HTLV-I/II Seroindeterminate Western Blot Reactivity in a Cohort of Patients with Neurological Disease

Samantha S. Soldan,1,2 Michael D. Graf,† Allen Waziri,1,a Alfred N. Flerlage,† Susan M. Robinson,† Taketo Kawanishi,†,4 Thomas P. Leist,† Tanya J. Lehky,†,a Michael C. Levin,†,a and Steven Jacobson†

The human T-cell lymphotropic virus type I (HTLV-I) is associated with a chronic, progressive neurological disease known as HTLV-I-associated myelopathy/tropical spastic paraparesis. Screening for HTLV-I involves the detection of virus-specific serum antibodies by EIA and confirmation by Western blot. HTLV-I/II seroindeterminate Western blot patterns have been described worldwide. However, the significance of this blot pattern is unclear. We identified 8 patients with neurological disease and an HTLV-I/II seroindeterminate Western blot pattern, none of whom demonstrated increased spontaneous proliferation and HTLV-I-specific cytotoxic T lymphocyte activity. However, HTLV-I tax sequence was amplified from the peripheral blood lymphocytes of 4 of them. These data suggest that patients with chronic progressive neurological disease and HTLV-I/II Western blot seroindeterminate reactivity may harbor either defective HTLV-I, novel retrovirus with partial homology to HTLV-I, or HTLV-I in low copy number.

The human T-cell lymphotropic virus type I (HTLV-I) is endemic in several regions of the world; there are clusters of high prevalence in the Caribbean, southern Japan, equatorial Africa, South America, and Iran [1]. Long established as the causative agent of both adult T-cell leukemia/lymphoma [2] and an inflammatory neurological disease termed HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [3], HTLV-I has more recently been implicated in other inflammatory diseases in a subset of patients, including HTLV-I-associated uveitis and infectious dermatitis [4, 5]. Although an estimated 15–25 million people worldwide are infected with HTLV-I, the vast majority are clinically asymptomatic [1]. Transmission of HTLV-I requires cell-to-cell contact with infected T cells and may occur via sexual contact, breast milk, and transfusion with infected blood products. An increased risk for developing HAM/TSP is associated with transfusion [6, 7]. Therefore, blood bank screening for HTLV-I seropositivity was initiated in Japan (1986), the United States (1988), France (1991), and the Netherlands (1993). The screening for HTLV-I in the Japanese blood supply has significantly decreased the number of new HAM/TSP patients in that country [6, 7].

Many serological procedures are available to detect HTLV-I antibodies, including EIA, particle agglutination, Western blot, radioimmunoprecipitation assay (RIPA), and immunofluorescent assays. In the United States, HTLV-I and HTLV-II seropositivity is based on positive EIA results, which are confirmed by RIPA or Western blot. Recombinant proteins specific for HTLV-I and HTLV-II Env glycoproteins are incorporated into Western blot strips to increase sensitivity and distinguish between antibody responses to HTLV-I and HTLV-II. The established HTLV-I/II Western blot criteria maintain that an infected individual must have an antibody response to all of the core bands and the respective recombinant glycoprotein. However, anomalous Western blot banding patterns have been described [8, 9] for sera from patients who are HTLV-I/II EIA positive but show a response only to some, not all, of the core HTLV-I Western blot bands. This HTLV-I/II seroindeterminate Western blot profile has been reported from throughout the world, more frequently in tropical areas and especially among the Pygmies from the tropical forests of southern Cameroon [8–10]. However, its significance remains unclear.

Received 21 October 1998; revised 30 April 1999; electronically published 9 August 1999.


Informed consent was obtained from patients, and human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of clinical research.

No author has a commercial or other association that might pose a conflict of interest.

† Present affiliations: Laboratory of Viral and Demyelinating Diseases, Department of Neurology, University of Tennessee Medical Center, Memphis (M.C.L.); HIV Research Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Rockville, MD (T.J.L.); Kansai Denryoku Hospital, Osaka, Japan (T.K.).

A.W. is in the Howard Hughes Medical Institute–NIH Research Scholar Program.

