	Negative	
4	Day 61	Day 63	29	119	1 (66)	0	Negative	
5	Day 31	Day 32	106	76	25 (5)	47,000*	Positive	ND
		Day 81	37	75	17 (82)	4	Positive: 100	B
		Day 102	32	84	2 (96)	1	Negative	
6	Day 25	Day 25	64	74	7 (64)	138	Positive	ND
		Day 37	44	59	7 (85)	3	Negative	
7	Day 32	Day 33	48	71	16 (92)	30*	Positive: >999,000†	B
		Day 45	50	64	7 (88)	0	Positive: <1,000†	B
		Day 66	81	62	7 (90)	23	ND	
8	Day 14	Day 25	28	59	3 (58) 27% monocytes	18	Positive: <1000§	B
9	Day 18	Day 19	42	78	2 (44)	0	Positive: 203,000‖	B
		Day 34	49	112	3 (92)	44	Positive: <1,000	B
		Day 46	42	148	1 (91)	2	Negative	

Table 3   CSF analysis and human herpesvirus-6 (HHV6) PCR results

*Specimen obtained through a traumatic lumbar puncture yielding pink CSF.
†Plasma sample on Day 36 showed a HHV6 viral load of 186,000 copies/mL.
‡Plasma sample on Day 45 showed a HHV6 viral load of <1,000 copies/mL.
§Plasma sample on Day 17 showed a HHV6 viral load >999,999 copies/mL. Foscarnet was begun, and a follow-up viral load was 1,430 copies/mL (Day 30).
‖Plasma sample on Day 22 showed a HHV6 viral load of 4,070 copies/mL.
¶Plasma sample on Day 33 did not reveal HHV6 viremia.
PALE = post-transplant acute limbic encephalitis; LP = lumbar puncture.

months after symptom onset, making it unlikely the culprit pathogen in the limbic encephalitis. Initial CSF samples from Patients 5 and 7 were also blood-tinged, but their second samples were untainted and still positive. Other microbiologic studies performed on CSF varied due to clinician preference, season, and CSF availability, but all patients were negative for HSV 1 and 2, CMV, EBV, JCV, and VZV by PCR. Bacterial and fungal cultures, stains for acid fast bacilli, and VDRL were unrevealing. Patients tested for paraneoplastic autoantibodies (anti-Hu, anti-Ma/Ta, anti-CV2) in the CSF (Patients 1 and 3) or serum (Patient 6, anti-Hu and anti-Ma/Ta only) were found negative.

Neuropathology. Brain autopsy was performed only on Patient 4, who died 3 months after his encephalitic episode due to unrelated causes (table 2). Microscopic examination (figure 4) revealed severe neuronal loss and gliosis in CA1 and CA4 regions of the hippocampus and the amygdala, with milder gliosis of CA2, CA3, subiculum, fornices, insula, and cingulate. The hippocampus showed no immunopositivity for HSV, CMV, or VZV antigens. A single microglial nodule was seen in the fornix. No other focal abnormalities were identified. HHV6 immunohistochemistry was not performed.

Clinical database findings: CSF HHV6 PCR results over a 2-year period. Between March 17, 2003, and March 31, 2005, CSF aliquots from 41 lumbar punctures performed on 27 post-HSCT patients were submitted for HHV6 detection, corresponding to 6.4% of patients transplanted during this period. Twelve samples corresponded to Patients 5 through 9 of the present series. HHV6 DNA was detected in none of the 29 samples from the remaining 22 patients. From these patients, initial specimens were obtained a median of 51 days after HSCT (range, 16 to 733 days). Indications for CSF sampling were an

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Gross findings (A, B) included atrophy of the hippocampus, right worse than left, and amygdala (not shown). Microscopic examination of the hippocampus (C–E) revealed profound neuronal loss in CA1 and CA4, accompanied by astrocytic gliosis (E). CA2 and 3 were also injured, though less severely (C, D). Hematoxylin & eosin stain. Scale bars correspond to 100 μm (C), 50 μm (D), and 20 μm (E). Regions boxed in A and C are magnified in B, D, and E. The left side of each image corresponds the left side of the brain. Dg = dentate gyrus.

