A Chronic Illness Characterized by Fatigue, Neurologic and Immunologic Disorders, and Active Human Herpesvirus Type 6 Infection

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■ *Objective:* To conduct neurologic, immunologic, and virologic studies in patients with a chronic debilitating illness of acute onset.

■ *Design:* Cohort study with comparison to matched, healthy control subjects.

■ *Patients:* We studied 259 patients who sought care in one medical practice; 29% of the patients were regularly bedridden or shut-in.

■ *Main Outcome Measures:* Detailed medical history, physical examination, conventional hematologic and chemistry testing, magnetic resonance imaging (MRI) studies, lymphocyte phenotyping studies, and assays for active infection of patients' lymphocytes with human herpesvirus type 6 (HHV-6).

■ *Main Results:* Patients had a higher mean (± SD) CD4/CD8 T-cell ratio than matched healthy controls (3.16 ± 1.5 compared with 2.3 ± 1.0, respectively; $P < 0.003$). Magnetic resonance scans of the brain showed punctate, subcortical areas of high signal intensity consistent with edema or demyelination in 78% of patients (95% CI, 72% to 86%) and in 21% of controls (CI, 11% to 36%) ($P < 10^{-9}$). Primary cell culture of lymphocytes showed active replication of HHV-6 in 79 of 113 patients (70%; CI, 61% to 78%) and in 8 of 40 controls (20%; CI, 9% to 36%) ($P < 10^{-8}$), a finding confirmed by assays using monoclonal antibodies specific for HHV-6 proteins and by polymerase chain reaction assays specific for HHV-6 DNA.

■ *Conclusions:* Neurologic symptoms, MRI findings, and lymphocyte phenotyping studies suggest that the patients may have been experiencing a chronic, immunologically mediated inflammatory process of the central nervous system. The active replication of HHV-6 most likely represents reactivation of latent infection, perhaps due to immunologic dysfunction. Our study did not directly address whether HHV-6, a lymphotropic and gliotropic virus, plays a role in producing the symptoms or the immunologic and neurologic dysfunction seen in this illness. Whether the findings in our patients, who came from a relatively small geographic area, will be generalizable to other patients with a similar syndrome remains to be seen.

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We studied 259 patients who had an illness that was typically of abrupt onset, beginning with a "flu-like" syndrome that was followed by months or years of sometimes disabling chronic fatigue and impaired cognition. Enough cases occurred among family members, coworkers, and other close contacts to suggest the possibility of an infectious agent transmissible by casual contact. A few patients developed transient periods of apparent encephalitis, characterized by confusion, ataxia, paresis, and primary seizure disorders. Several unusual features have been revealed by immunologic testing, magnetic resonance imaging (MRI) studies of the brain, and virologic studies.

Methods

Initiation of the Study

In late 1984, two of us (DLP and PRC) who had a general medicine practice in Incline Village, Nevada, on the north shore of Lake Tahoe, began to see several patients with an unusual illness. By mid-1985, we were concerned that an epidemic might be unfolding. Two of us who were studying a similar illness in an academic general medicine practice in Boston (DB and ALK) heard of the events in Nevada. Together, the four of us began a formal study in January 1986. A team from Boston traveled to Nevada on several occasions. Many additional collaborators became involved. Research assistants in Lake Tahoe and Boston gathered detailed clinical and laboratory data and collected blood specimens, and all data were entered into a computerized database in Boston. Because a formal study was initiated a year after events unfolded, we were unfortunately not able to determine exactly how many patients were seen in this practice who had the symptoms of this unusual illness but were not entered into the study. However, we estimate that 85% of the patients seen in the practice who had this illness were enrolled.

Patients

One hundred and eighty-three patients lived in one of several communities near the California-Nevada border, in the vicinity of Lake Tahoe, and sought medical care between 1984 and 1987 for chronic fatigue at the internal medicine practice. The mean (± SD) age of this "Tahoe" group was 38.9 ± 12.3 years; 67% were female and 41% were college graduates.

Seventy-six other patients with chronic, debilitating fatigue came to the same practice during the same time period. These patients were from outside the Lake Tahoe area, typically from urban areas of California and Nevada. The mean age of this "non-Tahoe" group was 38.6 ± 11.4 years; 70% were female and 36% were college graduates. The mean age of all patients, including both the Tahoe and non-Tahoe groups, was 38.8 ± 12.0 years; 68% of patients were female, and 39% were college graduates.

The Tahoe group may have represented part of an epidemic, because most of these patients became ill within a 2-year

Table 1. Characteristics of Patients and Control Subjects by Individual Test*

Test	Patients	Control Subjects	*P* Value†
HHV-6 replication assay			
Number	113	40	
Mean age, y	39.5 ± 11.2	37.5 ± 10.7	> 0.2
Female, %	70	53	0.07
HHV-6 serologic testing			
Number	134	27	
Mean age, y	38.4 ± 12.3	47.5 ± 11.9	< 0.01
Female, %	63	48	0.23
Lymphocyte phenotyping			
Number	121	54	
Mean age, y	41.2 ± 11.7	32.4 ± 9.1‡	< 0.01
Female, %	70	60‡	0.21
MRI study			
Number	144	47	
Mean age, y	38.8 ± 10.8	36.9 ± 13.2	> 0.2
Female, %	72	68	> 0.2

* HHV-6 = human herpesvirus-6; MRI = magnetic resonance imaging. Mean values are expressed ± SD.

† $P < 0.05$ was considered statistically significant.

‡ Data on age and sex were available from only 35 of the 54 control subjects.

period and many had close contacts who became ill. Hence, they are also referred to as the "epidemic" group; however, the design of our study did not allow us to establish that a true epidemic had occurred. The non-Tahoe group is referred to as the "endemic" group because the patients did not know many other persons who were similarly affected; however, many patients knew at least one close contact who was allegedly affected.

The 259 patients enrolled in the study were asked to complete a detailed questionnaire, and data from each patient's medical record were abstracted. To be included in our study, a patient had to have chronic, debilitating fatigue of at least 3 months duration that was associated with at least two of the following symptoms (also of at least 3 months duration): fever, headache, sore throat, earache, rhinorrhea, cough, diarrhea, or myalgias. At the time of enrollment in the study, the median duration of illness had already reached 1.3 years.

Data were obtained from several different groups of healthy control subjects. These control groups are described in the following sections and in Table 1, with reference to the diagnostic testing for which they served as controls.