Reprints or correspondence: Dr. Steven Jacobson, Viral Immunology Section, National Institute of Neurological Disorders and Stroke, Building 10, Room 5B-16, Bethesda, MD 10892 (stevej@helix.nih.gov)

The Journal of Infectious Diseases 1999;180:685–94
© 1999 by the Infectious Diseases Society of America. All rights reserved.
0022-1899/1999/18003-0015$02.00
Here we describe 8 individuals who were referred to the Clinical Center of the National Institutes of Health (NIH) for evaluation of neurological disease in conjunction with an HTLV-I/II seroindeterminate Western blot pattern. We undertook a series of immunological and molecular studies to determine whether these HTLV-I/II seroindeterminate Western blot patterns were associated with prototypic HTLV-I infection. Peripheral blood lymphocytes (PBL) from seroindeterminate patients were tested for spontaneous lymphoproliferation, an immunological hallmark of HTLV-I infection, and for HTLV-I–specific cytotoxic T lymphocyte (CTL) activity. HTLV-I tax sequences were also periodically amplified by nested polymerase chain reaction (PCR) from PBL and confirmed by sequence analysis. Possible explanations for an HTLV-I/II seroindeterminate Western blot pattern include infection with defective HTLV-I, a novel retrovirus with some homology to HTLV-I, or prototypic HTLV-I in low copy number.

Materials and Methods

Study population. All patients had been screened for HTLV-I/II antibodies by EIA as part of their diagnostic evaluation after presenting with a variety of neurological diseases, before they were referred to us at NIH. When possible, these patients underwent neuroimaging and physical examinations. Six patients from the United States with an HTLV-I/II seroindeterminate Western blot pattern were identified and evaluated at NIH (table 1), and 2 additional Japanese patients with chronic progressive neurological disease and a seroindeterminate Western blot pattern were identified and examined by T. Kawanishi in Japan. All patients presented with neurological signs and/or symptoms in their third or fourth decade of life. Three of the 8 patients carry the diagnosis of multiple sclerosis (MS; 1 with relapsing-remitting MS and 2 with primary progressive MS); magnetic resonance imaging revealed bilateral white-matter lesions in each of the 3 patients, consistent with the diagnosis of MS. Five of 6 individuals tested had abnormalities in their cerebrospinal fluid (CSF). All patients except patient 6 had risk factors for HTLV-I infection or were from areas where the virus is endemic. All 8 patients were HIV negative.

Serological analysis. Serum samples were tested for the presence of HTLV-I/II antibodies by HTLV-I/II EIA (Abbott Laboratories, North Chicago, IL), according to the manufacturer’s instructions. Serum and CSF samples from HTLV I/II EIA–positive individuals were also tested with an HTLV-I/II modified Western blot assay (HTLV Blot 2.4, Genelabs Technologies, Singapore). Nitrocellulose strips were derived from an HTLV-I–infected T-cell line (HUT 102) lysate; the strips included the recombinant protein rgp21 (representing a conserved epitope between HTLV-I and HTLV-II), the HTLV-I–specific recombinant protein rgp46-I, and the HTLV-II–specific recombinant protein rgp46-II. HTLV-I/II Western blots were run according to the instructions in the kit. Serum was used at concentrations of 1 : 200 and 1 : 100. CSF samples were tested at concentrations of 1 : 200, 1 : 100, 1 : 10, and neat.

Spontaneous lymphoproliferation assay. Spontaneous lymphoproliferation assays were performed on PBL from all 8 seroindeterminate patients and on PBL from seronegative and HAM/TSP control patients, as described previously [11]. The PBL were re-suspended in RPMI medium (Life Technologies, Gaithersburg, MD) and subjected to 3 different serum conditions: 5% AB serum (Sigma, St. Louis), 5% autologous patient serum, and 5% AB serum with phytohemagglutinin (Sigma) at a 1 : 50 solution. PBL were plated in triplicate on a 96-well plate at a concentration of

