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The Complete Genomic Sequence of an HTLV-II Isolate from a Guahibo Indian from Venezuela

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A polyclonal $CD3^+$, $CD8^+$ T-cell line, G2, was derived from the peripheral blood of a seropositive, PCR-positive, HTLV-IIB infected Guahibo Indian from Venezuela. The cell line is productively infected with HTLV-IIB. The entire HTLV-II G2 proviral DNA was sequenced via PCR using overlapping HTLV-II primer pairs. Phylogenetic analyses indicate that HTLV-II G2 is the most divergent HTLV-IIB strain identified to date. Characterization of its deduced proteins and its relationship to other members of the PTLV/BLV genus of retroviruses are discussed.

The human T-cell lymphoma/leukemia viruses I and II (HTLV-I and HTLV-II) belong to a group of complex oncogenic retroviruses (Kalyanaraman *et al.,* 1982; Poiesz *et al.,* 1980). HTLV-I is associated with a variety of clinical disorders, including T-cell lymphomas and leukemias, neurodegenerative disease, polymyositis, arthritis, and uveitis (Bhagavati *et al.,* 1988; Mochizuki *et al.,* 1992; Poiesz *et al.,* 1980; Sherman *et al.,* 1995). HTLV-II has been increasingly associated with rare lymphocytic neoplasms and cases of neurodegenerative disease (Harrington *et al.,* 1993; Hjelle *et al.,* 1992; Kalyanaraman *et al.,* 1982; Loughran *et al.,* 1992; Lowis *et al.,* in press; Rosenblatt *et al.,* 1988).

HTLV-I and HTLV-II are ≈60% homologous at the genetic level (Seiki *et al.,* 1983; Shimotohno *et al.,* 1985). Together with a variety of related retroviruses detected in nonhuman primates, they share a common ancestor with bovine leukemia virus (BLV) with which they form a genus (Dube *et al.,* 1997). The strains of this genus found in nonhuman primate hosts are often referred to as simian T-cell lymphoma leukemia viruses (STLV). However, because of many incidences of cross-species transmission, the HTLV and STLV do not segregate phylogenetically according to host species of origin but rather according to geography. Hence, it is easier to refer to HTLV and STLV collectively as the primate T-cell lymphoma/leukemia viruses (PTLV) and discuss their phylogenetic subgroupings as PTLV-I, PTLV-II, etc. (Dube *et al.,* 1997).

There are two major subtypes of HTLV-II, A and B, which are about 94% homologous to each other (Dube *et al.,* 1993; Hall *et al.,* 1992). Most North Americans infected with HTLV-II, including intravenous drug users in whom the virus is endemic, are infected with subtype A, whereas most Paleo-Amerindians from Florida, Central America, and South America are infected with subtype B (Dube *et al.,* 1993; Ferrer *et al.,* 1996; Hall *et al.,* 1992; Leon-Ponte *et al.,* 1998; Neel *et al.,* 1994). Interestingly, the Indians of the Amazon river basin are infected with a variant of subtype A (Ishak *et al.,* 1995; Switzer *et al.,* 1996) and HTLV-II is endemic among some African pygmy groups (Goubau *et al.,* 1992).

Recently, we have shown that HTLV-II is endemic among some, but not all, Venezuelan Amerindians (Echeverria de Perez *et al.,* 1992, 1993; Leon-Ponte *et al.,* 1996, 1998). A particular isolate, HTLV-IIB G2, obtained from a Guahibo Indian, was found to be the most divergent HTLV-IIB strain identified to date (Leon-Ponte *et al.,* 1998). We decided to fully sequence this unique isolate in order to compare it phylogenetically to other members of its genus and to have a structural blueprint for correlation with observed *in vitro* and *in vivo* functional phenomena.

RESULTS

Cell line production

After 4 weeks of culture, peripheral blood mononuclear cells (PBMCs) from G2 developed the features of an HTLV-II infected cell line with syncitial formation characterized by large bi- and multinucleated cells and some "hairy" cytoplasmic projections (Popovic *et al.,* 1983). Cells proliferated rapidly with a doubling time of less than 24 h and were split each third to fourth day. HTLV p19 antigen was detected in the culture supernatants of the G2 cell line at week 4 (Fig. 1). HTLV antigens were

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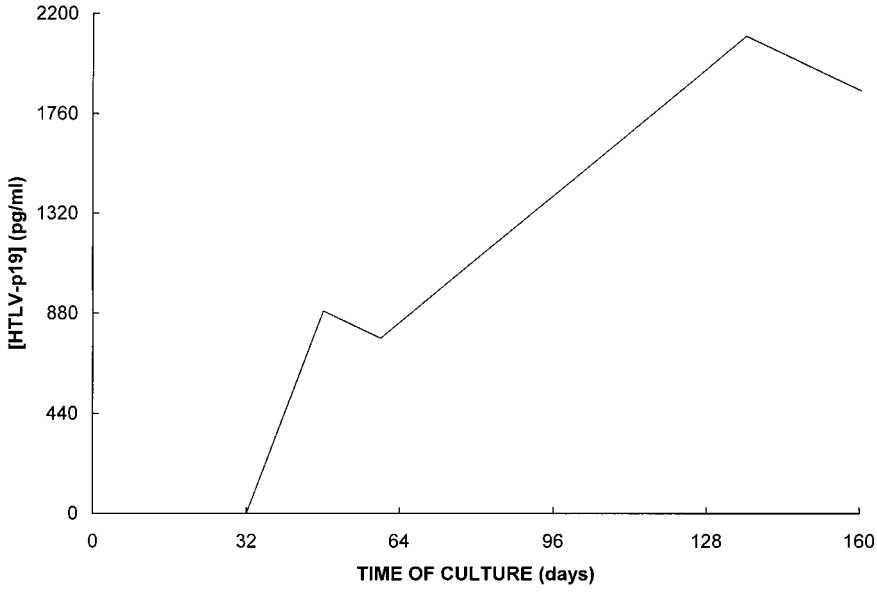

FIG. 1. HTLV p19 antigen levels in the conditioned medium of G2 PBMC continuously cultured over time.

detected in the G2 cultured cells by indirect immunofluorescence using polyvalent human anti-HTLV-II antibodies after 2 months in culture; 60 to 85% of the cells were fluorescent, showing the typical pattern of HTLV antigens when incubated with the HTLV-II-positive sera. Controls were as expected, with no fluorescence for H9 and strong positivity for both MT2 and clone 19. The cell line is still dependent on exogenous IL-2.

Cell line phenotypic and molecular analyses

After 3 months in culture, cell surface phenotype analyses showed that the G2 cell line was $CD3^+$, $CD4^-$, $CD8^+$, $CD11a^+$, $CD25^+$, and $CD45RO^+$ and also expressed high levels of HLA-DR antigen. This phenotypic analysis demonstrated that the cell line consisted of mature, activated, suppressor/cytotoxic T-cells of the killer effector type. Polymerase chain reaction (PCR) analysis indicated that at 3 months of culture the cell line was polyclonal with respect to T-cell receptor β-gene rearrangements (data not shown).