Measurements

Standard Laboratory Tests

Complete blood counts, erythrocyte sedimentation rates, standard serum chemistry tests, various tests for collagen vascular diseases, and thyroid function tests were ordered when indicated clinically and not according to a study protocol.

Cerebrospinal Fluid Examination

Analysis of cerebrospinal fluid obtained by lumbar puncture was done in six patients who had developed acute neurologic changes (confusion, marked cognitive deficits, delirium, ataxia, transient paresis, or primary seizure).

Magnetic Resonance Imaging of the Brain

Magnetic resonance imaging studies were done by one of us (RB) at Reno Diagnostics Center. One hundred and forty-four patients had at least one MRI study. These patients had somewhat greater debility but were otherwise generally similar to the 115 patients in whom MRI studies were not done (Table 2). The MRI findings in patients were compared with those in 47 control subjects who did not have chronic fatigue and who were similar to the patients in mean age and gender distribution (see Table 1). Forty-two of these controls had been asked to undergo scanning as part of a study to assess the resolution of a new machine with a larger magnet; four patients had sustained head trauma and one patient had a suspected cervical herniated disk. The images from the controls were obtained on the same brand of machine, with the same size magnet, by Dr. Victor Haughton of Milwaukee, Wisconsin. The images from patients could be distinguished from those of control subjects by the logos of the two radiology departments (Reno and Milwaukee); thus, blind interpretation was not possible. However, the images were interpreted independently by two of us (RB and FAJ), and inter-rater reliability was determined.

The patient studies were done using a 1.5-tesla superconducting Signa magnetic resonance imager (General Electric Medical, Milwaukee, Wisconsin). Images included a T1-weighted sagittal scan followed by T2-weighted transaxial spin echo scans of the brain. These axial scans were obtained at 5-mm intervals from the foramen magnum to the vertex, with a skip of 1 to 2 mm. The repetition time was between 2000 and 2500 ms and the echo delay time was between 20 and 30 ms for the first echo and between 50 and 80 ms for the second echo.

Lymphocyte Phenotyping

Two of us (SBW and JBP) did the phenotyping studies. Two hundred and thirty-nine measurements were done in 121 patients who were slightly older but were otherwise generally similar to the 139 patients not tested (Table 2). Studies were also done on 270 occasions in 54 healthy control subjects (laboratory personnel) who were somewhat younger but similar in gender distribution to the patients (*see* Table 1). Phenotyping of coded specimens was done concurrently and in blinded fashion by the same technicians using the same reagents and flow cytometer.

Mononuclear cells were separated by Ficoll-Hypaque centrifugation from 20 to 40 mL of heparinized venous blood and were then washed and stained with the T-cell monoclonal antibodies CD2, CD4, CD8 (Ortho Pharmaceuticals, Raritan, New Jersey), and CD5 (Boehringer Mannheim, Chicago, Illinois) and the B-cell monoclonal antibody CD20 (Coulter Immunology, Hialeah, Florida), using standard indirect immunofluorescence techniques. Isotype-matched myeloma protein was used in place of these monoclonal antibodies as a staining control; fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Tago, Inc., Burlingame, California) was the secondary reagent.

Assay for Active Replication of Human Herpesvirus Type 6

An assay was conducted by one of us (BH) to detect active replication of HHV-6 in peripheral mononuclear cells. Assays were done on at least one occasion in 113 patients. These patients were similar to the 146 patients not tested (*see* Table 2). The only criterion in selecting patients for these studies was the availability of the virology technician to receive and process the blood sample immediately.

Heparinized blood samples were obtained from 40 healthy blood donors and laboratory personnel who served as controls and were similar to the patients in mean age and in gender distribution (*see* Table 1). The control samples were tested according to identical techniques in two laboratories (those of BH and DVA). The control samples were tested late in the study, when initial results from patients had suggested a high frequency of active replication. Control samples were then intermixed with patient samples, and the testing was done in blinded fashion by the same technicians using the same reagents.

The method used to detect the presence of HHV-6 in human peripheral blood lymphocytes was similar to that previously described by one of us (SZS) (1). Briefly, peripheral mononuclear cells were separated by Ficoll-Hypaque centrifugation at 1200 *g* for 45 minutes. The cells were collected, washed once in RPMI 1640 medium, placed in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum and penicillin-streptomycin, and incubated at 37 °C for 24 hours under 5% CO_2. Cells were then washed, exposed to phytohemagglutinin (5 μg/mL) for 48 hours, collected by centrifugation, and resuspended in media containing dexamethasone (5 μg/mL). The cell cultures were then observed daily.

We used modification of the standard immunofluorescence technique to confirm infection with HHV-6. Briefly, the patients' cells were fixed in ethanol, then centrifuged and resuspended in RPMI 1640 medium containing 50% fetal calf serum. The desired number of cells was placed on a slide, fixed for 30 minutes in cold methanol, and air-dried. The fixed cells were then exposed to the desired dilution of test and control sera, followed by exposure to a fluorescein-labeled secondary human antibody (Chemicon International, Inc., Temecula, California). Test sera contained high levels of antibody to HHV-6 and no detectable antibody to Epstein-Barr virus or human cytomegalovirus. Control sera had no detectable antibody to HHV-6 but did have antibody to Epstein-Barr virus or human cytomegalovirus, or both, as determined by standard immunofluorescence assays. The presence of infected cells was determined by the degree of fluorescence relative to that seen in cell lines infected with Epstein-Barr virus (B95-8 and Raji) and a cell line infected with human cytomegalovirus (HEL).

A "positive bioassay" for active HHV-6 infection was defined by the following findings (1): 1) After 4 to 8 days in culture, refractile "giant cells" developed that increased in number and then underwent cytolysis, the cytopathic effect typical of HHV-6; 2) these giant cells showed diffuse fluorescence (typically not limited to either the nucleus or the membrane) when incubated with HHV-6-positive serum and then with fluorescein-labeled antibody; 3) the giant cells did not demonstrate fluorescence when incubated with control sera (as defined above); and 4) normal-sized cells that did not exhibit the characteristic cytopathic effect also did not show fluorescence when incubated with HHV-6-positive serum. When multiple longitudinal samples were obtained, a patient was considered to have a "positive bioassay" if the number of positive results (as defined above) was equal to or greater than the number of negative results.