### Table 1. Demographics, symptoms, and laboratory findings for 8 human T-cell lymphotropic virus type I or II (HTLV-I/II) seroindeterminate patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years), sex, race or ethnicity</th>
<th>Symptoms or diagnosis</th>
<th>Cerebrospinal fluid</th>
<th>MRI findings</th>
<th>Risk factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>35, F, AA</td>
<td>Relapsing, remitting MS</td>
<td>5-WBC, OCB+, high IgG index</td>
<td>Bilateral WM lesions</td>
<td>Residence in southern US</td>
</tr>
<tr>
<td>2</td>
<td>47, M, AA</td>
<td>Primary progressive MS, pan uveitis</td>
<td>42-WBC, OCB+, normal protein</td>
<td>Bilateral WM lesions</td>
<td>Intravenous drug user</td>
</tr>
<tr>
<td>3</td>
<td>44, F, WH</td>
<td>Mild quadraparesis, dysarthria, dysphagia, memory loss</td>
<td>2-WBC, normal protein</td>
<td>Normal brain, C5C6 fusion</td>
<td>Residence in southern US</td>
</tr>
<tr>
<td>4</td>
<td>46, F, WH</td>
<td>Complaint of memory loss and leg weakness, normal neurological examination</td>
<td>No data</td>
<td>Normal spinal cord, normal brain</td>
<td>Sex worker</td>
</tr>
<tr>
<td>5</td>
<td>32, M, WH</td>
<td>Myalgias, arthralgias, normal neurological examination</td>
<td>No data</td>
<td>Normal brain, bulging disk</td>
<td>Homosexual</td>
</tr>
<tr>
<td>6</td>
<td>30, M, WH</td>
<td>Abnormal gait, dementia, cerebellar dysfunction</td>
<td>13-WBC, high IgG index</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>Japan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>40, M, JP</td>
<td>Primary progressive MS</td>
<td>OCB+, pleocytosis, high IgG index</td>
<td>WM lesions</td>
<td>Residence in Okayama</td>
</tr>
<tr>
<td>8</td>
<td>40, M, JP</td>
<td>Chronic inflammatory polyradiculoneuropathy</td>
<td>OCB+, pleocytosis, high IgG index</td>
<td>No data</td>
<td>Residence in Hyogo</td>
</tr>
</tbody>
</table>

NOTE. MRI, magnetic resonance imaging; AA, African American; WH, white American; JP, Japanese; WM, white matter; WBC, white blood cells; OCB, oligoclonal bands; MS, multiple sclerosis.
3 × 10⁷ cells/well. Three identical plates were prepared for harvest on days 3, 4, and 5. Each well was pulsed with 1 μCi of H (Du Pont NEN, Boston) and incubated at 37°C for 4 h before harvesting with a cell harvester. Tritiated thymidine incorporation was measured on a Beta-plate counter (Wallac, Gaithersburg, MD).

Cytotoxic T-cell assay. Cytotoxic T-cell assays were performed as described [12] on PBL from 7 of 8 HTLV-I/II seroindeterminate patients and, as controls, on PBL from HAM/TSP patients and asymptomatic carriers. Briefly, 2 × 10⁵ Epstein-Barr virus–transformed autologous B-cell targets were infected with various HTLV-I vaccinia recombinants [12] at a multiplicity of infection of 10 for 12 h and subsequently incubated with 0.2 μCi of Na¹ⁱ⁵Cr (Amersham Corp., Arlington Heights, IL) for 90 min. Targets were then plated at a concentration of 3.5 × 10⁴ cells/well and incubated with fresh PBL at the indicated effector : target ratio for 4 h at 37°C. Percentage of specific lysis was calculated as described elsewhere [12].

DNA extraction. DNA was extracted from PBL, B cell lines, and T cell lines using a commercial DNA kit provided by Qiagen (Santa Clara, CA). The kit protocol was followed, using 5 × 10⁴ PBL.

PCR and Southern blotting. Primary PCRs were used to amplify HTLV-I sequences from the HTLV-I LTR, env, pol, tax, and gag regions, using 1–3 μg of each DNA sample. The procedure was performed by use of a 50-μL volume that contained the DNA sample, 10× buffer (100 mM Tris-HCl, 15 mM MgCl₂, and 500 mM KCl, at pH 8.3), 0.5 μM primers (Genset, La Jolla, CA), 0.2 mM dNTP, 2.5 units polymerase (Boehringer Mannheim, Indianapolis), and an overlay of mineral oil (Perkin Elmer, Norwalk, CT). PCRs were performed in a Perkin Elmer thermal cycler as follows: 25 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 54°C, and extension for 1 min at 72°C, followed by a 10-min extension at 72°C after the final cycle.

