RAPID COMMUNICATION

High Frequency of Human T-Cell Leukemia-Lymphoma Virus Type II Infection in New Mexico Blood Donors: Determination by Sequence-Specific Oligonucleotide Hybridization

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The Albuquerque branch of the United Blood Services system was found to have an unusually high blood donor human T-cell leukemia/lymphoma virus (HTLV) seroprevalence (0.72 per 1,000). Many studies investigating HTLV seroprevalence and transmission have assumed that all seropositivity is due to HTLV type I (HTLV-I); recent data dispute this conclusion. We investigated the high prevalence of HTLV seropositivity in New Mexico by determining whether HTLV-I or HTLV-II is predominant in our donors.

Using polymerase chain reaction (PCR) amplification of proviral DNA from peripheral blood, followed by sequence-specific hybridization with oligonucleotide probes to distinguish the two viruses, we demonstrate that 9 of 10 Western blot-confirmed HTLV-seropositive blood donors from New Mexico are infected with HTLV-II. Implications of this finding for donors and the safety of the blood supply are discussed.

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use of stringent wash conditions allows these primers to selectively (104-fold preference) bind to their exact complement at the expense of the mismatched homolog, allowing determination of the type of virus infecting each of 10 seropositive blood donors. We found that 9 of 10 of our HTLV-positive donors were infected with HTLV II.

**MATERIALS AND METHODS**

**Genomic DNA.** Mononuclear cells (PBLs) from Ficoll-Hypaque gradients were prepared from 20 to 40 mL of anticoagulated blood. DNA was prepared as described.29 Infected cell lines harboring the HTLV-I or HTLV-II proviruses (MT4 and Mo, respectively) were used as positive controls. The Mo cell line, in later passages, contains three different proviral genomes, including two with large internal deletions. Since at least one of the Mo proviral genomes includes the regions amplified by our primer/probe sets, Mo DNA proved useful as an HTLV-II-infected control cell. Peripheral blood DNAs from uninfected individuals were used as negative controls.

**Oligonucleotide primers and probes.** Oligonucleotide primers with sequences as described by Lee et al11 (Genetic Designs, Houston, TX) were prepared and purified. These primers match with nearly perfect complementarity both HTLV-I and -II, and thus produce PCR amplification products from both viruses.11 We named these primers 53HT (5' CGGATACCCAGTCTACGT 3') and 35HT (5' GAGCCGATAACGCGTCCATCG 3').

Oligonucleotide probes were chosen to correspond to portions of the amplified product that have undergone maximal change between HTLV-I and HTLV-II. The degree of divergence of the HTLV-I-specific probe, HTI (5' TACAGGCGACTGGTGCCCCAT 3'), and the HTLV-II proviral sequence is 5 out of 22 basepairs (bp). The HTLV-II specific probe, HTII, has the sequence 5' TACAGGC-GATTGGTGTCCCGT 3'.

**PCR, blotting, and hybridization.** PCR conditions were as described11 except that 50 pmol, rather than 100 pmol, of each primer was used, and 3 mmol/L MgCl₂, rather than 5 mmol/L, was used. Two-microgram aliquots of genomic DNA were subjected to 35 to 45 cycles of denaturation (91° for 1 minute) followed by annealing/extension (65° for 2 minutes). Extensive precautions were taken to separate work areas and equipment used to handle PCR products from those used to prepare reagents for amplification.

Dot-blotting was essentially as described by Abbott et al16 except that 5 μL (out of a final PCR reaction volume of 25 μL), rather than 10 μL, was spotted onto nylon membranes (Sigma Chemical Co, St Louis, MO). All samples were spotted onto two filters to allow hybridization with each probe in separate hybridization bags. PCR procedures using the SK54/SK55 or SK58/SK59 primers and probes SK56 and SK60 were as described by Kwok et al.19

Prehybridization was in standard conditions26 for 1 to 12 hours at 37°C or 42°C. Hybridization was identical to prehybridization except that 5'-end-labeled oligonucleotide probes HTI or HTII (specific activity 10⁵ to 10⁶ cpm/pmol with γ-[³²P]-ATP) were added to a final concentration of 10⁴ cpm/mL. The same specific activity of HTI and HTII probes were always used in side-by-side hybridizations; when labeling reactions produced different specific activities, unlabeled probe was added to the higher specific activity probe to produce identical specific activities. All hybridizations were performed overnight at 37°C to 42°C.

Wash conditions were chosen to maximize the specificity of annealing between probes HTI and HTII and their respective proviral complements. Filters were rinsed for 1 to 2 minutes in 6X saline sodium citrate (SSC) at 0°C four times, then washed in 6X SSC at 70°C for 1 minute with shaking, then air-dried, and subjected to autoradiography (2 to 24 hours) with intensifying screens at -70°C.

**RESULTS**

Sequence-specific probes allow the reliable distinction between HTLV-I and -II. Figure 1A shows the results of
hybridization of PCR products from the infected cell lines MT4 (HTLV-I) and Mo (HTLV-II) and uninfected cell DNA (PBL) with probes HTI and HTII. It is clear from this figure that sequence-specific probes readily allow the distinction between the two integrated proviral DNAs.

In Fig 1B the signal produced from 2 µg of Mo DNA (far left panel) is compared with the signal obtained from serial 10-fold dilutions of MT4 DNA samples, using the HTI probe. The intensity of hybridization of 0.2 ng of MT4 DNA (fifth panel) was comparable with that produced by 2 µg of Mo DNA, suggesting that the oligonucleotide-specific probes detected their perfectly matched complement at a 10^4-fold lower concentration (of input DNA), compared with mismatched sequences.