Additional studies were done in patients with positive bioassays to confirm the presence of active HHV-6 infection. First, cord-blood lymphocytes were co-cultivated with supernatant from six randomly selected patients who had a positive bioassay. For purposes of control, cord-blood lymphocytes were also co-cultivated with supernatant from three persons who had a negative bioassay, and with supernatant from mock-infected HSB-2 cells, from Epstein-Barr-virus-infected producer (P3HR-1) and nonproducer (Raji) cell lines, and from cell-free human cytomegalovirus. Second, fluorescein-labeled monoclonal antibodies developed by one of us (NB) were used to stain cells from seven randomly selected patients who had a positive bioassay; for purposes of control, three samples from patients with a negative bioassay and uninfected HSB-2 cells were stained with the same monoclonal antibodies in the laboratory of three of us (DVA, SZS, and RCG). Three different monoclonal antibodies specific for different HHV-6 (GS isolate) epitopes were used in the immunofluorescence assay: 12B3G4, which recognizes a protein of 135 kDa; 6A5G3, which recognizes three glycoproteins of 54, 64 and 116 kDa; and 945D12, which recognizes proteins of 41 and 110 kDa (2). Third, polymerase chain reaction was used to detect HHV-6-specific DNA concurrently in cells from six randomly selected patients who had a positive bioassay; for purposes of control, the study was also done in cells from eight persons who had a negative bioassay. The specificity of the HHV-6 probe was also tested by determining whether it hybridized to cell DNAs (VERO and HEL) and other virus DNAs (human cytomegalovirus, herpes simplex virus types 1 and 2, varicella-zoster virus, and Epstein-Barr virus); and whether it produced a positive signal with a reaction involving the HHV-6 oligonucleotide primers, buffer, and Taq polymerase, but without a DNA template. The polymerase chain reaction assays were done according to the method of Saiki and colleagues (3). Briefly, reactions were conducted using an annealing temperature of 56 °C for 2.5 min, an extension temperature of 72 °C for 1.7 min, and a denaturation temperature of 94 °C for 1.7 min. A series of 40 amplification cycles was used for each reaction.

Serologic Testing

Coded sera from 134 patients were tested for IgG antibody reactivity against HHV-6-associated antigens by end-point dilution enzyme-linked immunosorbent assay (ELISA) in the laboratory of two of us (CS and RCG), according to previously described techniques (4). These 134 patients were similar to the remaining 125 patients not tested (*see* Table 2). Coded sera from 27 healthy control subjects from the same community who were somewhat older but similar in gender distribution to the patients (*see* Table 1) were also tested.

Serologic test panels for Epstein-Barr virus were done at least once in 249 of 259 patients (96%) at Nichols Institute Immunology Reference Laboratory, San Juan Capistrano, California, according to standard techniques (5, 6). (Specimens from 29 patients and control subjects were tested in the laboratory of the late Dr. Werner Henle at the Children's Hospital of Philadelphia, and values were within one tube dilution of those observed at the Nichols Institute.) Coded sera from 36 healthy control subjects (mean age, 47.4 ± 11.4 years; 56% female) from the same community were also tested for antibodies to Epstein-Barr virus.

Testing for human cytomegalovirus and *Toxoplasma gondii* was also done by standard immunofluorescence assay at Nichols Institute.

Human Retrovirus Studies

Testing for antibodies to human T lymphotropic virus type I (HTLV-I) was done using a conventional ELISA (DuPont,

Table 2. Characteristics of Tested and Untested Patients*

Test	Patients Tested	Patients Not Tested	*P* Value†
HHV-6 replication assay			
Number	113	146	
Mean age, *y*	39.5 ± 11.2	38.3 ± 12.6	> 0.2
Female, %	70	67	> 0.2
College graduates, %	37	42	> 0.2
Bedridden or shut-in‡, %	40	20	< 0.01
Chronic headaches‡, %	93	79	< 0.01
Difficulty in sleeping‡, %	44	56	< 0.01
Paresthesias‡, %	44	56	< 0.01
HHV-6 serologic testing			
Number	134	125	
Mean age, *y*	38.4 ± 12.3	39.2 ± 11.2	> 0.2
Female, %	63	73	0.11
College graduates, %	41	36	> 0.2
Bedridden or shut-in‡, %	21	29	< 0.01
Paresthesias‡, %	30	48	< 0.01
Lymphocyte phenotyping			
Number	121	139	
Mean age, *y*	41.2 ± 11.7	36.7 ± 12.0	0.01
Female, %	70	65	> 0.2
College graduates, %	43	36	> 0.2
MRI study			
Number	144	115	
Mean age, *y*	38.8 ± 10.8	38.7 ± 13.4	> 0.2
Female, %	72	62	0.10
College graduates, %	43	35	0.22
Unable to work full-time‡, %	67	37	< 0.01
Chronic headaches‡, %	91	78	< 0.01
Chronic nausea‡, %	63	38	< 0.01
Arthralgias‡, %	80	61	< 0.01
Paresthesias‡, %	75	47	< 0.01

* HHV-6 = human herpesvirus type 6. MRI = magnetic resonance imaging. Mean values are expressed ± SD.

† Alpha = 0.01 (*see* Tukey [9]).

‡ Symptom or descriptor (out of a total of 190 evaluated) for which a statistically significant difference was found between the patients tested and those not tested.

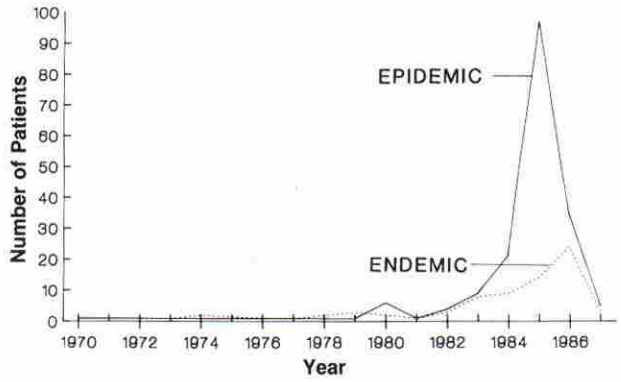

Figure 1. Onset of illness by year (1970 to 1987) in the "Tahoe" (*epidemic*) and "non-Tahoe" (*endemic*) groups. The Tahoe group was referred to as the "epidemic" group because most of the patients became ill within a 2-year period and many had close contacts who became ill. The non-Tahoe group was referred to as the "endemic" group because the patients did not know many other persons who were similarly affected.