Figure 4   Postmortem findings in Patient 4, 3 months after clinical encephalitis.
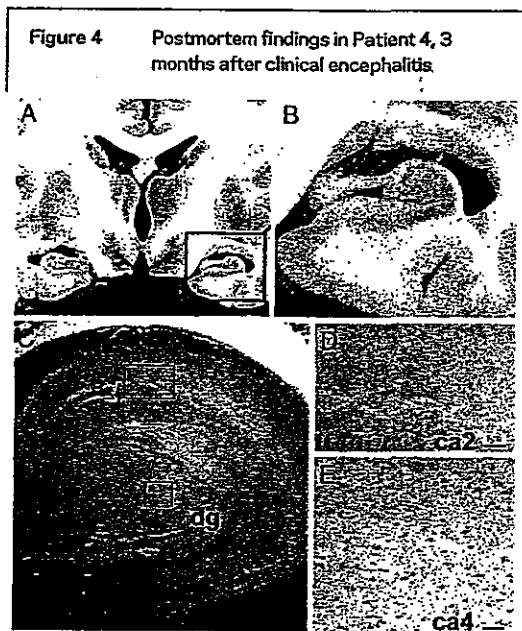
acute confusional state in nine, decreased sensorium in seven, seizures in three, and transverse myelitis, polyradiculitis, and a catatonic state in one each. Final diagnoses were encephalopathy due to sepsis, renal, or hepatic failure in nine, tacrolimus-associated reversible posterior leukoencephalopathy in three, and a case each of leukemic relapse, encephalomyelitis, focal occipital encephalitis vs glioma, Lyme neuroborreliosis,[29] oversedation, subarachnoid hemorrhage, seizure disorder, staphylococcal meningoencephalitis, herpes zoster myelitis, and depression. No additional cases of limbic encephalitis were noted. Therefore, during this 2-year period, CSF HHV6 PCR was positive only in the context of acute limbic encephalitis.

DISCUSSION Based on this series and prior reports,[15-21,23-25] we propose that patients undergoing allogeneic HSCT are at risk for post-transplant acute limbic encephalitis, or PALE, a distinct neurologic syndrome. Children and adults are susceptible. Men appear more vulnerable, as we have yet to observe the illness in women though they constitute 40 to 45% of our annual HSCT cohorts and 10 of 13 PALE patients reported in the literature have been men.[15-22,24] Consistent PALE clinical features include marked anterograde amnesia, seizures or temporal lobe EEG abnormalities, SIADH, and mild CSF pleocytosis. The syndrome is accompanied by an MR signature of bilateral, nonenhancing, medial temporal lobe T2/FLAIR/DWI hyperintensities sharply demarcated by parahippocampal gyrus sparing. PALE is associated with a short interval from transplantation (15 to 60 days) and may be more common after unrelated donor HSCT and

myeloablative conditioning. Due to reports of a nearly identical illness after solid organ transplantation[30,31] and the likelihood of a shared pathophysiology, we offer the term PALE for its unifying and descriptive qualities.

The natural history of PALE has not been previously described. None of the patients in the present series died as a direct result of encephalitis (table 2). Patients alive 6 months after HSCT (Patients 1, 3, 6, and 7) had persistent mild to moderate neuropsychological deficits largely confined to episodic memory. Factors influencing outcome remain uncertain, but early seizure control may significantly improve prognosis.

What is the pathophysiology of PALE? In this series, symptoms arose early in the post-HSCT course (<61 days) but always after engraftment. CSF profiles suggested increased blood–brain barrier permeability and mild pleocytosis, both attributable to low-grade inflammation, seizures, or both. Minor subarachnoid hemorrhage with xanthochromia, as seen with the necrotizing inflammation of HSV encephalitis, was absent. EEG often revealed the epileptogenic nature of the illness and supported the clinical impression of interictal as well as ictal temporal dysfunction. Serial MRI characteristics of PALE highlight its urgency. ADC maps at times showed restricted water diffusion in affected tissues, suggesting that acute DWI hyperintensities seen in all patients were not only due to "shine through" T2 prolongation effects from vasogenic edema. Known pathologic-biophysical correlates of reduced ADC include ischemic cytotoxic edema (with failure of energy dependent ion homeostasis), seizure-related excitotoxicity/hyperperfusion (with glucose utilization exceeding oxygen delivery, leading to cellular lactate accumulation and osmotic shifting of extrato intracellular water), and acute myelin vacuolization.[32,33] Although our patients were not clinically seizing at the time of imaging, subclinical epileptiform activity was often found when sought. Considering the prior report of PET hypermetabolism in children with PALE,[23] our DWI/ADC findings suggest a reversible metabolic supply/demand mismatch during the acute epileptogenic phase. Rising T2 and falling DWI signals of the early subacute phase suggest that vasogenic edema may linger even after seizures are controlled. Apparent hippocampal atrophy following PALE suggests that the excitotoxicity of the illness, though treatable, can lead to irreversible limbic injury.