Nested PCR studies were initiated for HTLV-I tax, gag, and LTR regions, using primer sequences internal to the original primer sets. We used 5 μL of the primary PCR product in the nested reactions. The same PCR conditions were used for both the primary and nested PCR experiments. Ten μL of the amplified products was then analyzed by electrophoresis in a 2% agarose gel (FMC Bioproducts, Rockland, ME) stained with ethidium bromide (Life Technologies, Gaithersburg). After alkaline denaturation and neutralization, the DNA samples were transferred on to nylon filter membranes (Nytran, Schleicher and Schuell, Keene, NH) for Southern blotting. The housekeeping gene β-actin was used to ensure that all extracted DNA was amplifiable. Enzyme chemoluminescence was performed using HTLV-I–specific fluorescein-labeled probes (Amersham Life Sciences, Little Chalsont, UK), according to the protocol provided.

The primer pairs used in PCR analysis and Southern blotting were as follows. Primary PCR for HTLV-I LTR was performed with BS1F (5′-AGGGGCGGACCACGGGCGCGTGATTCTGACATGACATGACC-3′) and BS2R (5′-GAGGAATTCGAAATCTATAGGATGCCGTGCCTGGC-3′) and followed by nested PCR with BS2F (5′-GAGGGCGGACCACGGGCGCGCGACATGACATGACC-3′) and BS2R (5′-GAGGAATTCGAAATCTATAGGATGCCGTGCCTGGC-3′). A product of 230 bp was obtained during primary PCR using M2 (5′-TCCGGCATTGCGAGCGACGCTTTGGA-3′) and M3 (5′-GCCAGAGTTGCGTATTCTTCGCT-3′) for the HTLV-I gag region. Nested PCR for HTLV-I gag was performed with M4 (5′-GACCTTCAGAGAGCTTGGTATGGGGAAGGAGT-3′) and M5 (5′-ATTGTTGGCTTGAGACCGAGGGGAA-3′). M2.3 (5′-CTTCCCTCACCCAGAGCAGCTAGATGACGAT-3′) was used as a probe for HTLV-I gag during Southern blot analysis. SK110 (5′-CACTCACCAAGTCACTGCGGTC-3′) and SK111 (5′-GTGGTGAGCTGGCATGGTGGTTT-3′) were used to amplify HTLV-I pol. Primary PCR for HTLV-I tax was performed with F205 (5′-CAACCTGCTTCTTTCCAGACCC-3′) and B225 (5′-CGTGCTCATCAGTTAAAGTCC-3′) and followed by nested PCR with F205I (5′-AGGGCTCCTGGTCTGGCATGAC-3′) and B225I (5′-AAAGGTTGGTGGCACAAGCT-3′). The P3.1 probe (5′-TTCTACCGAGACTGTTTGCCACCC-3′) was used in Southern blot analysis after nested PCR with the F205/B225 and F205I/B225 primer pairs. An additional set of primers, SK43 (5′-CGGATACCCAGTCTACGTGT-3′) and SK44 (5′-GACGGCGATAACCCGTTCCATCG-3′), was used to amplify HTLV-I tax sequences. The SK45 probe (5′-CGCCTACTGCGCCACCTGCTCCAGAGCATCGACATC-3′) was used in Southern blot analysis, following PCR amplification with SK43/SK44. The nested PCR assay for HTLV-I tax used in this study was demonstrated to be as sensitive as a quantitative TaqMan (PE Applied Biosystems, Foster City, CA) fluorescence energy transfer assay developed for the HTLV-I/II tax region. This HTLV-I/II tax–specific TaqMan assay detects as little as one copy of HTLV-I tax DNA per 1 × 10⁷ cells (David J. Waters, SAIC, Frederick, MD, personal communication).

Results

Sera from all patients were screened for HTLV-I/II antibodies by EIA. Upon confirmation by Western blot, these individuals were determined to be HTLV-I/II seroindeterminate. Unlike individuals with known HTLV-I– or HTLV-II–associated neurological disease, this cohort of patients lacked antibodies to the recombinant HTLV-I Env glycoproteins (rgp46-I and rgp-46II) and to HTLV-I gag p24. All patients had a strong serum antibody response to HTLV-I gag p19. Additionally, the majority had seroreactivity directed against HTLV-I gag p26, p28, p32, and p36. Although there are several Western blot reactivity patterns that are classified as “HTLV-I/II seroindeterminate,” the Western blot profiles of this cohort of patients with neurological disease were strikingly similar and are consistent with the HTLV-I gag–seroindeterminate Western blot profile recently described by Mauclère et al. [10] (figure 1). No HTLV-I/II seroindeterminate patients with indeterminate profiles other than that described, such as p24 alone, were referred to the Neuroimmunology Branch. Furthermore, selection of these patients was based on the presence of neurological disease and not on a specific HTLV-I/II indeterminate Western blot profile.