Wilmington, Delaware). Testing for HTLV-I was done in 49 patients whose mean age (38.1 ± 13.7 years), gender distribution (69% female), and education (39% college graduates) were not significantly different from those of patients not tested. Testing for antibodies to human immunodeficiency virus (HIV) was done in 39 patients whose mean age (39.5 ± 11.0 years), gender distribution (51% female), and education (44% college graduates) were not significantly different from those of patients not tested. These serologic retroviral studies were done in the laboratory of John L. Sullivan at the University of Massachusetts Medical Center, as well as at Nichols Institute.

One of us (TF) attempted to find evidence of a human retrovirus using heparinized blood from 14 patients. Peripheral mononuclear cells from the patients and from normal persons (laboratory personnel) were isolated and stimulated using a previously described method (7). After stimulation, patients' peripheral mononuclear cells were either cultured in media alone or co-cultured with normal stimulated peripheral mononuclear cells according to a previously described method (8). Supernatants from these cultures were tested for reverse transcriptase activity (7) over a 30-day period. The primer used for the reverse transcriptase assay was oligo dT; the template was poly rA. Both Mg^{++} and Mn^{++} were used as divalent cations, because the reverse transcriptase from different known human retroviruses has different cation dependency. The patients' cells were established in culture only once. The supernatants were tested at 3-day intervals for 1 month (approximately 10 tests for reverse-transcriptase activity per patient over the 1-month culture period).

Statistics

When percentages of patients and controls with a certain clinical or laboratory finding were compared, we used the chi-square or Fisher exact test. Intergroup comparisons of the distributions of continuous variables were done using either *t*-tests or Wilcoxon rank-sum tests (when distributions were non-normal). When a large number of similar comparisons was made between two groups (such as comparing the frequency of multiple symptoms), a P value for detecting type I errors was chosen according to the method of Tukey (9). When distributions were non-normal, we normalized them by log-transformation (base 10). When multiple values for a variable were obtained for an individual at different points in time, we calculated a mean value for the variable after first normalizing the distribution (if necessary). For example, a geometric mean titer for Epstein-Barr virus antibody was calculated for each patient, as described previously (10).

Results

Onset of Illness

Most patients experienced the onset of illness between 1984 and 1986 (Figure 1), although in some cases the illness had begun years before. No seasonal preponderance of cases was observed.

"Clustering" of Cases

In the Tahoe group, several groups of patients who had frequent close contact became ill within several months of each other: 10 of 31 teachers at one local high school (at least one student from the same school was similarly affected, but this student chose not to participate in the study); 5 of 28 teachers and 3 students at another local high school; 3 students and 1 teacher at a third high school; and 11 employees at a casino. The spouses or sexual contacts of six patients were similarly afflicted, and there were eight instances in which at least one parent and one child both had the illness. Clinical and laboratory findings in the "clustered" patients did not differ from those of the larger patient group. Altogether, 101 of 183 patients (55%) in the Tahoe group and 39 of 76 patients (51%) in the non-Tahoe group stated that a close contact was similarly affected.

Symptoms and Signs

The two groups were generally similar regarding symptoms and signs. In most patients, the chronic, debilitating illness was of sudden onset, beginning with a "flu-like" syndrome; 29% of the patients were regularly bedridden or shut-in. The symptoms (Table 3) were chronic and were experienced on a nearly daily basis in the months and years after the typically sudden onset of the illness. These chronic symptoms constituted a new experience for most patients: Very few of the patients reported these chronic symptoms in the years before illness onset. For example, difficulty in concentrating was experienced chronically by 3% of patients before the onset of the illness but by 82% of patients after the onset of illness; depression (as reported by patients) was experienced chronically in 6% of patients before onset but by 68% after onset. Patients in the non-Tahoe group were more frequently shut-in and had a slightly higher frequency of headaches, adenopathy, arthralgias, paresthesias, and rash, but generally the two groups were similar (Table 3). No statistically significant differences were found when patients who experienced the onset of illness before 1984 were compared with those who had a later onset of illness. No statistically significant differences were found between patients whose illness began abruptly with "flu-like" symptoms and the few patients who experienced a more insidious onset, except that abdominal pain was more frequent in the former group.

Twenty-two patients (8%) had evidence of an acute neuropathic process, which generally occurred within several weeks of the acute onset. Acute neurologic symptoms included primary seizure disorder (7 patients); profound, transient ataxia of acute onset (10

patients); and transient paresis (8 patients). Ataxia and paresis were confirmed by neurologic examination (done by PRC and DLP) and typically lasted 1 to 4 weeks; none of the patients sustained a permanent deficit. The frequency of acute neurologic events was similar in the Tahoe and non-Tahoe groups (*see* Table 3).

Table 3. Clinical Findings

Findings	Tahoe Group (N = 183)	Non-Tahoe Group (N = 76)
	n (%)	
History of fatigue		
Severity of the fatigue		
Bedridden (can do virtually nothing)	11 (6)	3 (4)
Shut-in (cannot do even light housework or its equivalent or carry out family responsibilities)	32 (17)*	28 (37)*
Can work on only a part-time basis	51 (28)	22 (29)
Can fulfill all home or work responsibilities but is much more easily fatigued from such activities (no energy left for anything else)	89 (49)*	23 (30)*
Chronic fatigue started suddenly with a "flu," "cold," or virus†	156 (85)	69 (91)
Associated symptoms		
Myalgias	151 (83)	69 (91)
Headaches	149 (81)	71 (93)
Difficulty in concentrating	144 (79)	67 (88)
Recurrent pharyngitis	134 (73)	63 (83)
Swollen lymph glands	130 (71)	65 (86)
Difficulty in sleeping	121 (66)	42 (55)
Anxiety	129 (70)	54 (71)
Depression or unusual mood changes	122 (67)	55 (72)
Joint pain	122 (67)	62 (82)
Cough	99 (54)	38 (50)
Recurrent fevers at home	79 (43)	41 (54)
Nausea	88 (48)	47 (62)
Stomach ache	83 (45)	39 (51)
Loss of appetite	70 (38)	27 (36)
Odd sensations in the skin	73 (40)*	52 (68)*
Intermittent swelling of the fingers	81 (44)	42 (55)
Diarrhea	60 (33)	32 (42)
Weight gain of more than 4.54 kg (10 lb)	44 (24)	26 (34)
Eczema	37 (20)*	30 (39)*
Other rash	54 (30)	33 (43)
Vomiting	29 (16)	9 (12)
Weight loss of more than 4.54 kg (10 lb)	23 (13)	12 (16)
Neurologic events		
Primary seizure disorder	4 (2)	3 (4)
Transient ataxia	7 (4)	3 (4)
Transient paresis	3 (2)	5 (7)
Physical examination		
Anterior cervical adenopathy (enlarged or tender nodes)	118 (66)	44 (64)
Posterior cervical adenopathy (enlarged or tender nodes)	91 (51)	36 (52)
Temperature of more than 37.6°C	10 (6)	2 (3)

* $P < 0.01$; threshold for significance given that more than 20 comparisons were made (*see* Tukey [9]).