Viral nucleic acid studies

Serial endpoint dilutions indicate that there is at least one copy of HTLV-II per cell in the G2 culture. PCR amplification and Southern blot detection were successful for each of the primer pair/probe groups shown in Fig. 2. Some primer pair/probe systems not listed were not successful, usually because of G2-specific mutations (data not shown). PCR using the primer pair HTII 916-934^+/HTII 1375-1394^- in the 5′ end of the HTLV-II *gag* gene (Fig. 2) resulted in two distinct amplified products; both were sequenced (Fig. 3). The larger (minority) product represents the wild-type HTLV-II sequence, while the smaller (majority) product contains a 66-base deletion in the 5′ end of the HTLV-II G2 *gag* gene. We will refer to these deleted sequences as HTLV-II G2 del. The deletion would not be expected to produce a frameshift and would not alter the protease cleavage site involved in processing the Gag polyprotein. It would result in a smaller p19 gag protein. The complete sequence of HTLV-II G2 was obtained from these overlapping amplified products (GenBank Accession No. AF074965). Enough fresh PBMC DNA was available to validate that most of the HTLV-II sequences obtained from G2 cell line DNA were also present *in vivo;* unfortunately, none was left to ascertain whether HTLV-II G2 del was present *in vivo* or was an artifact of culture. However, when fresh PBMCs from three HTLV-II infected individuals (two subtype A and one subtype B) and cell culture DNA from G2 and the HTLV-IIA infected cell line MoT were amplified, all had both the wild-type and the deleted sequences (data not shown). Interestingly, in the two cell cultures, the deleted sequence was the dominant species present, while in the *in vivo* specimens, the opposite was the case (data not shown).

Comparative analyses of HTLV-II G2 indicate that it belongs to the HTLV-IIB subtype (Figs. 3, 4, and 5). It is 94.8, 98.4, and 62.6% homologous to the prototypical HTLV-IIA MoT, HTLV-IIB NRA, and HTLV-I ATK strains, respectively. Its LTR sequences (Fig. 4) are intact, containing the usual three 21-bp repeat enhancer sequences 5′ to the TATA box promoter typical of all HTLV. These regions interact with cellular CREB/ATF proteins complexed with the viral Tax protein to upregulate viral transcription. HTLV-II G2 genomic RNA would be predicted to have a typical 3′ poly(A) signal, a Rex response element, and a 5′ proline tRNA primer binding site. The

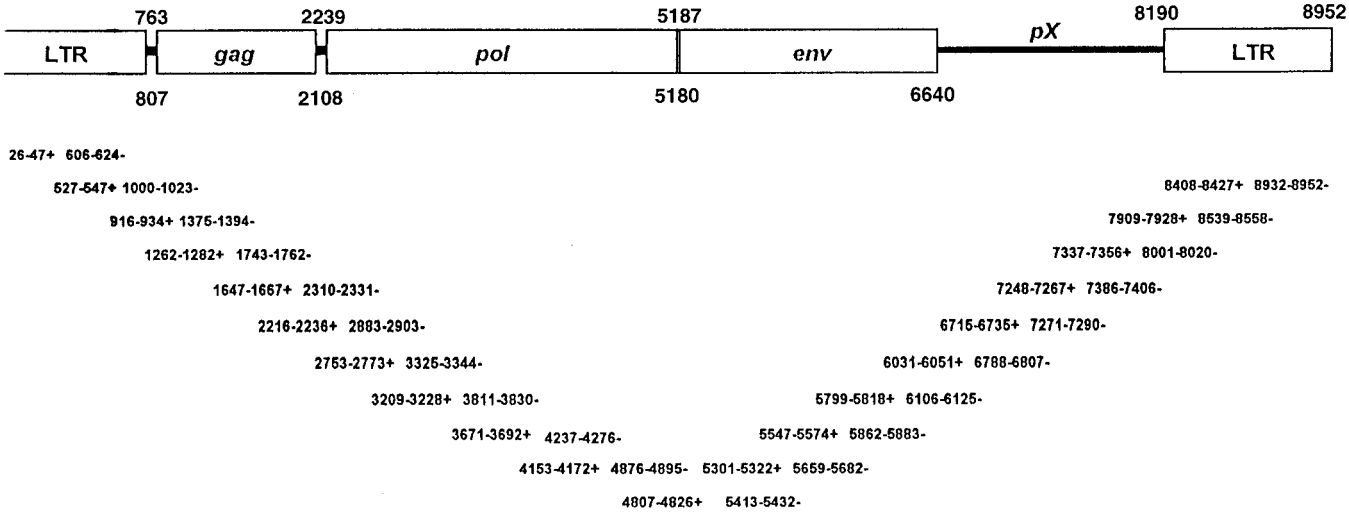

FIG. 2. Cartoon of HTLV-II proviral DNA showing its various functional and structural genes. The bases shown above and below are from the HTLV-IIA isolate MoT (Shimotohno *et al.,* 1985). The primers overlapping oligonucleotide pairs utilized for PCR are shown below the proviral DNA. Their inclusive base numbers are also derived from the HTLV-IIA isolate MoT. By convention, we refer to each primer as HTII for HTLV-II and then the list inclusive base numbers and the polarity of the DNA strand. For example, the first primer is HTII 26-47^{+}.

LTR proviral DNA contains 10 potential methylation sites (one more than found in prototype strains) 5′ to the TATA box promoter.

As stated above, the deduced amino sequences for the HTLV-II G2 p19 gag protein show both a wild-type and a mutant form, with the mutant being 22 amino acids shorter on its carboxy-terminus but still retaining its myristylation site (Fig. 3). Whether this mutant protein is produced *in vitro* or *in vivo* and whether it is functional are currently unknown. Of note, the HTLV-II G2 p19 protein is more divergent from that of the HTLV-IIB NRA strain than it is from the HTLV-IIA Mot strain (Fig. 3). It is 61.8% homologous to its HTLV-I counterpart (data not shown). The deduced p24 gag, p15 gag, and protease amino acids of HTLV-II G2 again are typical of an HTLV-IIB sequence and are 86.4, 69.8, and 53.9% homologous to those of HTLV-I (Fig. 3, and data not shown). Both the HTLV-I and the HTLV-II proteases contain aspartyl-specific recognition motifs. The cognate proteins of the *pol* gene of HTLV-II G2, namely those that encode for RNase H, reverse transcriptase, and integrase functions, show that they are typical of an HTLV-IIB strain and are smaller than and 60.6% homologous to those of HTLV-I (Fig. 3, and data not shown).

The amino acid sequences of the HTLV-II G2 gp46 and gp21 envelope proteins are also characteristic of an HTLV-IIB isolate with reasonable, but not complete, conservation of the immunodominant HTLV-II gp46-specific epitope GH2K15 (Fig. 3) utilized in HTLV-I/II discriminatory and confirmatory antibody detection assays and also complete conservation of the HTLV-II gp46 neutralizing domain (Fig. 3) (Lipka *et al.,* 1992; Sherman *et al.,* 1993). Also preserved are the GD21 and BA21 epitopes of the HTLV gp21 env transmembrane protein (Fig. 3). Seroreactivity against an HTLV-I GD21 peptide among infected humans and animals is highly sensitive and specific for infection with a member of the BLV/PTLV genus, whereas reactivity to an HTLV-I BA21 peptide is less frequent in truly infected humans and is often positive in uninfected individuals (Hadlock *et al.,* 1995; Loughran *et al.,* 1998). The HTLV-I GD21 peptide is now included in highly sensitive and specific Western blot assays for HTLV antibodies. The HTLV-II GD21 epitope is 85% homologous to its HTLV-I counterpart (data not shown). Taken together the HTLV-II G2 env proteins are 63.5% homologous to their HTLV-I counterparts.