No HTLV-I/II antibodies were found by Western blot in the CF of 3 seroindeterminate patients tested (data not shown). Of the 3 seroindeterminate patients (patients 1, 2, and 6) in whom CSF was analyzed, HTLV-I/II antibodies were found by Western blot in the CSF of 3 seroindeterminate patients tested (data not shown). No HTLV-I/II antibodies were found for Western blot in the CF of 3 seroindeterminate patients tested (data not shown). Of the 3 seroindeterminate patients (patients 1, 2, and 6) in whom CSF was analyzed for HTLV-I/II Western blot reactivity,
2 (patients 1 and 2) were also HTLV-I \textit{tax} PCR reactive. With the exception of the husband of patient 3, we were unable to obtain serology on HTLV-I/II seroindeterminate patients’ family members. The husband of patient 3 was HTLV-I/II seronegative by both EIA and Western blot analysis. Additionally, we were unable to amplify HTLV-I/II \textit{tax} sequences from his PBL (data not shown).

An immunological hallmark of HTLV-I seropositive individuals is the capacity of their PBL to lymphoproliferate spontaneously in the absence of exogenous stimulation (figure 2A). In addition, PBL from HAM/TSP patients demonstrate high levels of HTLV-I-specific CTL activity (figure 2B). It was therefore of interest to determine if PBL from patients with neurological disease and HTLV-I/II seroindeterminate Western blot profiles also exhibit these HTLV-I specific immunological responses. As shown in figure 2A, PBL from all these 8 seroindeterminate patients did not spontaneously lymphoproliferate, and HTLV-I-specific CTL activity could not be demonstrated for 7 of 8.

To determine whether PBL from these 8 patients contained HTLV-I proviral DNA, PCR was used to amplify retroviral sequences. Although primary PCR failed to amplify HTLV-I \textit{tax}, \textit{pol}, \textit{env}, and \textit{gag}, nested PCR periodically amplified HTLV-I \textit{tax} sequence in PBL from 4 of 8 patients (figure 3). We were able to amplify \textit{tax} sequence from HTLV-I/II seroindeterminate patients 1, 2, 3, and 4 in at least 1 of 3 attempts. As suggested by others [13–15], this may have been possible because of low copy numbers of HTLV-I and the highly sensitive HTLV-I \textit{tax} primer pairs used. The specificity of the HTLV-I \textit{tax} amplification by PCR was confirmed by Southern blot, using probes specific to the HTLV-I \textit{tax} region. In addition, the HTLV-I \textit{tax} region (202 bp) was sequenced from seroindeterminate patient 3 and was found to be 99% homologous to prototypic HTLV-I \textit{tax} sequenced from HUT102; there was only one nucleotide difference (data not shown).

HTLV-I EIA data were collected from serial serum samples obtained during follow-up examinations of the 8 seroindeterminate patients. Antibody titers to HTLV-I in these samples
Figure 2. **A.** Spontaneous lymphoproliferation from representative human T-cell lymphotropic virus type I (HTLV-I) seroindeterminate patients, a patient with HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and a normal donor who is HTLV-I/II negative by polymerase chain reaction, as measured by tritiated thymidine incorporation. Spontaneous lymphoproliferation was observed in peripheral blood lymphocytes (PBL) from HAM/TSP patients but not in PBL from HTLV-I/II seroindeterminate individuals with neurological disease or from HTLV-I/II PCR negative individuals in the absence of mitogenic stimulation. **B.** Autologous B cell lines in peripheral blood lymphocytes (PBL) infected with human T-cell lymphotropic virus type I (HTLV-I). Vaccinia recombinants were used as targets to detect HTLV-I-specific cytotoxic T-lymphocyte (CTL) responses to HTLV-I proteins by use of polymerase chain reaction (PCR). Although patients with HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) have a strong HTLV-I-specific CTL response, an HTLV-I-specific CTL response was not demonstrated in HTLV-I/II seroindeterminate patients with neurological disease, as represented by the absence of HTLV-I-specific CTL activity in HTLV-I/II seroindeterminate patient 4.
Figure 3. Human T-cell lymphotropic virus type I (HTLV-I) tax was detected by primary polymerase chain reaction (PCR) with the tax-specific primer pair SK43/44 (155 bp PCR product) from peripheral blood lymphocytes (PBL) of patients with HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (lane 1). HTLV-I tax detected in PBL of HTLV-I/II seroindeterminate patients with neurological disease by performing nested PCR (117 bp PCR product) with an additional primer pair internal to SK43/44 (lanes 5 and 6). HTLV-I tax was not detected by nested PCR in PBL from seronegative individuals (lane 3) or from distilled water controls (lane 2). HTLV-I tax was periodically amplified from PBL from 4 of 8 seroindeterminate patients with chronic progressive neurological disease, as demonstrated by the detection of HTLV-I tax sequence in 1 of 2 times in patient 2 in this experiment (lanes 5 and 6).