† Characterized by at least two of the following symptoms: fever, headache, myalgias, sore throat, earache, congestion, runny nose, cough, diarrhea, and fatigue.

Measurements

Standard Laboratory Tests

Laboratory tests generally yielded unremarkable results. However, 31 of 172 patients (18%) had atypical lymphocytosis (mean percentage of atypical cells, 6.5%). In addition, 21 of 81 patients (27%) tested had an antinuclear antibody titer of 1:20 (only 1 patient had a titer as high as 1:320); no patient had clinical evidence of systemic lupus erythematosus. The frequency of these laboratory abnormalities was similar in the Tahoe and non-Tahoe groups.

Cerebrospinal Fluid Examination

Of the six patients whose cerebrospinal fluid was studied, one had pleocytosis (leukocyte count, 8×10^6/L). Glucose and protein levels were unremarkable in all cases. Oligoclonal bands were absent. No bacterial organisms were cultured from any patient. In one instance, Epstein-Barr virus antibodies were measured: Neither viral capsid antigen IgG nor viral capsid antigen IgM was detected. Unfortunately, no cerebrospinal fluid was available for HHV-6 isolation or serologic studies.

Magnetic Resonance Imaging of the Brain

Foci of high signal intensity on T2-weighted images, typically punctate and occasionally larger patchy areas, were seen in 113 of 144 patients (78%) but in only 10 of 47 matched healthy controls (21%) ($P < 10^{-9}$) (Table 4). The films were interpreted independently by two neuroradiologists (RB and FAL), who agreed about the presence or absence of abnormal signal in 97% of the patients. Representative images are shown in Figure 2. The subcortical white matter was affected most often, but white matter elsewhere in the central nervous system was also affected. A relation was seen between the anatomic area affected and the clinical presentation: One patient with ataxia had high signal intensity areas involving the cerebellum (Figure 2, *panel D*), seven patients with visual symptoms had high signal intensity areas involving the occipital cortex, and one patient with paresis had a high signal intensity area involving the contralateral internal capsule. In several cases, MRI studies were repeated, and the scans showed that areas of high signal intensity persisted even after symptoms resolved.

Lymphocyte Phenotyping Studies

The CD4/CD8 ratio was higher in each of the two patient groups when compared with the control group (Tahoe group, 3.11 ± 1.6; non-Tahoe group, 3.19 ± 1.40; control group, 2.30 ± 1.00 [$P < 0.003$]). The higher CD4/CD8 ratios in the patient groups were accounted for by both higher numbers of CD4+ cells (934.4 ± 319.6 cells/mm^3 for the patients and 871.9 ± 389.0 cells/mm^3 for the control subjects; $P = 0.07$) and lower numbers of CD8+ cells (370.2 ± 215.1 cells/mm^3 for the patients and 452.0 ± 257.6 cells/mm^3 for the control subjects; $P = 0.01$). No significant differences were noted in the total T-cell or B-cell number.

Table 4. Results of the Magnetic Resonance Imaging Studies of the Brain and of the Bioassay for Replication of Human Herpesvirus Type 6 in the Tahoe Group, the non-Tahoe Group, and the Control Group*

Finding	Tahoe Group	Non-Tahoe Group	Control Group	*P* Value†
	← *n/n(%)* →			
Hyperintense signal in white matter on MRI study of the brain	71/91 (78)	42/53 (79)	10/47 (21)	$<10^{-9}$
Positive bioassay	45/71 (63)	34/42 (81)	8/40 (20)	$<10^{-8}$

* The Tahoe group comprised patients from the Lake Tahoe area near the Nevada-California border; this patient group was also referred to as the "epidemic" group because most of the patients had many contacts who also became ill. The non-Tahoe group comprised patients from outside the Lake Tahoe area; this patient group was also referred to as the "endemic group" because patients did not have many contacts who became ill. The control group comprised healthy persons. MRI = magnetic resonance imaging.

† *P* value for the comparison of two patient groups with the control group.

Assays for Active Replication of Human Herpesvirus Type 6

Two hundred and thirty-five specimens were obtained from 113 patients. A positive bioassay for HHV-6 (Figure 3) was seen in 70% of the patients and 20% of the control subjects ($P < 10^{-8}$) (*see* Table 4). Most of the assays in 27 of the 34 patients (79%) who had serial testing for HHV-6 indicated active replication of HHV-6. When, later in the study, samples from control subjects were intermixed with those from patients, the rate of positivity among patients was as high as it had been before control samples were intermixed, indicating that the earlier reading of patient samples was accurate.

Confirmatory studies for HHV-6 replication were also done. Co-cultivation of cord-blood lymphocytes with supernatant from primary cultures yielded a positive cytopathic effect in each of the six patients tested; no cytopathic effect was seen in any of the controls. In two patients showing a positive cytopathic effect in the primary co-cultivation study, secondary co-cultivation studies using supernatant from the presumably infected cord-blood lymphocyte cultures were done. The characteristic cytopathic effect was seen in the new cord-blood lymphocyte cultures in each of the two patients tested. Monoclonal antibody studies were positive with all three monoclonal antibodies in all seven patients studied, confirming that HHV-6-specific antigens were present in patients who had a positive bioassay. Monoclonal antibody staining was not observed in the controls. Polymerase chain reaction studies were shown to be specific for HHV-6, as described in the Methods section; the studies were positive in all six patients who had a positive bioassay and in none of the controls, confirming that HHV-6-specific nucleic acid sequences were also present in patients with a positive bioassay. As reported elsewhere, Southern blot examination was also done in three patients (11), which confirmed the presence of HHV-6 in all three instances.

Of the 113 patients studied for HHV-6 replication, 84 (74%) underwent MRI study; 68 of these 84 patients (81%) had MRI abnormalities, a proportion similar to that observed for all patients who had MRI study (79%). Patients with abnormalities were not more likely to have evidence of actively replicating HHV-6 ($P = 0.6$).

