RAPID COMMUNICATION

Detection of Human T-Cell Leukemia/Lymphoma Virus, Type II, in a Patient With Large Granular Lymphocyte Leukemia

By Thomas P. Loughran, Jr, Thomas Coyle, Michael P. Sherman, Gordon Starkebaum, Garth D. Ehrlich, Francis W. Ruscetti, and Bernard J. Poiesz

We studied a patient with large granular lymphocyte (LGL) leukemia for evidence of human T-cell leukemia/lymphoma virus (HTLV) infection. Serum from this patient was positive for HTLV-I/II antibodies by enzyme-linked immunosorbent assay (ELISA) and was confirmed positive in Western blot and radioimmunoprecipitation assays. Results of a synthetic peptide-based ELISA showed that the seropositivity was caused by HTLV-II and not HTLV-I infection. Analyses of enzymatic amplification of DNA from bone marrow sections using the polymerase chain reaction (PCR) were positive for HTLV-II specific gag, pol, env, and pX gene sequences. Cloning and sequencing of amplified products showed that the HTLV-II pol and pX sequences in patient DNA differed from the sequences of 17 other HTLV-II isolates examined in our laboratory. HTLV infection may have a role in some patients in the pathogenesis of LGL leukemia.

Large granular lymphocyte (LGL) leukemia results from a chronic proliferation of either CD3+ or CD3- LGL.1 T-cell receptor gene rearrangement studies have shown that, if not, all patients with CD3+ LGL leukemia have a clonal disorder.2,3 The etiology of the clonal LGL proliferation is not known. Our previous study demonstrated that 6 of 12 LGL leukemia patients had antibodies to human T-cell leukemia/lymphoma virus (HTLV), type I/II gag proteins p19 or p24.4 In this study, we further investigated the possible role of HTLV infection in this disease. A patient with LGL leukemia was HTLV-I/II seropositive as confirmed by Western blot assay and radioimmunoprecipitation assay. Using enzymatic DNA amplification, we detected HTLV-II gag, pol, env, and pX gene sequences in DNA from bone marrow mononuclear cells from this patient.

MATERIALS AND METHODS

Patient. The clinical, hematologic, and immunopathologic findings in this patient have been described previously.5 LGL counts were increased markedly, with a CD3+, CD8+, CD57+ phenotype. Clonal disease was demonstrated by the findings of a clonal cytogenetic abnormality (trisomy 8) in unstimulated cultures of peripheral blood mononuclear cells.6 Marrow aspiration and biopsy results were normal; in particular, there was no evidence for lymphocytic infiltration. The patient had not received blood transfusions, did not have a history of intravenous drug use, and had not lived in an HTLV-I/II endemic region, although he had been a merchant sailor in the Far East many years previously. A detailed sexual history was not obtained. Subsequent to our initial report, the patient died at the age of 89, presumably of natural causes, in a nursing home; an autopsy was not obtained.

Antibody studies. These assays included an HTLV-I enzyme-linked immunosorbent assay (ELISA) (Cellular Products, Buffalo, NY), an HTLV-II ELISA, an HTLV-I/II radioimmunoprecipitation assay (RIPA), and an HTLV I/II Western blot assay performed as described previously.6 To discriminate HTLV-I from HTLV-II infection, a synthetic peptide-based ELISA (SELECT-HTLV; Coulter Corp, Hialeah, FL) was used according to the manufacturer’s instructions.