(from both asymptomatic carriers and patients with HAM/TSP), as measured by EIA optical density values, were consistently above 2.2, a value well above the 0.52 seropositivity cutoff (figure 4). However, HTLV-I/II EIA values varied significantly among HTLV-I/II seroindeterminate patients from whom serum samples were available in a longitudinal series. Although each of the individuals in our cohort initially screened positive for HTLV-I/II antibodies by EIA, patients 1 and 5 were later determined to be HTLV-I/II EIA negative (figure 4). Significantly, these 2 individuals remained HTLV-I/II seroindeterminate by Western blot at times when their sera would be considered negative by HTLV-I/II EIA. As represented in figure 5, serum from patient 1 was clearly HTLV-I/II EIA positive at her initial visit (February 1995) but demonstrated a Western blot seroindeterminate pattern; later serum samples fluctuated at the HTLV-I/II seropositive cutoff range. Sera from this patient were demonstrated to be HTLV-I/II Western blot seroindeterminate even at times when they were HTLV-I/II EIA negative (figure 5).

Discussion
Herein we describe a cohort of patients who have an HTLV-I/II seroindeterminate Western blot reactivity and were screened...
Figure 4. Patients with human T-cell lymphotropic virus type I (HTLV-I) confirmed by Western blot consistently had HTLV-I EIA values above 2.2. Optical density (OD) values obtained by HTLV-I/II EIA were lower for HTLV-I/II seroindeterminate patients than for tested patients with HTLV-I-associated myelopathy/tropical spastic paraparesis or for HTLV-I asymptomatic carriers. Furthermore, in the 4 patients for whom multiple serum samples were available (patients 4, 3, 1, and 5), HTLV-I/II EIA OD values varied over time, with 2 patients (1 and 5) having OD values below the 0.52 seropositivity cutoff of this assay at particular sampling points.

at the Clinical Center of the National Institutes of Health for chronic progressive neurological disease. All patients presented with a variety of neurological signs and symptoms, and 3 met the clinical criteria for MS. However, these HTLV-I/II Western blot seroindeterminate patterns were demonstrated in a select group of patients, which does not suggest an association between HTLV-I seroreactivity and neurological disease. None of these patients have the typical immunological profile of a HAM/TSP patient—that is, an increase in spontaneous lymphoproliferation [16], the presence of HTLV-I-specific CD8+ CTL in PBL [17–19], and an increase in antibodies to HTLV-I in serum and CSF [3]. However, the detection of HTLV-I tax sequence by nested PCR and the sequencing of prototypic tax sequence from PBL suggest the possibility that these individuals harbor an HTLV infection.

The significance of an HTLV-I/II seroindeterminate Western blot remains unclear. Several theories have been proposed to explain these serological reactivities, including cross-reactivity with other infectious agents such as *Plasmodium falciparum* [20, 21] and the presence of autoantibodies to endogenous retroviruses with homology to HTLV-I [10]. However, none of our 8 HTLV-I/II seroindeterminate individuals with neurological disease live in a region where malaria is endemic or, with the exception of patient 2, have traveled to such a region. The inability to detect HTLV-I sequences by primary PCR from these HTLV-I/II seroindeterminate individuals is consistent with studies in Central Africa that have shown similar HTLV-I/II Western blot reactivities, which have been termed the “HTLV-I gag–indeterminate Western blot profile” [10]. It has also been suggested that HTLV-I/II seroindeterminate Western blot reactivity is associated with specific epitopes of endogenous retroviral sequences related to HTLV-I, which may be reactivated by stimulation with microbial antigens [22]. However, it is unlikely that the tax region amplified by nested PCR and confirmed by sequence analysis from the PBL of HTLV-I/II seroindeterminate patients was from an endogenous human DNA sequence, since regions of homology between endogenous retroviruses and HTLV-I are found not in the tax region but in the LTR, gag, and pol regions of HTLV-I [22].