Serologic Testing

Median optical density values for the HHV-6 IgG ELISA in patients and control subjects were 1905 and 1288, respectively ($P = 0.08$, one-tail).

Ninety-three percent of patients were seropositive for Epstein-Barr virus; only two patients had serologic evidence of primary Epstein-Barr virus infection. The reciprocal geometric mean titers (±SD) for the Tahoe and non-Tahoe groups were significantly higher than those of the control group for Epstein-Barr virus viral capsid antigen IgG (138.0 ± 2.6 for the Tahoe group, 154.9 ± 3.0 for the non-Tahoe group, and 67.6 ± 4.4 for the control group; $P < 0.0001$); and for early-antigen R antibody (40.7 ± 2.6 for the Tahoe group, 30.9 ± 2.2 for the non-Tahoe group, and 12.6 ± 6.0 for the control subjects, $P < 0.0001$); there were no statistically significant differences in values for viral capsid antigen IgM, early-antigen D antibody, and Epstein-Barr nuclear-antigen antibody.

Of 92 patients tested, 45 (49%) had cytomegalovirus IgG antibody levels of more than 1:20 and 10 had low levels of cytomegalovirus IgM antibody. Low levels of IgG and IgM antibody to *Toxoplasma gondii* were found in 7 of 29 (24%) and in 1 of 11 (9%) patients tested, respectively.

Human Retrovirus Studies

Serologic test results for antibodies to HTLV-I and antibodies to HIV were negative in the patients tested. The 14 culture supernatants tested were negative for reverse transcriptase activity by both assays (Mg^{++} and Mn^{++}).

Discussion

The illness we have observed shares many features with the entities variably called "postinfectious" or "postviral" fatigue syndrome (12); myalgic encephalomyelitis, Royal Free disease, or Icelandic disease (13-19); primary fibromyalgia (or fibrositis) (20-22); chronic mononucleosis (23-25); chronic active Epstein-Barr virus infection syndrome (26-28); and the chronic fatigue syndrome (29, 30). Perhaps these differently named entities are the same illness and share a common etiologic agent; perhaps they are clinically similar but separate illnesses, with each triggered by a particular and different etiologic agent; or perhaps these entities represent one illness that can be triggered by various factors and that manifests different clinical features in different patients (perhaps because of host factors) but causes chronic fatigue and certain common pathophysiologic features in all patients. We are more inclined to the last view.

We cannot say precisely if the illness we witnessed from 1984 to 1986 meets criteria for chronic fatigue syndrome because the case definition for that illness developed in 1988 (30) includes some symptoms (for

example, a greater than 50% reduction in function) that we did not explicitly ask the patients about, using the same language. Nevertheless, most patients probably would have met the case definition for chronic fatigue syndrome because the illness began suddenly in 87% of patients, the median duration of the illness at time of study entry was 1.3 years, 57% of patients were bedridden, shut-in, or unable to work full-time, and most had the key symptoms required by the case definition (*see* Table 3).

For most patients in our study, the illness consisted largely of subjective and nonspecific symptoms, and the results of common laboratory tests were often normal. Initially, it was unclear to us whether the patients were experiencing an organic illness or the somatic manifestations of a psychological illness. The several objective neurologic, immunologic, and virologic findings make a diagnosis of purely psychological illness unlikely, although it remains possible that these findings are biologic concomitants of primary psychiatric illness. Studies of that possibility are in progress.

We frequently saw areas of abnormal signal intensity in the white matter of the central nervous system. Without studies of central nervous system tissue, we cannot be certain about the meaning of these findings. Progress in characterizing white matter abnormalities using magnetic resonance imaging has been severely inhibited by the absence of tissue. In normal persons, for example, hyperintense signal on T2-weighted images is seen anterior to the frontal horns (31). Similar signal characteristics are seen in the periventricular white matter lesions of patients with multiple sclerosis; because no signal feature can be used to differentiate these MRI signal abnormalities, only their spatial distribution identifies them. Many different diseases in addition to multiple sclerosis are associated with areas of high signal intensity caused by white matter edema or demyelination, or both: Alzheimer's disease; vascular dementia (Binswanger disease, lacunar state, multi-infarct dementia); normal pressure hydrocephalus; metastatic disease; trauma; leukodystrophies; secondary Wallerian degeneration from any brain damage; and post-irradiation, post-chemotherapy, toxic, or metabolic leukoencephalopathies. Viral infection (for example, with the retroviruses HIV [32] or HTLV-I [33]) also commonly produces similar white matter changes. As was the case in our study, such punctate areas of high signal intensity can also be seen in apparently healthy persons of all ages.

The clinical significance of these "incidental" areas of high signal intensity in the white matter is not known (34-37); their prevalence appears to rise with age and with the presence of cerebrovascular risk factors. Particularly ambiguous is the interpretation of punctate areas of high signal intensity seen mostly in the subcortical white matter. The distribution of this finding