DNA analyses. The only source of DNA was paraffin-embedded bone marrow biopsy sections. Ten- or 20-μm slices were cut from these sections and paraffin removed with xylene, which was then removed with 95% ethanol. The sample was resuspended in water and subjected to polymerase chain reaction (PCR) after 20 minutes of boiling. Analyses of enzymatically amplified DNA using the PCR were performed in a Perkin-Elmer Cetus Thermal Cycler (Norwalk, CT) with the thermostable DNA polymerase, Taq (a kind gift of Cetus Corp), isolated from Thermus aquaticus for 30 cycles, as described.6 Amplified DNA was assayed using the liquid hybridization format described previously.6 Oligonucleotide PCR primers and probes are designated by a two-letter initial for HTLV and a numeral for the type (1 or II), followed by an abbreviation for the proviral region, followed by the numerical region it was homologous to in a 5’ to 3’ orientation, followed by a “+” or “−” to indicate which DNA strand it resembled, and finally a “d” is placed whenever the oligonucleotide was used as a detector. Primers and probes are as follows: primer pair 1 HTIILTR(26-47)+/HTIILTR(355-320)− and probe a HTIILTR(255-275)+d; primer pair 2 HTIILTR(255-275)+/HTIILTR(622-604)− and probe b HTIILTR(355-320)−d; primer pair 3 HTIILTR(91-109)+/HTIILTR(782-760)− and probe c HTIILTR(283-324)+; primer pair 4 HTIIPOL(4735-4756)+/HTIIPOL(4920-4897)− and probe d HTIIPOL(4880-4899)+d; primer pair 5 HTIIPOL(4198-4217)+/HTIIPOL(4300-4281)− and probe e HTIIPOL(4276-4237)+d; primer pair 6 HTIIPOL(4735-4756)+/HTIIPOL(4300-4281)− and probe f HTIIPOL(4880-4899)+d; primer pair 7 HTIIPOL(501-5322)+/HTIIPOL(5682-5659)− and probe g HTIIPOL(5521-5560)+d; primer pair 8 HTIIPOL(5799-5818)+/HTIIPOL(6125-6106)+ and probe h HTIIPOL(5841-5880)+; and primer pair 9 HTIIPX(7248-7267)+/HTIIPX(7406-7386)+d and probe i HTIIPX(7337-7376)+d. Of note, primer pairs 6 and 9 will amplify pol and pX gene sequences, respectively, which are common to both

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HTLV-I and HTLV-II. Probe i will detect either HTLV-I or HTLV-II pX sequences amplified by primer pair 9. Probe f will detect specifically the HTLV-II pol sequence whereas probe j will detect specifically the HTLV-I pol sequence whereas probe k will detect specifically the HTLV-I gene sequences amplified by primer pair 6. For purposes of cloning and sequencing, hyperamplified PCR products were ligated into the M13mp18 vector (Bethesda Research Laboratories, Bethesda, MD), as described. Clones containing the correct inserts, as detected by hybridization to the PCR detector probes, were sequenced by the dideoxy method using 32P end-labeled universal primer and the sequenase DNA sequencing kit, version 2.0 (United States Biochemical Corp, Cleveland, OH), as described.

RESULTS

Antibody studies. Serum from the patient tested positive in both the HTLV-I and HTLV-II ELISA. We reported previously that serum from this patient was positive in an HTLV-I/II Western blot assay (patient 10 of that report). Serum from the patient was also confirmed positive in the HTLV-I/II RIPA (Fig 1). Serum from the patient was HTLV-I negative and HTLV-II positive when tested in the synthetic peptide-based ELISA (Table 1).

Table 1. Results of Synthetic Peptide-Based ELISA to Discriminate HTLV-I From HTLV-II Infection

<table>
<thead>
<tr>
<th>Serum</th>
<th>Absorbance Values</th>
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<tbody>
<tr>
<td></td>
<td>HTLV-I Plate</td>
<td>HTLV-II Plate</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>0.052</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>0.047</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>0.048</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Mean of negative controls</td>
<td>0.049</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>HTLV-I+ control</td>
<td>0.671</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>HTLV-II+ control</td>
<td>0.053</td>
<td>0.449</td>
<td></td>
</tr>
<tr>
<td>Cutoff value for HTLV-I+</td>
<td>0.149</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Cutoff value for HTLV-II+</td>
<td>—</td>
<td>0.119</td>
<td></td>
</tr>
<tr>
<td>LGL leukemia patient</td>
<td>0.035</td>
<td>1.096</td>
<td></td>
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</table>

