Comparison of a human T-cell lymphotropic virus type I strain from cerebrospinal fluid of a Jamaican patient with tropical spastic paraparesis with a prototype human T-cell lymphotropic virus type I

(enzyme-linked immunosorbent assay/Southern blotting/immunoblotting/dementia)

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ABSTRACT The isolation and characterization of a human T-cell lymphotropic virus type I (HTLV-I) from cerebrospinal fluid of a Jamaican patient with tropical spastic paraparesis is described. The virus isolate is a typical type C retrovirus as seen by electron microscopy and is related to prototype HTLV-I isolated from patients with adult T-cell leukemia but is not identical to this prototype HTLV-I as seen by restriction enzyme mapping.

Human T-cell lymphotropic virus type I (HTLV-I) has been shown to be the etiological agent of tropical spastic paraparesis (TSP) in several parts of the world. We have compared a strain isolated from the cerebrospinal fluid of a Jamaican TSP patient with a prototype HTLV-I isolated from a patient with adult T-cell leukemia (ATL), by restriction enzyme mapping.

TSP is a neurological disorder characterized by paraparesis and spasticity of the lower limbs, which is sometimes associated with posterior column and superficial sensory change. Endemic TSP occurs in diverse geographical regions (1), and recently IgG antibodies to HTLV-I were found in several Caribbean islands (2–4), South America (3, 5, 6), the Seychelles islands (7), parts of Africa (8), and also in patients in the temperate climate of southern Japan, where an identical syndrome called HTLV-I-associated myelopathy (HAM) has been described (9). HTLV-I is a type C retrovirus that is endemic in the Caribbean and southern Japan and has been isolated from patients with ATL in these regions (10, 11).

MATERIALS AND METHODS

Patient and Source of Tissues. The patient CH is a 66-year-old Jamaican female who was first seen at the University Hospital in 1984, approximately 13 years after onset of the disease. She developed progressive difficulty in walking, stiffness of the legs, and back pain. Neurological examination showed positive signs of spastic paraparesis with extensor plantar responses and ankle clonus. There was generalized hyperreflexia, more marked in the lower than the upper limbs, and no cortical, cranial nerve, or sensory deficiencies were present. Routine hematological and biochemical laboratory investigations were carried out and samples of hepaticized blood, serum, and cerebrospinal fluid (CSF) were obtained for serological, immunological, and virus-isolation studies. The detection of antibodies to HTLV-I in serum and CSF of this patient was done by ELISA and electrophoretic immunoblot (Western blot) techniques (12). The densitometric measurements on Western blot strips were done with a Dynatech densitometer.

Coculture of CSF or Peripheral-Blood Mononuclear Cells. CSF removed from the patient was spun to obtain mononuclear cells. The CSF mononuclear cells were suspended in RPMI 1640 medium containing 20% (vol/vol) fetal calf serum and were cocultivated with peripheral-blood or umbilical-cord-blood mononuclear cells at a concentration of 5 × 10⁵ cells in the presence of 0.1% phytohemagglutinin (PHA), 10% (vol/vol) interleukin 2, and 5 μg of hydrocortisone per ml. After 72 hr the cells were fed with culture medium without PHA and subsequently were fed every 4 days. Fresh PHA-activated lymphocytes were added as needed to maintain the cell count. Virus expression was determined by measuring reverse transcriptase, HTLV-I p19, and HTLV-I p24 production by immunofluorescence; the presence of virus particles was determined by electron microscopy. For coculture with patient’s mononuclear cells from peripheral blood, the cells were separated by Ficoll/Hypaque centrifugation and cultured at a concentration of 5 × 10⁵ cells per ml in the presence of 0.1% PHA in RPMI 1640 medium containing 20% fetal calf serum, 10% interleukin 2, and 5 μg of hydrocortisone per ml. After 3 days the cells were cocultivated with PHA-stimulated peripheral-blood lymphocytes or with PHA-stimulated umbilical-cord-blood mononuclear cells. The cultures were examined for virus expression by measuring reverse transcriptase and viral antigen production by indirect immunofluorescence and by electron microscopy. The virus isolate was finally transmitted into H9 and MOLT-3 cells by cocultivation.