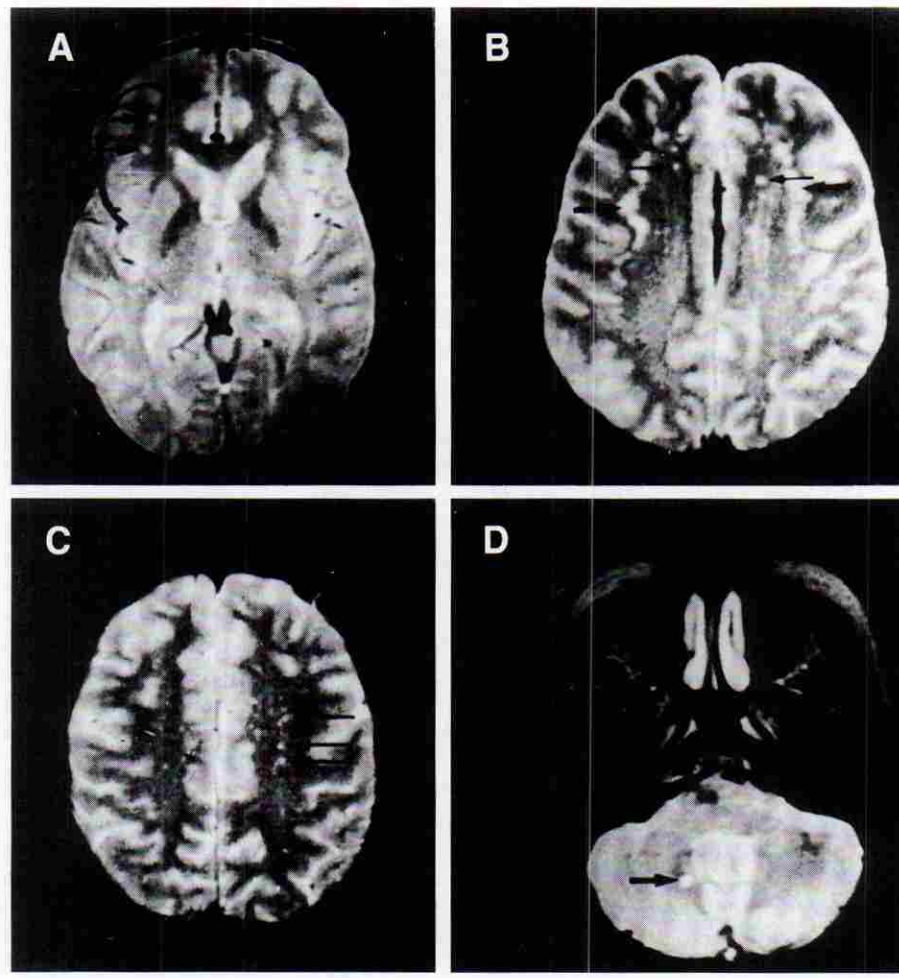

Figure 2. Magnetic resonance images of brain. Panel A. Transaxial section in a 44-year-old woman with cognitive impairment. Two punctate areas of high signal intensity (*arrows*) are seen in the deep, right frontal white matter on a T2-weighted image. These areas probably represent white matter abnormalities. **Panel B.** Transaxial section from a 50-year-old woman with disorientation and paresis. Taken at the level of the centrum semiovale, the image shows multiple patchy areas (*large arrows*) of high signal intensity in the subcortical white matter (an uncommon finding in our patient group) and smaller punctate lesions (*small arrows*) in the subcortical and deep white matter (a common finding in our patients). **Panel C.** Transaxial section from a 32-year-old woman with cognitive impairment. Taken at the level of high convexity, the image shows multiple punctate areas of high signal intensity (*arrows*) in the subcortical white matter. These findings were most typical of our patient group and may correspond to prominent or enlarged perivascular spaces. **Panel D.** Posterior fossa cut from a 34-year-old patient with recurrent ataxia and disorientation. The image shows an isolated high-signal lesion (*arrow*) in the cerebellar white matter, an unusual location for high signal in our patient group. The patient also had the more typical punctate areas of high signal intensity in the subcortical white matter (not shown).

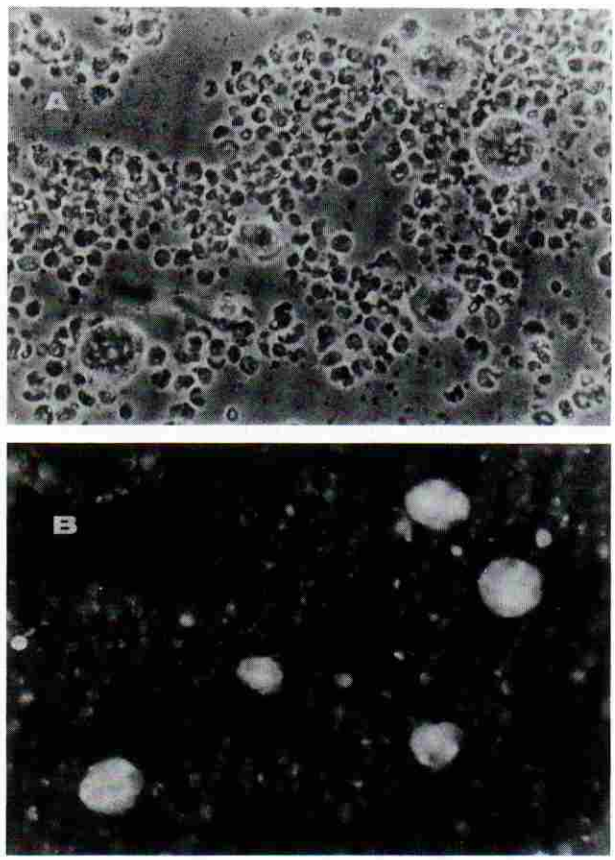

Figure 3. Positive bioassay for human herpesvirus type 6. Panel A. After 4 to 8 days in culture, an outgrowth of large, refractile "giant cells" is seen. **Panel B.** These cells show fluorescence after incubation with serum positive for human herpesvirus type 6 and negative for Epstein-Barr virus and human cytomegalovirus, followed by incubation with fluorescein-labeled antibody.

corresponds anatomically to the cerebrospinal-fluid-filled perivascular (Virchow-Robin) spaces that can be seen on high-resolution magnetic resonance images. The enlarged Virchow-Robin spaces may be a normal variant or may be the result of either cerebrospinal fluid penetration into ischemic white matter or cellular infiltration within the dilated perivascular spaces as part of an inflammatory process (38, 39). The areas of high signal intensity seen in our patients and control subjects varied in size and location and may arise from different causes. The much greater frequency of high signal intensity in the white matter of both the Tahoe and non-Tahoe groups than in the control group ($P < 10^{-9}$), the presence in some patients of larger and deeper white matter lesions, and the correlation between the anatomic area of involvement and clinical symptoms all suggest that the patients were experiencing a genuine but as yet undefined pathologic process.

We cannot judge how generalizable the MRI findings might be to other patients with a similar syndrome. In our preliminary studies of patients with chronic fatigue syndrome from New England, MRI abnormalities were seen less commonly (40% to 50% of patients) than in this patient group. We do not recommend routine use of MRI studies in patients with suspected chronic fatigue syndrome; the value of the test first needs to be assessed in other populations. We and others are currently evaluating magnetic resonance imaging and other neuroimaging techniques in chronic fatigue syndrome.