DNA analyses. DNA from paraffin-embedded bone marrow sections from the patient was positive when using PCR primers that amplify pX and pol gene sequences shared by HTLV-I and HTLV-II. The amplified pol product hybridized to the HTLV-II specific probe and not to the HTLV-I specific probe. Using HTLV-II specific primer pairs, we detected hybridization to another HTLV-II pol gene region, an HTLV-II gag gene region, and an HTLV-II env gene region (Table 2, Fig 2). The inability to detect HTLV-II sequences in patient DNA using LTR probes a, b, and c and ENV probe 7 may be due to lesser sensitivity of LTR primers, inefficiency of amplification from paraffin-embbeded material, or sequence heterogeneity of HTLV-II infecting LGL leukemia patient compared with the MO-T isolate. Use of multiple primer pairs tends to obviate concern of false-positive results through carryover of amplified product. Results of cloning and sequencing of amplified products also argue against false-positive results because we found HTLV-II pol and pX sequences in patient DNA that differed from MO-T and 16 other HTLV-II

Table 2. Detection of HTLV-II Gene Sequences by PCR Analyses of LGL Leukemia DNA

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>HTLV-II Region</th>
<th>LTR</th>
<th>GAG</th>
<th>POL</th>
<th>ENV</th>
<th>pX</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTLV-II control; MO-T*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HTLV-I control; HSC-CTCL-11B</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>LGL patient</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>+</td>
</tr>
</tbody>
</table>

*DNA from MO-T was amplified and detected with an internal probe by liquid hybridization down to an input of 10 copies, except for the LTR primers where 100 copies were necessary because of heterogeneity of the regions homologous to the primer pairs (1 and 2) and the relatively lengthy PCR product (primer pair 3).
of 12 patients with LGL leukemia had IgG antibodies to HTLV-I/II gag proteins, p19 or p24. Antibodies to HTLV-I/II p19 or p24 antigens were also found in sera of 7 of 27 patients with LGL leukemia studied in Italy. Also, Sohn et al described a case of chronic T-cell leukemia associated with antibodies to HTLV-I/II, which had many features similar to LGL leukemia, including a CD3+, CD8+ phenotype of leukemic cells. In the current study, serum from our patient was positive in an HTLV-I and an HTLV-II ELISA; results of HTLV-I/II Western blot assay and HTLV-I/II RIPA confirmed seropositivity for this patient.

Routine serologic studies cannot discriminate HTLV-I from HTLV-II infection. However, synthetic peptide-based ELISA tests are 100% specific in differentiating between the two retroviruses. Using such an assay, we

isolates (unpublished data, April 1992) sequenced in our laboratory (Fig 3).

DISCUSSION

Results of our serologic and DNA analyses show that this patient with LGL leukemia was infected with HTLV-II and not HTLV-I. Our previous study showed that serum from 6
showed that seropositivity was caused by HTLV-II, and not HTLV-I, infection. PCR studies of DNA from the patient also showed that the retrovirus was HTLV-II. HTLV-II has been isolated from only a few patients with clinical disease (T-cell variant of hairy cell leukemia), although HTLV-I/II seropositive intravenous drug abusers are often infected with HTLV-II and not HTLV-I. This increased recognition of HTLV-II infection in intravenous drug abusers makes it important to become aware of diseases that may be associated with HTLV-II infection.

The epidemiology of retroviral infection in patients with LGL leukemia is obscure. Infection with HTLV-I has been documented for one patient with LGL leukemia and pure red blood cell aplasia. In this case, the patient had received blood transfusions before onset of disease and subsequently received multiple transfusions before retroviral detection. It is well established that HTLV-I infection can be transmitted through blood transfusion. Our patient had not received blood transfusions, was not an intravenous drug user, and lived in an area not endemic for HTLV-I/II.

It is not possible to ascertain from our data the role of HTLV-II infection in the pathogenesis of clonal LGL proliferation in our patient. Clonal integration of HTLV-II in leukemic cells would provide strong evidence of a direct role in disease causation. Unfortunately, the only tissue available for our study was bone marrow, which was not infiltrated by leukemic LGL. Therefore, using Southern blot analyses to detect retroviral integration would not be informative. Large-scale serologic and nucleic acid analyses are underway to establish the prevalence and possible pathogenetic role of retroviral infection in patients with LGL leukemia.

REFERENCES

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