Sensitivity of PCR detection of HTLVs. Figure 1B demonstrates that HTLV-I sequences can be amplified from as little as 0.2 ng of genomic DNA from the infected cell line MT4. Assuming that no episomal HTLV-I DNA is present in this preparation, and that HTLV-I is present in a single copy per diploid genome, then it appears that the primer pair we used can detect as little as 30 molecules of HTLV proviral DNA.

Optimization of conditions for specific detection of HTLV-I and HTLV-II. In Fig 2 we examined the effect of increasing the number of PCR cycles from 35 to 45 on an HTLV-positive blood donor (determined to be positive for HTLV-II by previous PCR analysis). The number of PCR cycles was varied while keeping other factors constant. This experiment demonstrates that the degree of discrimination between HTLV-I- and HTLV-II-specific hybridization was improved by increasing the cycle number, with the maximum discrimination becoming apparent at 40 to 45 cycles.

Use of the hybridization assay for HTLV typing in infected blood donors. In the first 10 months of screening for HTLV-I/II antibodies at UBS-Albuquerque, 30 donations from 27 donors (from 41,657 total donations) were confirmed-positive by Western blot and/or radioimmune precipitation assay. Attempts have been made to obtain anticoagulated blood for PCR testing from all 27 positive donors. Ten samples were obtained by having seropositive donors return for a second blood sample. University of New Mexico Human Subject Research Committee approval was obtained, in accord with an assurance filed with and obtained by the Department of Health and Human Services.

All seropositive donor samples produced a PCR product detectable by one of the specific oligonucleotide probes (results from six donors are shown in Fig 3). Nine of the 10 donors’ DNAs had HTLV-II sequences, with only one having HTLV-I. All but one sample was available in sufficient quantity to replicate the hybridization results. Negative control spots were prepared with any of four different DNAs from uninfected persons in each experiment. Four of the donors’ amplification products were also tested for the appearance of a band on an ethidium bromide-stained agarose gel of the appropriate size (159 bp); all four produced the predicted band (data not shown), although nonspecific products were also apparent in donors’ and negative control DNAs.

Verification of HTLV-I or -II infection by second PCR procedure. Because PCR has a high potential for false positive diagnoses, we used a second PCR procedure to confirm the diagnoses rendered by the tax primer pair (data not shown). The combination of HTLV-I pol-specific primers SK54 and SK55 with HTLV-I specific probe SK56 was found to produce an amplification product only with DNA from the donor determined to have HTLV-I infection by the 53HT/35HT primer pair. We also confirmed the 53HT/35HT viral typing result in a majority (six) of our nine donors diagnosed with HTLV-II. We found the SK58/59 primers were less sensitive in our hands than were the 53HT/35HT primers, in that two donors’ DNA samples did not produce amplification product with the SK58/59 primer set. One HTLV-II–positive DNA was not available in sufficient quantity for confirmation.

DISCUSSION

A method for HTLV typing in seropositive donors and patients. We describe the use of oligonucleotide hybridization probes to detect HTLV-I- or -II–specific sequences of an amplified domain common to both proviruses. A similar technique has been used by Kwok et al., but the sensitivity of primers described by that group when applied to patient peripheral blood mononuclear cells (PBMCs) is not known.

This assay was able to allow assignment of virus type to
each of our 10 seropositive donors. We never failed to produce amplification products from undiluted samples of infected cell line DNA, or from seropositive donors. Our first attempts at amplifying HTLV-I/II from donor PBMCs sometimes failed to produce signal in some trials, but these events became very rare after we optimized parameters for amplification.

Because PCR-based techniques have potential for difficulties with specificity due to their susceptibility to laboratory contamination, we verified our assignment in as many samples as possible with infected cell line DNA, or from seropositive donors. Our first because blood donors. A known risk factor for HTLV infection, including travel to HTLV endemic areas and intravenous drug abuse. They came from diverse locations throughout central New Mexico. Although we are still in the process of studying the epidemiologic characteristics of HTLV-positive donors in our region, preliminary results suggest an overrepresentation of Hispanics and American Indians among our seropositive donors, including those shown to be infected with HTLV-II.

We have demonstrated two instances of transmission of HTLV-II via transfusion (Hjelle B, Mills R, Mertz G, Swenson S, unpublished results, February 1990), raising the possibility that previous examples of HTLV-I transmission by transfusion in the United States may have included cases of HTLV-II transmission.\textsuperscript{13,21,22} It is important to determine which virus was transmitted in these instances, for the purpose of advising the recipient correctly.

Our finding that 9 of 10 HTLV-seropositive donors were infected with HTLV-II, usually considered to be the more rare of the two HTLVs, is in agreement with the results of Lee et al.,\textsuperscript{11} and supports the hypothesis that HTLV-II infection is more frequent in the United States than previously recognized.\textsuperscript{15,17} Distinction of the two virus types should be performed in other regions with high seroprevalence.

REFERENCES


Fig 3. HTLV typing of New Mexico blood donors. Results of amplification-hybridization of six blood donors' peripheral mononuclear cell DNAs are shown. For this figure, dots were excised from various autoradiograms with different sets of controls. However, typical control experiments are represented in Fig 1.