Immunologic (lymphocyte phenotyping) studies revealed a significantly increased CD4/CD8 ratio in each of the two patient groups when compared with the control group ($P < 0.003$), because patients had both a higher number of CD4+ T cells and a reduced number of circulating CD8+ T cells. Landay and colleagues (40) have also found a reduced number of CD8+ cells in clinically similar patients, although some studies of clinically similar patients have not found a similar increase in the CD4/CD8 ratio (22, 40, 41). We did not examine T-cell subsets, but Gupta and colleagues (41) as well as Straus and Strober (Unpublished data) studied patients with an apparently similar syndrome and reported elevated numbers of activated CD4+ cells. Klimas and coworkers (22) and Landay and coworkers (40) have found an elevated number of activated CD8+ cells, decreased numbers of CD4+ CD45RA+ "suppressor-inducer" cells (22), and decreased numbers of CD8+ 11b+ "suppressor" cells (40). We did not measure T-cell function, but Klimas and colleagues (22), Murdoch (42), and Lloyd and colleagues (43) have found evidence of cutaneous anergy or T-cell dysfunction, or both, as reflected by conventional mitogen stimulation assays, in patients with a similar chronic illness. Gupta and colleagues (41) found T-cell dysfunction in response to challenge with soluble antigens. Increased B-cell numbers have been reported in some patients with chronic fatigue syndrome (22, 44) but not in others (40, 41). An increase in the B-cell subset (CD20+ CD5+) that may be dedicated to the production of autoantibodies (45) has been reported by Klimas and colleagues (22). Increased numbers of activated monocytes have been observed (41). We have reported previously that natural killer cell phenotypic and functional abnormalities are also present in patients with chronic fatigue syndrome: Such patients show reduced numbers of the NKH1+ T3− subset, and their natural killer cells demonstrate defective cytolytic activity against target cell lines (46). Other investigators have also found defective natural killer cell function (22, 47-49). Indeed, defective natural killer cell function may be the most commonly reported abnormality in patients with chronic fatigue syndrome. Taken together, the controlled studies cited above and many others summarized elsewhere (50) seem to indicate an immune system chronically responding to a "perceived" antigenic challenge.

The virologic studies we have done thus far indicate that two human herpesviruses may be actively replicating more often in patients than in control subjects. The first is Epstein-Barr virus, and the indirect evidence for its active replication in patients with chronic fatigue syndrome comes from the Epstein-Barr virus antibody profiles, which are characterized by higher levels of IgG antibody to both viral capsid antigens and early antigens. The second such virus is HHV-6, which was recently discovered in one of our laboratories (RCG) (1, 51, 52). With HHV-6, the evidence of active viral replication is direct: When lymphocytes from the two patient groups were placed in primary cell culture, a cy-

topathic effect typical of HHV-6 was seen in 70% of patients but in only 20% of control subjects ($P < 10^{-8}$) (*see* Table 2 and Figure 3). The use of three different monoclonal antibodies specific for HHV-6 epitopes confirmed that HHV-6 protein was present in cells exhibiting cytopathic effect, and the application of polymerase chain reaction using probes specific for HHV-6 nucleic acid sequences also confirmed the presence of HHV-6. The only other known human virus that might also have been detected with these assays was the closely related, recently discovered human herpesvirus type 7 (53). Finally, in every instance, supernatant from the primary cell culture produced the same cytopathic effect in cord-blood lymphocytes, demonstrating that a transmissible agent was indeed present in the patients' lymphocytes and that this agent was HHV-6.

We do not know how generalizable these findings may be. Several other groups have found elevated antibodies to Epstein-Barr virus (23, 26-28). After our first report (54), several groups reported elevated antibody levels to HHV-6 (40, 55-57) in patients with a similar illness. As for our finding of active HHV-6 replication in patients' lymphocytes, although one group has been unable to induce active replication of HHV-6 in the lymphocytes of healthy persons (57), others (58, 59; Gillespie D. Personal communication; Yamanishi K. Personal communication) have been able to do so with a frequency similar to that which we report. Like us, some investigators (59; Gillespie D. Personal communication) also have found evidence of inducible active HHV-6 replication in patients with chronic fatigue syndrome. At this time, because the assays are difficult to do and because their value has yet to be tested in other populations, we do not recommend the routine use of HHV-6 testing in patients with suspected chronic fatigue syndrome.

It appears that most patients acquire primary infection with HHV-6 early in life (4). Therefore, the active replication of HHV-6 seen in most patients probably represents reactivation of an old, latent infection rather than a new, primary infection with the virus. This reactivation of HHV-6 could be nothing more than an epiphenomenon, secondary to immune dysfunction or transactivation by another unrecognized virus, and could have no relation to patients' symptoms. Alternatively, whatever the mechanism of reactivation, HHV-6 might contribute to producing the morbidity associated with this illness. Such a hypothesis is plausible because HHV-6 is tropic for T cells; B cells; and glial, neuroblastoma, and intestinal cell lines (60, 61).

Our study had several limitations. We could not systematically survey a random sample of the denominator population in the community; hence, we could not assess disease prevalence nor could we examine more closely the possible clustering of cases. Magnetic resonance imaging lymphocyte phenotyping, and the assays for HHV-6 replication were not done in all 259 study patients; nevertheless, the large samples tested were sufficiently similar to the total group (*see* Table 2 and the Results section) that the striking differences between patients and healthy control subjects are unlikely to be explained by a sampling bias. The MRI findings were interpreted independently by two neuroradiologists, with very high agreement. However, because films from patients could be distinguished from those of control subjects by the logos on each image, interpretation was non-blinded.

We do not argue that these observations identify the cause of this illness, nor can we be sure our findings will be confirmed in other populations of patients with apparently similar illnesses. Indeed, as stated earlier, we think that this probably is a heterogenous illness that can be triggered by multiple different genetic and environmental factors (including stress, toxins, and exogenous infectious agents), all of which can lead to immune dysfunction and the consequent reactivation of latent viruses (29). Several different exogenous and endogenous infectious agents may be involved in this illness, acting singly in some cases and collaboratively in others. In addition to HHV-6 and Epstein-Barr virus, the enteroviruses (62-65), *Borrelia burgdorferi* (66), and other infectious agents (67) may be involved in some cases. Preliminary evidence suggests that a novel retrovirus may be involved in this illness (68); at least one other retrovirus, HIV, is known to act synergistically with HHV-6 (69, 70). In our study, the apparently "epidemic" Tahoe cases may have been triggered by different agents than the apparently "endemic" non-Tahoe cases; however, the remarkably similar clinical and laboratory findings in the two patient groups suggest a final common pathogenetic pathway involving immune dysregulation. We hope that further investigation generated by these observations may lead to a better understanding of this often devastating illness.

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