Reverse Transcriptase Assays. Reverse transcriptase assays were carried out as described (13, 14). Briefly the culture supernatant (5 ml) was treated with 30% (wt/vol) polyethylene glycol and 0.4 M NaCl, allowed to stand overnight at 4°C, and centrifuged to pellet the precipitate. The viral precipitate was suspended in 300 μl of buffer containing 50 mM Tris-HCl (pH 7.5), 5 mM dithiothreitol, 250 mM KCl, and 0.25% Triton X-100. Reverse transcriptase activity was analyzed in a 50-μl reaction mixture containing 10 μl of the disrupted virus suspension, 50 mM Tris-HCl (pH 7.8), 5 mM dithiothreitol, 100 mM KCl, 0.01% Triton X-100, 10 μl of dT123A as template-primer, 10 mM MgCl₂, and 15 μM [3H]dTTP (15

Abbreviations: HTLV-I, human T-cell lymphotropic virus type I; TSP, tropical spastic paraparesis; ATL, adult T-cell leukemia; CSF, cerebrospinal fluid; PHA, phytohemagglutinin.

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Ci/mm; 1 Ci = 37 GBq). After incubation for 60 min at 37°C and subsequent addition of 50 μg of yeast tRNA, the incorporation of [3H]dTMP into trichloracetic acid-insoluble fraction was assayed (13, 14). Assays were done in duplicate.

**Immunofluorescence Assays.** The percentage of cells expressing HTLV-I p19 and p24 was determined by indirect immunofluorescence analysis on fixed cells with monoclonal or polyclonal antibodies to HTLV-I p19 or p24 as described (13). Briefly, the cultured cells were plated on toxoplasmosis slides and fixed with methanol/acetone, 1:1 (vol/vol), for 30 min at room temperature. The slides were stored in sealed plastic bags at −20°C until ready for use. HTLV-I p19 monoclonal or HTLV-I p24 rabbit antibodies were added to duplicate wells, incubated at room temperature in a humid chamber for 1 hr, and washed with phosphate-buffered saline containing 0.25% Triton X-100 for 2 hr. The cells were then exposed to fluorescein-labeled goat anti-mouse or anti-rabbit IgG (Capell Laboratories) for 1 hr and washed with phosphate-buffered saline containing 0.25% Triton X-100 overnight. The cells were counterstained with Evan's blue, the slides were mounted with 50% (vol/vol) glycerol, and fluorescence was observed under a Zeiss fluorescence microscope.

**Electron Microscopy.** Electron microscopic examination was carried on cultured cells by Bernhard Kramarsky (Electro-Nucleonics).

**Restriction Enzyme Analysis.** Restriction enzyme mapping of the prototype HTLV-I isolate (C91/PL) DNA and the CSF virus isolate DNA was carried out with several restriction enzymes by the Southern hybridization technique as described (15). Briefly, DNA was extracted from the infected cells by incubating with lysis buffer containing 10 mM Tris-HCl (pH 8), 10 mM NaCl, 10 mM EDTA, 0.5% sodium dodecyl sulfate, and 100 μg of proteinase K per ml at 60°C for 2 hr. After phenol/chloroform extraction, DNA was precipitated with ethanol and dissolved in buffer containing 10 mM Tris-HCl (pH 8) and 1 mM EDTA. DNA was digested with

![Image]( attachment://image.png)

**FIG. 1.** Light-microscopic examination of peripheral blood from patient CH. The figure shows the presence of a giant cell with lobulated nuclei typical of patients with ATL.
different restriction enzymes, and the digested DNA was electrophoresed on a 0.8% agarose gel, transferred to a nitrocellulose membrane, and hybridized with a radiolabeled HTLV-I probe.