Characterization of the deduced HTLV-II G2 regulatory proteins shows that the Tax transactivator protein is highly conserved and of the characteristic larger size of most HTLV-IIB, HTLV-I, and HTLV-IIA Kayapo strains relative to other HTLV-IIA strains (Fig. 3). HTLV-II G2 Tax is 80.0% homologous to that of HTLV-I (data not shown). Similarly, the Rex protein, which regulates viral RNA splicing, is also highly conserved among the HTLV-II strains (Fig. 3). HTLV-II G2 Rex is 62.7% homologous to the HTLV-I Rex (data not shown). Several other proteins, p10^{XI}, p28^{XII}, and p22^{XIII}/20^{XIII}, and p11^{XV}, encoded by the regulatory genes of the HTLV have been identified (Ciminale *et al.,* 1995). Their functions remain unknown, but there are only minor differences in G2 relative to other HTLV-II strains (Fig. 3). The HTLV-II p10^{XI}, p28^{XII}, and p22^{XIII}/p20^{XIII} peptides are 43.4, 77.6, and 60.0% homologous to their counterparts in HTLV-I (data not shown). There is no p11^{XV} equivalent expressed in HTLV-I.

Phylogenetic comparisons of the HTLV-II G2 *pol* DNA sequence to other published BLV and PTLV sequences indicate that G2 belongs to the HTLV-IIB subtype (Fig. 5). The data support the interpretation that the BLV and

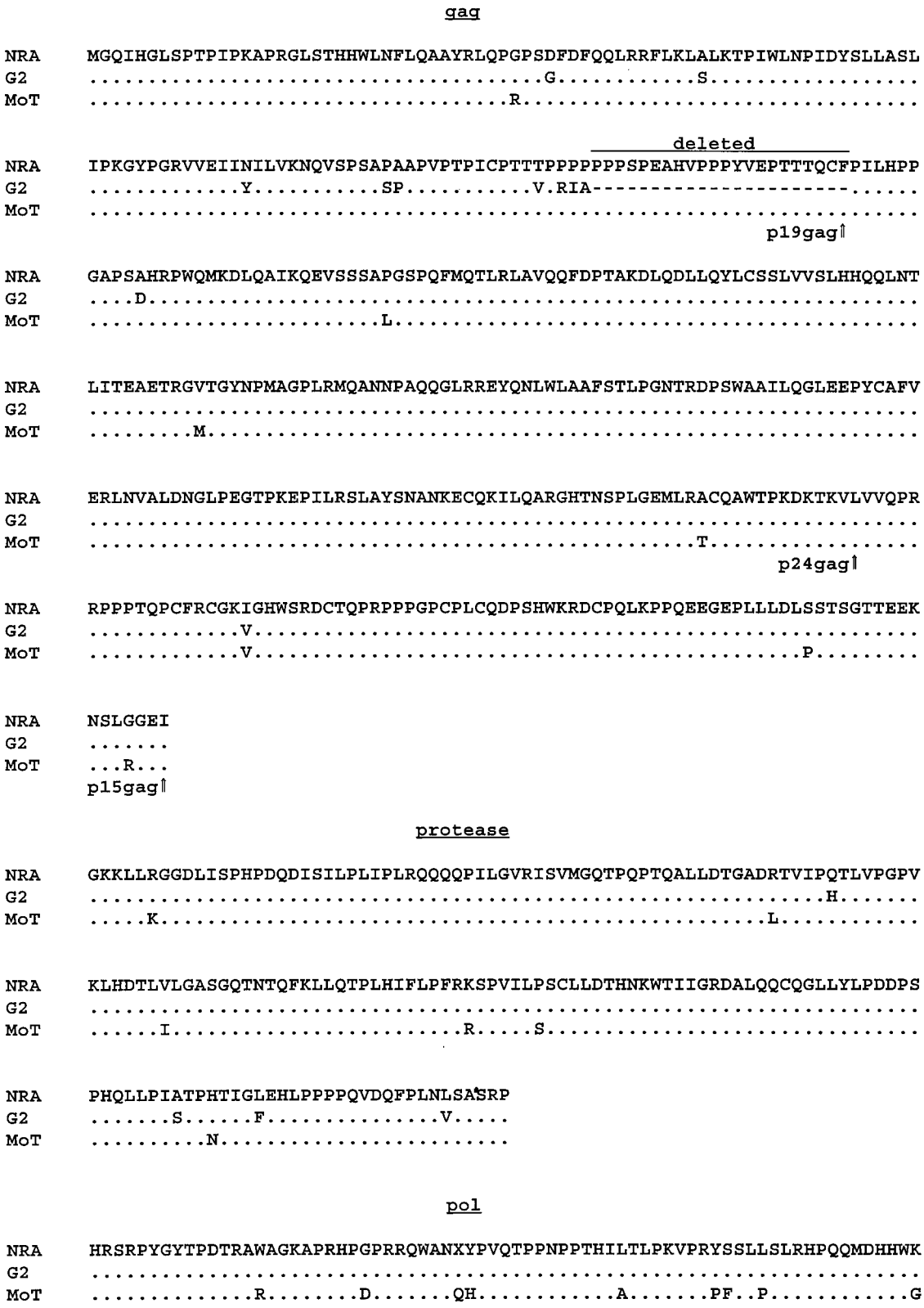

FIG. 3. Amino acid sequence of the various proteins of HTLV-II G2 compared to those of the prototypical HTLV-IIA isolate MoT and the prototypical HTLV-IIB isolate NRA. The bullet shows areas of homology while the amino acid substitutions are as indicated. The ends of the various proteins are indicated by the ↑ symbol. The wild-type p19 sequence is shown but the deleted region is indicated by a line above the sequence. The interesting immunoreactive regions of the gp46 and gp21 env proteins discussed in the text are also indicated.

```
NRA    RRPTTMPGASIPSRRSQPPSIAANSHSTHHRPRTPSPTAPGGPISFKPERLQALNDLVSKALEAGHIEPYS
G2     ..................P....I......L.......A.......R.......................
MoT    ..........P..P....P.......K.........S.S.............................

NRA    GPGNNPVFPVKKPNGKWRFIHDLRATNAIATTLTSPSPGPPDLTSLPTALPHLQTIDLTDAFFQIPLPKQF
G2     ............................................................................
MoT    ............................T.....................................Y

NRA    QPYFAFTIPQPCNYGPGTRYAWTVLPQGFKNSPTLFEQQLAAVLNPMRKMFPTSTIVQYMDDILLASPTNK
G2     ............................................................................
MoT    ............................................................................E

NRA    ELQQLSQLTLQALTTHGLPISQEKTQRTPGQIRFLGQVISPNHITYESTPAIPIKSQWTLTELQVILGEIQ
G2     ..............................................................RK..
MoT    ........................Q.....................T...................

NRA    WVSKGTPILRKHLQSLYSALHGYRDPRACITLTPQQLHALHAIQQALQHNCRGRLDPTLPLLGLISLSTSG
G2     ..........R.....................................................
MoT    ..........................................N.A.....................

NRA    TTSVIFQPKQNWPLAWLHTPHPPTSLCPWGHLLACTILTLDKYTLQHYGLLCQSFHHNMSKQALCDFLRNS
G2     ....................................P....F......Q..................
MoT    ................................................Q..................

NRA    PHPSVGILIHHMGRFHNLGSQPSGPWKTLLHLPTLLQEPRLLRPIFTLSPVVLDTAPCLFSDGSPQKAAYV
G2     ............................................................................
MoT    ............................................................................

NRA    LWDQTILQQDITPLPPHETNSAQKGELLALIYGLRAAKPWPSLNIFLDSKYLIKYLHSLAIGAFLGTSAHQ
G2     ................V.............C.....................................
MoT    ..............S...H...........C.....................................

NRA    TLQAALPPLLQGKTIYLHHVRSHTNLPDPISTFNEYTDSLIVAPLVPLTPQGLHGLTHCNQRALVSFGATP
G2     ............................................................................
MoT    ....................................L.....................................
                                      RNase/RT⇑

NRA    KEAKSLVQTCHTCQIINSQHHMPQGHIRRGLLPNHIWQGDVTHYKYKKYKYCLHVWVDTFSGAVSVSCKKK
G2     ............................................................................
MoT    R............T........R.X.....................................................

NRA    ETSCETISAFLQAISLLGKPLHINTDNGPAFLSQEFQEFCTSYHIKHSTHIPYNPTSSGLVERTNGIIKNL
G2     ............................................................................
MoT    .........V.................................R.....................V....
```