The pathophysiology of PALE is compatible with an infectious or inflammatory etiology. This study provides modest support for the alleged role of HHV6 in the syndrome, with the caveat that dif-

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ferent HHV6 PCR assays were employed during the study period as clinical practice evolved. Nonetheless, six of nine cases (Patients 1, 5 through 9) had a positive initial CSF HHV6 PCR assay. When available, quantitative HHV6 PCR showed rapid treatment-related reductions in HHV6 viral load not always accompanied by clinical improvement. CSF HHV6 PCR from Patients 2 through 4 was negative or indeterminate. Therefore, despite the clinical consistency of this series, CSF HHV6 DNA was detected in only two-thirds of the patients. This incomplete association of HHV6 with PALE should trigger several important questions about this clinical syndrome.

Is PALE caused by HHV6 and, if so, what are the sensitivity and specificity of CSF PCR assays? PALE is a newly described, infrequent, and under-recognized syndrome, making the test characteristics of HHV6 CSF PCR difficult to evaluate, even at centers that are attuned to the diagnosis. In the only prior study to explore the significance of HHV6 PCR in immunocompromised patients, HHV6 was found in CSF from 1/107 asymptomatic control patients who underwent standard non-HSCT treatment for leukemia, 0/11 post-HSCT patients with known causes of their "CNS symptoms," and 5 of 11 post-HSCT patients with symptoms for which no cause was found.[34] None of the 5 HHV6 PCR-positive cases had a syndrome typical of PALE. During a 2-year interval at our institution, quantitative CSF PCR assays for HHV6[27] were positive in all patients (5/5) with a clinical diagnosis of PALE and negative among 22 HSCT patients who lacked core features of PALE but were tested in the context of other acute neurologic syndromes.

How often does PALE arise without HHV6? To our knowledge, Patients 2 through 4 are the only HHV6-negative PALE cases on record. Each developed PALE between 2001 and 2002 when a non-quantitative, less sensitive assay was in use at our institution. Nonetheless, their results suggest that HHV6-PCR-negative PALE can occur and that scattered cases may have gone unreported at our center or elsewhere. Awareness of PALE is limited, and such cases could have been wrongly attributed to herpes simplex encephalitis, even in the face of a negative CSF HSV PCR assay. In a recent longitudinal study of three HSCT patients with encephalitis, high levels of HHV6 DNA and mRNA were detected in postmortem hippocampus and other limbic regions, even though CSF HHV6 DNA had fallen to undetectable levels in the weeks before death.[35] Further prospective studies are needed to more completely assess HHV6 PCR test characteristics in the context of PALE.

If PALE is caused by HHV6, what is the range of clinical phenomena that HHV6 can effect? Two reviews of post-transplant HHV6-associated encephalitis conducted in 2000[14,15] demonstrated heterogeneous syndromes and a paucity of neuroimaging abnormalities. Only three of the combined 14 patients described had clinical and imaging patterns characteristic of PALE. Therefore either HHV6 has protean manifestations or its detection can be nonspecific, perhaps reflecting an innocent bystander brought forth by an unidentified primary disease. PALE could reflect HHV6 reactivation, as the virus resides latently in at least 85% of non-neurologic autopsy control brains[12] and has a predilection for the medial temporal lobes.[4]

Limbic encephalitis (LE) has become an important and multifaceted syndrome in neurology. Apart from the rapidity with which it evolves, PALE bears a strong clinical resemblance to paraneoplastic LE, classically a subacute illness associated with occult small cell lung carcinoma and anti-Hu antibodies.[36] During the past decade, LE has been linked to multiple underlying malignancies and autoantibodies[37] and can occur without cancer, at times accompanied by autoantibodies to voltage-gated potassium channel isoforms expressed by hippocampal neurons.[38,39] The related construct of non-herpetic acute limbic encephalitis (NHALE) has emerged in the Japanese literature,[40] occurs in immunocompetent hosts after routine viral illness, causes hippocampal DWI hyperintensity, and can respond to immunosuppressive therapy.[41] In view of this gathering complexity, PALE provides a useful new model of LE by occurring in a context unfit for most forms of autoimmunity. Conversely, studies of autoimmune LE could reveal parainfectious immune mechanisms that prove relevant to PALE. Many LE variants focus their attack on limbic structures through mysterious means; whether HHV6 plays a role in all, some, or none of them remains an open question.

PALE is a clinically recognizable entity that requires a low threshold for diagnosis. Post-transplant patients with acute alterations in mental status, prominent amnesia, or unexplained seizures should undergo MRI with diffusion-weighted imaging, lumbar puncture, and CSF PCR testing for HHV6 and other potential culprit viruses. Seizures should be assumed ongoing or impending and treated early to prevent irreversible hippocampal injury. The pathogenesis of PALE deserves further investigation, and prospective antiviral treatment trials for PALE are needed. We hope this series will stimulate clinicians to report PALE cases whether or not an identifiable viral etiology is found.

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