The seroindeterminate Western blot pattern and the detection of HTLV-I tax in this cohort of patients could also be explained by infection with either defective HTLV-I or a novel retrovirus with partial homology to HTLV-I. Some evidence supports these theories, including the absence of spontaneous lymphoproliferation or HTLV-I–specific CTL activity, the ability to detect some (tax) but not all (pol, gag, LTR) HTLV-I sequences by PCR, and the incomplete antibody response to HTLV-I antigens. It has been demonstrated that patients harboring defective HTLV-I do not show a characteristic immune response to HTLV-I [23, 24]. Alternatively, a novel retrovirus could have enough homology with HTLV-I tax to be amplified by tax–specific primer pairs (SK43/44) yet have enough novel sequence to escape detection by other methods specific for HTLV-I and HTLV-II. Recently, 2 new simian T-lymphotropic viruses (STLVs) have been identified that give rise to HTLV-I/II indeterminate serology, and it has been suggested that the human HTLV-I/II seroindeterminate Western blot may be the result of infection by a close relative of one of these STLVs or divergent subtypes of HTLV-I [15, 25–27].

An explanation also compatible with the results presented in this study is that an extremely low copy number of HTLV-I may result in HTLV-I/II seroindeterminate Western blot patterns. A low HTLV-I virus load is consistent with the following observations presented in this report: decreased HTLV-I antibody responses to some but not all HTLV-I antigens, lack of spontaneous lymphoproliferation and HTLV-I–specific CTL, and periodic amplification of HTLV-I sequences from PBL by nested PCR techniques. The ability to amplify some but not all HTLV-I genes (particularly HTLV-I tax) has been reported
Figure 5. Human T-cell lymphotropic virus (HTLV) type I/II EIA optical density (OD) values sampled over time from HTLV-I/II seroindeterminate patient 1. Although the OD values fluctuated around the positivity cutoff at certain times (October 1996, November 1996, and January 1997), an HTLV-I/II seroindeterminate Western blot pattern could still be shown.

by others [10, 14, 15] and may reflect the sensitivity of primer/pair sets to this region. If low HTLV-I virus load explains HTLV-I/II Western blot seroreactivity, then the longitudinal findings presented in this study, which demonstrate HTLV-I/II Western blot seroindeterminate reactivity in sera obtained at times when PBL were HTLV-I/II EIA negative, have profound implications for the extent of HTLV-I infection worldwide. The isolation of full length virus from an HTLV-I/II seroindeterminate individual would further support this theory.

HTLV-I/II seroindeterminate Western blot patterns are prev-
alent worldwide [10] and are found in countries where neither HTLV-I nor HTLV-II is considered endemic. Whereas some argue that the presence of HTLV-I/II seroindeterminate individuals in nonendemic areas suggests that these individuals do not carry HTLV-I or HTLV-II [9, 10], others caution that these data may indicate that the prevalence of HTLV-I is grossly underestimated [14, 24, 28]. It has been reported that the seroprevalence of HTLV-I, on the basis of HTLV-I \( \text{tax} \) sequences detected in HTLV-I/II EIA negative US blood donors, may be higher than currently estimated [14, 29]. Support for this includes the presence of HTLV-I provirus in a large percentage of seronegative individuals with mycosis fungoides [14, 24, 28] and in a minority of other seronegative individuals who are HTLV-I/II PCR positive [8, 15, 23, 28–30]. Therefore, the association of the HTLV-I/II seroindeterminate Western blot pattern with low copy number of HTLV-I would further suggest that the seroprevalence of HTLV-I may be higher than is currently reported. This study also suggests that blood products from HTLV-I/II seroindeterminate individuals screened for HTLV-I/II antibodies by EIA may be reported as HTLV-I/II negative at times when HTLV-I/II EIA values are below the HTLV-I seropositivity cutoff. However, the clinical consequence of receiving blood products from HTLV-I/II seroindeterminate individuals has yet to be determined, especially in light of reports demonstrating that the individuals who receive blood screened for HTLV-I/II antibodies have an extremely low probability of seroconverting [31–33].

Acknowledgments

We would like to thank Renaud Mahieux for critical review of this manuscript and Renu Lal for the gift of primers to the LTR.

References