FIG. 3—*Continued*

PTLV evolved from a common ancestor. Shortly after the PTLV-I separated from the PTLV-II, a branch represented by PTLV-L (from an Eritrean baboon) (Van Brussel *et al.,* 1997) diverged from STLV-II (from an African pygmy chimp) (Digilio *et al.,* 1997) and from the branch we currently refer to as HTLV-II. Much later, the PTLV-I branched into African and Asian/Austronesian subgroups and the HTLV-II into the A and B subgroups.

DISCUSSION

HTLV-II is a member of a genus of pathogenic retroviruses that cause a variety of malignant and autoimmune diseases in cattle, humans, and nonhuman primates. To date, prototypic HTLV-II has been found only in humans, while the PTLV-I (HTLV-I and STLV-I) are found in both humans and nonhuman primates, with evidence

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NRA    LNKYLLDCPNLPLDNAINKALWTLNQLNVMNPSGKTRWQIHHSPPLPPIPEASTPPKPPSKWFYYKLPGLT
G2     .......................................................................
MoT    ...........................................................P...........

NRA    NQRWKGPLQSLQEAAGAALLSIDGFPRWIPWRFLKKAACPRPDASEPAEHAATDHQHHG
G2     ..............G............................................
MoT    ........................S.....................L............
                                                                IN⇑

                                   env

NRA    SRCTLTVGISSYHSSPCSPTQPVCTWNLDLNSLTTDQRLHPPCPNLITYSGFHKTYSLYLFPHWIKKPNRQ
G2     ......................................I................................
MoT    ......I................................................................

NRA    GLGYYSPSYNDPCSLQCPYLGCQSWTCPYTGPVSSPSWKFHSDVNFTQEVSQVSLRLHFSKCGSSMTLLVD
G2     .......................................................G.....G.........
MoT    .......................A..SA...........................................

                         GH2 k15
NRA    APGYDPLWFITSEPTQPPPTPPPLVHDSDLEHVLTPSTSWTTKMLKFIQLTLQSTNYSCMVCVDRSSLSSW
G2     ..............I.L...................P.........................M........
MoT    ....................S......................I...........................
                                ↑___↑
                         neutralizing domain

NRA    HVLYTPNISIPQQTSSRTILFPSLALPAPPFQPFFPWTHCYQPRLQAITTDDCNNSIILPPFSLAPVPPPAT
G2     ..........................................G.......N.....................
MoT    ..............................S...................N.....................

                                                                  GD21
NRA    RRRRAVPIAVWLVSALAAGTGIAGGVTGSLSLASSKSLLFEVDKDISHLTQAIVKNHQNILRVAQYAAQNR
G2     .......................................................................
MoT    .......................................L...............................
      gp46⇑

                                      BA21
             GD21
NRA    RGLDLLFWEQGGLCKAIQEQCCFLNISNTHVSVLQERPPLEKRVITGWGLNWDLGLSQWAREALQTGITIL
G2     .......................................................................
MoT    .......................................................................

NRA    TLLLLVILFGPCILRQIQALPQRLQNRHSQYALINQETML
G2     ............................N......P....
MoT    A...........................N..S...P....
                                         gp21⇑

                                   tax

NRA    MAHFPGFGQSLLYGYPVYVFGDCVQADWCPVSGGLCSTRLHRHALLATCPEHQLTWPDIDGRVVSSPLQYL
G2     .......................................................................
MoT    .......................................................................
```

FIG. 3—*Continued*

of multiple cross-species transmissions over the past thousands of years. All worldwide HTLV-II sequences described to date have high homology to those strains identified in the New World, including those from very isolated Paleo-Amerindian groups, like the HTLV-II G2 isolate described herein. This suggests that they represent a relatively recent dissemination from the Western Hemisphere. Presumably, the introduction of HTLV-II into

```
NRA   IPRLPSFPTQRASKTLKVLTPPTTPVSPKVPPAFFQSMRKHTPYRNGCLEPTLGDQLPSLAFPEPGLRPQN
G2    ............................................................................
MoT   ............................................................................

NRA   IYTTWGKTVVCLYLFQLSPPMTWPLIPHVIFCHPRQLGAFLTKVPLKRLEELLYKMFLHTGAVIVLPEDDL
G2    ............................................................................
MoT   ..............Y.............................................................

NRA   PTTMFQPVRAPCIQTAWCTGLLPYHSILTTPGLIWTFNDGSPMISGPCPKAGQPSLVVQSSLLIFEKFQTK
G2    ...........................................................K.....
MoT   ..................................................Y.....................E..

NRA   AFHPSYLLSHQLIQYSSFHNLHLLFDEYTNIPVSILFNKEEADDNGDQPPEPAAQGESSTQKVRPSHTNNPK
G2    ............................................................................
MoT   ..............................................-------------------------
```

rex

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NRA    MPKTRRQRTRRARRNRPPTPWPISQDSDRASYMDTPSTCLAIVYRPIGVPSQVVYVPPAYIDMPSWPPVQS
G2     ............................................................................
MoT    ........................L...................................................

NRA    TSSPGTPSMDALSALLSNTLSLASPPSPPREPPRPSRSLPLPPLLSPPRFHPPSSNQCESTPPIAMDAWNQ
G2     ...........................................................T........
MoT    .N.............................QG..................L..F.........TE......

NRA    PSGISSPPSPSLNLASVPKTSTPPGEKP
G2     ............................
MoT    ...........P................
```

p10[xI]

```
NRA    MPKTRRQRTRRARRNRPPTPWGLFFLFQEIHILKQITKPIKTQESYTLQLLMPLFPLPALLILFPQALLSA
G2     ..................................................F.........F.........
MoT    ......................D.L.P.........V............................L.........

NRA    PLPLLTLLQKL
G2     ...I........
MoT    ...L......F
```

p28[xII]

```
NRA   MFHPPTSTCPPGHLSRAPAHLGPHRWTRCQLSSPIPYPSPPLLPHPENRQDPQGPYPSHHSCLPQGSTRLLP
G2    ..............................................L.........................
MoT   ..................T.............................LK..................C..S
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FIG. 3—*Continued*

humans came from an ancient transmission of a virus with a common ancestor to both PTLV-L and STLV-II as the Paleo-Amerindians migrated into the New World. To these authors, the distance in separation between HTLV-II and PTLV-L is so great that it might make more taxonomic sense to refer to the PTLV-L strain as belonging to "PTLV-III." To date, no humans have been shown to be infected with a retrovirus with high homology to either the PTLV-L or the STLV-II strains. Obviously, this is an extremely interesting area for further research, as is the hypothesis that there await to be identified modern examples of side branches to the relatively uncomplicated BLV arm of the PTLV/BLV evolutionary tree (Fig. 5).

BLV and the PTLV-I are clearly pathogenic to their

```
NRA   INAKAHPLSQWMPGTNPRGSAPLPRLP*TWPPSPKHLHHLGKNRSVPVPIPAFPTHDLATYTPCHILPPKTI
G2    ........P.................*.........................................
MoT   ........PK.................R.................M.I.........M...........Q..