RESULTS AND DISCUSSION

With the detection of HTLV-I-related antibodies in the serum and CSF of patients with TSP (1), attempts have been made to isolate and characterize the virus. Recently, the isolation of an HTLV-I-like virus from the peripheral-blood lymphocytes of TSP patients has been reported (9, 16, 17), and, as a result of Southern blot analysis, this virus isolate is considered to be identical to HTLV-I from patients with ATL (18).

This report presents evidence for the isolation and characterization of an HTLV-I strain from the CSF of a patient from Jamaica. Examination of the peripheral blood of patient CH showed the presence of multinucleated giant cells with lobulated nuclei, which are commonly seen in peripheral-blood lymphocytes of patients with ATL (Fig. 1). The peripheral blood of patient CH showed a preponderance of OKT4⁺ helper/inducer T cells (43%) compared with OKT8⁺ suppressor/cytotoxic T cells (12%). Both serum and CSF of this patient showed the presence of HTLV-I-related antibodies, as seen by ELISA and Western blot analysis (Fig. 2).

Cocultures of CSF mononuclear cells from patient CH with peripheral-blood lymphocytes or umbilical-cord-blood lymphocytes resulted in (i) the expression of a retrovirus that was characterized by the presence of reverse transcriptase in culture supernatants and (ii) the expression of HTLV-I p19 and p24 antigens as determined by immunofluorescence analysis with monoclonal or polyclonal antibodies to HTLV-I p19 or p24. Electron microscopic examination showed the presence of virus particles typical of type C retroviruses with rounded nucleoid cores as seen in HTLV-I (Fig. 3).

A comparison of the restriction enzyme maps of this virus isolate with eight restriction enzymes (Fig. 4) shows that, although the restriction maps are similar to those of HTLV-I, they are not identical as seen in the restriction patterns with Pst I, Kpn I, Sma I, and Xba I. These results suggest that there are differences between the prototype HTLV-I from ATL patients and the HTLV-I strain isolated from the CSF of a TSP patient. Further analysis of this virus isolate by gene cloning and sequence analysis is needed to establish the
Fig. 3. (a) Electron-microscopic examination of cultured cells showing typical HTLV-I-like type C virus particles with rounded nucleoid cores. (b and c) Higher magnifications of areas shown by arrows in a. Bars show the magnification.

Fig. 4. Comparison of the restriction maps of a prototype HTLV-I DNA with DNA isolated from cells infected with virus from CSF of patient CH. (Left) Restriction maps with EcoRI, Pst I, Sst I, and HindIII. Lanes: 1, 3, 5, and 7, maps from prototype HTLV-I (C91/PL); 2, 4, 6, and 8, patterns from the CSF virus isolate. (Right) Restriction maps with BamHI, Kpn I, Sma I, and Xba I. Lanes: 1, 3, 5, and 7, maps from prototype HTLV-I; 2, 4, 6, and 8, maps from the CSF virus isolate.

identity or differences between the CSF isolate from the TSP patient and the HTLV-I prototype from ATL patients.

Our studies show that an HTLV-I strain can be obtained from cocultures of mononuclear cells from CSF and either peripheral-blood or umbilical-cord blood. This virus isolate is similar to HTLV-I but not identical to the HTLV-I prototype isolated from ATL patients. These studies show that HTLV-I or an HTLV-I-related retrovirus may be important in the development of neurological dysfunction in patients with TSP. In support of this suggestion, we have recently observed that the patient MJ from whom the first HTLV-I virus isolate was obtained (19) developed a TSP-like myeloneuropathy shortly before his death. However, we were unable to isolate a virus from the CSF of this patient that could have been useful in deciphering the changes, if any, that may have occurred in the virus during the course of the disease from ATL to the suspected TSP. Further studies with viral isolates from the CSF of TSP patients should help to determine the neurovirulence of HTLV-I-related retroviruses and to explain the basic mechanisms involved in this chronic progressive neurological dysfunction.

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