NRA   GSLPHQGASKTTRRTSIQNVPTHRSGHSPPGGRPTHHNVPARKGSLYPDCLVYRTSPLSLHPNNPRPNMDLQ
G2    ............................................................................
MoT   R..........I............DS..............T.E..................L....S.....

                                p22^XIII/20^XIII

NRA    MDTPSTCLAIVYRPIGVPSQVVYVPPAYIDMPSWPPVQSTSSPGTPSMDALSALLSNTLSLASPPSPPREP
G2     ............................................................................
MoT    ..........................................N.................................

NRA    PRPSRSLPLPPLLSPPRFHPPSSNQCESTPPIAMDAWNQPSGISSPPSPSLNLASVPKTSTPPGEKP
G2     ..............................T.....................................
MoT    QG.................L..F........TE.................P................

                                p11^xv

NRA    MGPFFPLPRNPHNPEANHKTHQNPGVLYTPTADASLPSPGAFDPFPAGAPFCAAPAPHAPAEALRSPAAPP
G2     ...................................F........L...S.......D..............
MoT    ...S....G.......S.........................P...G.............V........

NRA    PTASDESLAPAR
G2     .........H..
MoT    ..V.........
```

FIG. 3—*Continued*

hosts, causing disease in approximately 4% of infected individuals (Poiesz, 1995). Perinatal infection with HTLV-I is associated with a 10% incidence of $CD4^+$ leukemia/lymphoma over the hosts' lifetimes (Kondo *et al.,* 1985; Murphy *et al.,* 1989). Currently, it is not clear whether the STLV-II or PTLV-L strains are pathogenic in their respective hosts. HTLV-II is known to be associated with clonal expansions of human T-cells *in vivo,* with infection being skewed, but not restricted, to $CD8^+$ lymphocytes (Cimarelli *et al.,* 1996; Ijichi *et al.,* 1992; Lal *et al.,* 1995; Love *et al.,* 1998). Also, HTLV-II has been associated with rare T-cell malignancies, including large granular lymphocyte (LGL) leukemias, which, when typed, have usually been shown to be of the $CD8^+$ phenotype (Kalyanaraman *et al.,* 1982; Loughran *et al.,* 1992; Rosenblatt *et al.,* 1988; Zucker-Franklin *et al.,* 1992). In some but not all of these cases, HTLV-II proviral DNA has been shown to be monoclonally integrated into the host DNA (Kalyanaraman *et al.,* 1982; Rosenblatt *et al.,* 1988). The fresh PBMCs of donor G2 showed no evidence for $CD8^+$ lymphocytosis but were never evaluated for LGL lymphocytes or the presence of T-cell clonal expansion. The G2 cell line is a polyclonal $CD8^+$ culture; hence, it is impossible to speculate whether donor G2 had any evidence of leukemia.

As is the case with HTLV-I, defective copies of HTLV-II have been identified *in vivo,* but the clinical impact of this finding is unknown (Loughran *et al.,* 1998). The finding of a deleted HTLV-II p19 mutant in the G2 cell line is not unique. Others have also found the same deletion, but not the wild-type sequence, in two HTLV-IIB-positive cell lines (Takahashi *et al.,* 1993). Our data herein and the published HTLV-IIB NRA sequence indicate that HTLV-IIB strains can contain the full-length wild-type sequence. However, serology studies indicate that the linear epitope contained in the deleted 22 amino acids may be expressed in HTLV-IIA infected individuals but not in HTLV-IIB infected individuals (Takahashi *et al.,* 1993). Our data would suggest that the wild-type sequence may be the predominant species *in vivo,* with the deleted sequence dominating *in vitro.* Whether this is truly the case, and why this should be so, will require further investigation.

Current serologic assays for HTLV-I or HTLV-II infection are based primarily on HTLV-I antigen preparation. Only recently have HTLV-II antigens been included in the assays. HTLV-II G2 is only the fourth fully sequenced HTLV-II strain. As shown herein, this sequence further extends our knowledge of the breadth of HTLV-II amino acid diversity. Comparative PCR and serologic assays with defined HTLV peptides in well-characterized HTLV infected populations will be required to fully evaluate the

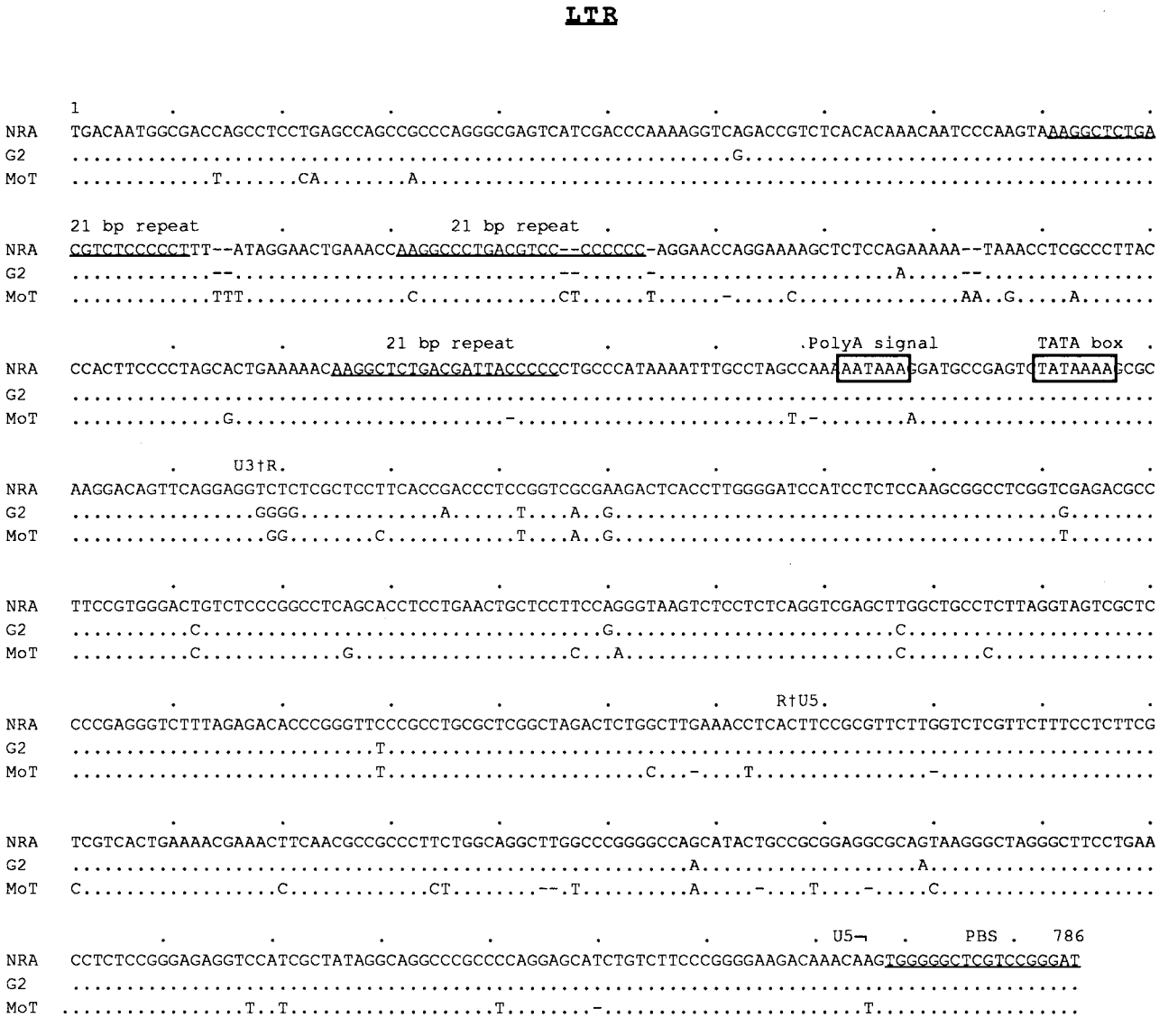

FIG. 4. The LTR DNA sequence of HTLV-II G2 compared to the prototypic HTLV-IIA (MoT) and HTLV-IIB (NRA) strains. Base substitutions are shown and the bullet symbol indicates identical bases, while the dashes indicate deletions. The first and last bases are numbered above the sequence and the boundaries of the U3, R, and U5 regions are shown. The three 21-bp repeat enhancer elements, the TATA box promoter, the poly(A) signal, and the tRNA primer binding sites (PBS) are shown.

relative sensitivities and specificities of various HTLV detection strategies.

The G2 cell line, the HTLV-II G2 isolate, and the Guahibo Indian group offer ideal opportunities to study the biology of HTLV-II in humans. G2 represents a rare cell line that should be an invaluable tool for understanding the impact of HTLV-II expression on the biology of $CD8^+$ T-lymphocytes. Subclones of the cell line, as well as infectious clones of the wild-type and mutant HTLV-II G2 strains, could be produced and examined for differences in their cellular and viral biology. Finally, continued clinical monitoring of the Guahibo and other Paleo-Amerindian groups should allow for further clarification of the pathogenesis of HTLV-II and evaluation of the performance of various HTLV detection techniques.

MATERIALS AND METHODS

Cell culture

In December 1995, fresh PBMCs were obtained from an HTLV-II seropositive, PCR-positive Venezuelan Guahibo Indian identified as G2 (Leon-Ponte *et al.,* 1998). Ten million cells were cryopreserved for further DNA extraction and sequencing analysis. At the time the sample was obtained, the donor had 5.9×10^3 white blood cells per microliter of peripheral blood with a differential of 36% lymphocytes, 10% monocytes, 48% neutrophils, 6% eosinophils, and 0% basophils. No assessment of LGL numbers was made at that time. Absolute lymphocyte subset counts were as follows: $CD3^+$ (1338/μl); $CD3^+$/$CD4^+$ (765/μl); and $CD3^+$/$CD8^+$ (510/μl). This last value

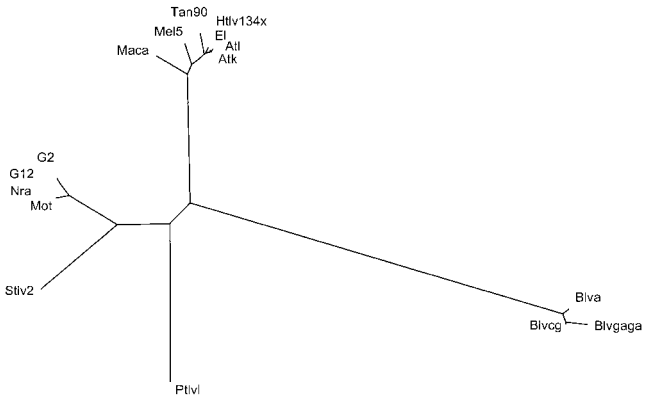

FIG. 5. A neighbor-joining tree showing the phylogenetic relationship between the *pol* DNA sequences of three BLV strains (Blvcg, Blva, and Blvgaga), seven PTLV-I strains (Maca from an Asian macaque, Mel5 from a Melanesian human, Tan 90 from an African tantalus monkey, HTLV 134 from a Caribbean human, El from a Congolese human, Atl from a Japanese human, and Atk from a Japanese human), and six PTLV-II strains [Ptlvl from an Eritrean baboon, Stlv2 from an African pygmy chimp, the HTLV-IIA strain MoT from an American human (lower branch), and the HTLV-IIB strains Nra from an American human, G12 from a Panamanian human, and G2 from a Venezuelan human (all on the upper branch)]. The bootstrap values for each branch are 100%.

was no different than the median $CD3^+/CD8^+$ count (504/μl) of non-HTLV-II infected Indians.

Five million PBMCs were placed in a 50-ml tissue culture flask (Falcon, Becton Dickinson and Co., Franklin Lakes, NJ) containing 5 ml of RPMI 1640 (Gibco BRL Inc., Gaithersburg, MD) supplemented with 20% heat-inactivated fetal bovine serum (FBS) (Gibco BRL) and 100 U/ml penicillin and streptomycin (Gibco BRL). Cells were stimulated with 5 μg/ml phytohemagglutinin (Wellcome Beckenham, UK) for 3 days. The cells were then cultivated in RPMI 1640 containing 15% FBS, 100 U/ml penicillin and streptomycin, and 50 U/ml recombinant human interleukin 2 (rhIL-2), which was kindly donated by Dr. Craig Reynolds of the Biological Response Modifiers Program of the National Cancer Institute (Frederick, MD). The medium was changed each third or fourth day, and the cell density was maintained below 1×10^6/ml.

HTLV antigen production

HTLV virus production in the culture supernatants was determined by using a p19 antigen capture assay (Cellular Products Inc., Buffalo, NY) according to the manufacturer's instructions (Papsidero *et al.,* 1990). Indirect immunofluorescence was also used to detect viral antigens in cultured cells, as previously described (Gallo *et al.,* 1991). Briefly, 5 μl of a cell suspension (3×10^6 cells/ml) was dried at room temperature on each well of 10-well HTC blue slides (Celline Associates Inc., Newfield, NJ). The slides were placed in acetone for 10 min. Fixed cells were incubated at 37°C for 20 min with a strong HTLV-II-positive serum diluted 1:10 in PBS. After the cells were washed with PBS, bound antibody was detected by incubation at 37°C for 20 min with goat anti-human immunoglobulin (FAB fraction) conjugated fluorescein isothiocyanate (Cappel Research Products, Durham, NC) and fluorescence was scored in a Zeiss microscope. Controls included HTLV-I (MT2) and HTLV-II (clone 19) infected cell lines, as well as an uninfected T-cell culture (H9).

Flow cytometry

The two-color flow cytometry analysis of cultured cells was performed in a Epics Profile II (Coulter Electronics, Miami, FL) flow cytometer. Cultured cells were treated with fluorochrome conjugated murine monoclonal antibodies to the CD3, CD4, CD8, CD11a, CD45RA, CD45RO, CD25, and HLA DR antigens (Coulter MaAb, Hialeah, FL, and Becton Dickinson MaAb, San Jose, CA).

Nucleic acid studies

DNA was organically extracted from fresh or cultured G2 cells and amplified via PCR, as previously described (Dube *et al.,* 1993). One microgram of each DNA sample was amplified with one of the overlapping HTLV-II primer sets shown in Fig. 2. The amplified products were detected by Southern blot hybridization using ^{32}P-labeled oligonucleotide probes located between the flanking primers. Amplified specific products were cloned into a TA cloning vector (Invitrogen, San Diego, CA) and sequenced using an automated sequencer (Applied Biosystems, Foster City, CA). Several clones were sequenced for each primer pair and sequences were obtained for both strands of DNA. The overlapping sequences were used to obtain the G2-specific regions homologous to the primers and to double-check the accuracy of sequences. Sequences were aligned (Needleman and Wunsch, 1970) and the neighbor-joining method utilizing the maximum likelihood technique to determine distance matrices (Felsenstein, 1989) was used to compare new and previously published retroviral sequences. Both distance and bootstrap (100 replications) trees were generated. The G2 cell line was analyzed for T-cell β-gene receptor rearrangements using a PCR assay as previously described (Love *et al.,* 1998).

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REFERENCES

Bhagavati, S., Ehrlich, G., Kula, R. W., Kwok, S., Sninsky, J., Udani, V., and Poiesz, B. J. (1988). Detection of human T-cell lymphoma/leukemia virus type I DNA and antigen in spinal fluid and blood of patients with chronic progressive myelopathy. *N. Engl. J. Med.* **318**(18), 1141–1147.

Cimarelli, A., Duclos, C. A., Gessain, A., Casoli, C., and Bertazzoni, U.

(1996). Clonal expansion of human T-cell leukemia virus type II in patients with high proviral load. *Virology* **223**(2), 362–364.

Ciminale, V., D'Agostino, D. M., Zotti, L., Franchini, G., Felber, B. K., and Chieco-Bianchi, L. (1995). Expression and characterization of proteins produced by mRNAs spliced into the X region of the human T-cell leukemia/lymphotropic virus type II. *Virology* **209**(2), 445–456.

Digilio, L., Giri, A., Cho, N., Slattery, J., Markham, P., and Franchini, G. (1997). The simian T-lymphotropic/leukemia virus from *Pan paniscus* belongs to the type 2 family and infects Asian macaques. *J. Virol.* **71**(5), 3684–3692.

Dube, D. K., Sherman, M. P., Saksena, N. K., Bryz-Gornia, V., Mendelson, J., Love, J., Arnold, C. B., Spicer, T., Dube, S., Glaser, J. B., *et al.* (1993). Genetic heterogeneity in human T-cell leukemia/lymphoma virus type II. *J. Virol.* **67**(3), 1175–1184.

Dube, S., Bachman, S., Spicer, T., Love, J., Choi, D., Esteban, E., Ferrer, J. F., and Poiesz, B. J. (1997). Degenerate and specific PCR assays for the detection of bovine leukaemia virus and primate T cell leukaemia/lymphoma virus pol DNA and RNA: Phylogenetic comparisons of amplified sequences from cattle and primates from around the world. *J. Gen. Virol.* **78**(Pt 6), 1389–1398.

Echeverria de Perez, G., Leon-Ponte, M., Noya, O., Botto, C., Gallo, D., and Bianco, N. (1993). First description of endemic HTLV-II infection among Venezuelan Amerindians. *J. Acquired Immune Defic. Syndr.* **6**(12), 1368–1372.

Echeverria de Perez, G., Loreto, O., Bianco, N. E., Mendez-Castellanos, H., Burczak, J. D., and Lee, H. (1992). Reappraisal of human retroviral infection in Venezuela. *AIDS Res. Hum. Retroviruses* **8**(2), 219–220. [Letter].

Felsenstein, J. (1989). PHYLIP—Phylogeny inference package. *Cladistics* **5,** 164–166.

Ferrer, J. F., Esteban, E., Dube, S., Basombrio, M. A., Segovia, A., Peralta-Ramos, M., Dube, D. K., Sayre, K., Aguayo, N., Hengst, J., and Poiesz, B. J. (1996). Endemic infection with human T cell leukemia/lymphoma virus type IIB in Argentinean and Paraguayan Indians: Epidemiology and molecular characterization. *J. Infect. Dis.* **174**(5), 944–953.

Gallo, D., Penning, L. M., and Hanson, C. V. (1991). Detection and differentiation of antibodies to human T-cell lymphotropic virus types I and II by the immunofluorescence method. *J. Clin. Microbiol.* **29**(10), 2345–2347.

Goubau, P., Desmyter, J., Ghesquiere, J., and Kasereka, B. (1992). HTLV-II among pygmies. *Nature* **359**(6392), 201. [Letter]

Hadlock, K. G., Goh, C. J., Bradshaw, P. A., Perkins, S., Lo, J., Kaplan, J. E., Khabbaz, R., and Foung, S. K. (1995). Delineation of an immunodominant and human T-cell lymphotropic virus (HTLV)-specific epitope within the HTLV-I transmembrane glycoprotein. *Blood* **86**(4), 1392–1399.

Hall, W. W., Takahashi, H., Liu, C., Kaplan, M. H., Scheewind, O., Ijichi, S., Nagashima, K., and Gallo, R. C. (1992). Multiple isolates and characteristics of human T-cell leukemia virus type II. *J. Virol.* **66**(4), 2456–2463.

Harrington, W. J., Jr., Sheremata, W., Hjelle, B., Dube, D. K., Bradshaw, P., Foung, S. K., Snodgrass, S., Toedter, G., Cabral, L., and Poiesz, B. (1993). Spastic ataxia associated with human T-cell lymphotropic virus type II infection. *Ann. Neurol.* **33**(4), 411–414.

Hjelle, B., Appenzeller, O., Mills, R., Alexander, S., Torrez-Martinez, N., Jahnke, R., and Ross, G. (1992). Chronic neurodegenerative disease associated with HTLV-II infection. *Lancet* **339**(8794), 645–646.

Ijichi, S., Ramundo, M. B., Takahashi, H., and Hall, W. W. (1992). In vivo cellular tropism of human T cell leukemia virus type II (HTLV-II). *J. Exp. Med.* **176**(1), 293–296.

Ishak, R., Harrington, W. J., Jr., Azevedo, V. N., Eiraku, N., Ishak, M. O., Guerreiro, J. F., Santos, S. B., Kubo, T., Monken, C., Alexander, S., *et al.* (1995). Identification of human T cell lymphotropic virus type IIa infection in the Kayapo, an indigenous population of Brazil. *AIDS Res. Hum. Retroviruses* **11**(7), 813–821.

Kalyanaraman, V. S., Sarngadharan, M. G., Robert-Guroff, M., Miyoshi, I., Golde, D., and Gallo, R. C. (1982). A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. *Science* **218**(4572), 571–573.

Kondo, T., Nonaka, H., Miyamoto, N., Yoshida, R., Matsue, Y., Ohguchi, Y., Inouye, H., Komoda, H., Hinuma, Y., and Hanaoka, M. (1985). Incidence of adult T-cell leukemia-lymphoma and its familial clustering. *Int. J. Cancer* **35**(6), 749–751.

Lal, R. B., Owen, S. M., Rudolph, D. L., Dawson, C., and Prince, H. (1995). In vivo cellular tropism of human T-lymphotropic virus type II is not restricted to $CD8^+$ cells. *Virology* **210**(2), 441–447.

Leon-Ponte, M., Echeverria de Perez, G., Bianco, N., Hengst, J., Dube, S., Love, J., and Poiesz, B. J. (1998). Endemic infection with HTLV-IIB in Venezuelan Indians: Molecular characterization. *J. Acquired Immune Defic. Syndr. Hum. Retroviral* **17**(5), 458–464.

Leon-Ponte, M., Noya, O., Bianco, N., and Echeverria de Perez, G. (1996). Highly endemic human T-lymphotropic virus type II (HTLV-II) infection in a Venezuelan Guahibo Amerindian group. *J. Acquired Immune Defic. Syndr. Hum. Retrovirol* **13**(3), 281–286.

Lipka, J. J., Miyoshi, I., Hadlock, K. G., Reyes, G. R., Chow, T. P., Blattner, W. A., Shaw, G. M., Hanson, C. V., Gallo, D., Chan, L., *et al.* (1992). Segregation of human T cell lymphotropic virus type I and II infections by antibody reactivity to unique viral epitopes. *J. Infect. Dis.* **165**(2), 268–272.

Loughran, T. P., Jr., Coyle, T., Sherman, M. P., Starkebaum, G., Ehrlich, G. D., Ruscetti, F. W., and Poiesz, B. J. (1992). Detection of human T-cell leukemia/lymphoma virus, type II, in a patient with large granular lymphocyte leukemia. *Blood* **80**(5), 1116–1119.

Loughran, T. P. J., Hadlock, K. G., Perzova, R., Gentile, T., Yang, Q., Foung, S. K. H., and Poiesz, B. J. (1998). Epitope mapping of HTLV envelope seroreactivity in LGL leukemia. *Br. J. Hematol.* **101,** 318–324.

Love, J. L., Marchioli, C. C., Dube, S., Bryz-Gornia, V., Loughran, T. P. J., Glaser, J. B., Esteban, E., Feldman, L., Ferrer, J. F., and Poiesz, B. J. (1998). Expansion of clonotypic T-cell populations in the peripheral blood of asymptomatic Gran Chaco Amerindians infected with HTLV-IIB. *J. AIDS Hum. Retrovirol.* **18,** 178–185.

Lowis, G. W., Sheremata, W. A., Wickman, P. R., Dube, S., Dube, D., and Poiesz, B. J. (in press). HTLV-II risk factors in Native Americans in Florida. *Neuroepidemiology.*

Mochizuki, M., Watanabe, T., Yamaguchi, K., Tajima, K., Yoshimura, K., Nakashima, S., Shirao, M., Araki, S., Miyata, N., Mori, S., *et al.* (1992). Uveitis associated with human T lymphotropic virus type I: Seroepidemiologic, clinical, and virologic studies. *J. Infect. Dis.* **166**(4), 943–944. [Letter]

Murphy, E. L., Hanchard, B., Figueroa, J. P., Gibbs, W. N., Lofters, W. S., Campbell, M., Goedert, J. J., and Blattner, W. A. (1989). Modelling the risk of adult T-cell leukemia/lymphoma in persons infected with human T-lymphotropic virus type I. *Int. J. Cancer* **43**(2), 250–253.

Needleman, S. B., and Wunsch, C. D. (1970). A general method applicable to the search for similarities in the amino acid sequence of two proteins. *J. Mol. Biol.* **48**(3), 443–453.

Neel, J. V., Biggar, R. J., and Sukernik, R. I. (1994). Virologic and genetic studies relate Amerind origins to the indigenous people of the Mongolia/Manchuria/southeastern Siberia region. *Proc. Natl. Acad. Sci. USA* **91**(22), 10737–10741.

Papsidero, L., Swartzwelder, F., Sheu, M., Montagna, R., Ehrlich, G., Bhagavati, S., Dosik, H., Sninsky, J., and Poiesz, B. (1990). Immunodetection of human T-cell lymphotropic virus type I core protein in biological samples by using a monoclonal antibody immunoassay. *J. Clin. Microbiol.* **28**(5), 949–955.

Poiesz, B. J. (1995). Etiology of acute leukemia: Molecular genetics and viral oncology. *In* "Neoplastic Diseases of the Blood" (P. H. Wiernik, G. P., Canellos, J. P. Dutcher, and R. A. Kyle, Eds.), 3rd ed., pp. 159–175. Churchill Livingstone, New York.

Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D., and Gallo, R. C. (1980). Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with

cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. USA* **77**(12), 7415–7419.

Popovic, M., Sarin, P. S., Robert-Gurroff, M., Kalyanaraman, V. S., Mann, D., Minowada, J., and Gallo, R. C. (1983). Isolation and transmission of human retrovirus (human T-cell leukemia virus). *Science* **219**(4586), 856–859.

Rosenblatt, J. D., Giorgi, J. V., Golde, D. W., Ezra, J. B., Wu, A., Winberg, C. D., Glaspy, J., Wachsman, W., and Chen, I. S. (1988). Integrated human T-cell leukemia virus II genome in $CD8^+$ T cells from a patient with "atypical" hairy cell leukemia: Evidence for distinct T and B cell lymphoproliferative disorders. *Blood* **71**(2), 363–369.

Seiki, M., Hattori, S., Hirayama, Y., and Yoshida, M. (1983). Human adult T-cell leukemia virus: Complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. *Proc. Natl. Acad. Sci. USA* **80**(12), 3618–3622.

Sherman, M. P., Amin, R. M., Rodgers-Johnson, P. E., Morgan, O. S., Char, G., Mora, C. A., Iannone, R., Collins, G. H., Papsidero, L., Gibbs, C. J., Jr., *et al.* (1995). Identification of human T cell leukemia/lymphoma virus type I antibodies, DNA, and protein in patients with polymyositis. *Arthritis Rheum.* **38**(5), 690–698.

Sherman, M. P., Dube, S., Spicer, T. P., Kane, T. D., Love, J. L., Saksena, N. K., Iannone, R., Gibbs, C. J., Jr., Yanagihara, R., Dube, D. K., *et al.* (1993). Sequence analysis of an immunogenic and neutralizing domain of the human T-cell lymphoma/leukemia virus type I gp46 surface membrane protein among various primate T-cell lymphoma/leukemia virus isolates including those from a patient with both HTLV-I-associated myelopathy and adult T-cell leukemia. *Cancer Res.* **53**(24), 6067–6073.

Shimotohno, K., Takahashi, Y., Shimizu, N., Gojobori, T., Golde, D. W., Chen, I. S., Miwa, M., and Sugimura, T. (1985). Complete nucleotide sequence of an infectious clone of human T-cell leukemia virus type II: An open reading frame for the protease gene. *Proc. Natl. Acad. Sci. USA* **82**(10), 3101–3105.

Switzer, W. M., Black, F. L., Pieniazek, D., Biggar, R. J., Lal, R. B., and Heneine, W. (1996). Endemicity and phylogeny of the human T cell lymphotropic virus type II subtype A from the Kayapo Indians of Brazil: Evidence for limited regional dissemination. *AIDS Res. Hum. Retroviruses* **12**(7), 635–640.

Takahashi, H., Zhu, S. W., Ijichi, S., Vahlne, A., Suzuki, H., and Hall, W. W. (1993). Nucleotide sequence analysis of human T cell leukemia virus, type II (HTLV-II) isolates. *AIDS Res. Hum. Retroviruses* **9**(8), 721–732.

Van Brussel, M., Goubau, P., Rousseau, R., Desmyter, J., and Vandamme, A. M. (1997). Complete nucleotide sequence of the new simian T-lymphotropic virus, STLV-PH969 from a Hamadryas baboon, and unusual features of its long terminal repeat. *J. Virol.* **71**(7), 5464–5472.

Zucker-Franklin, D., Hooper, W. C., and Evatt, B. L. (1992). Human lymphotropic retroviruses associated with mycosis fungoides: Evidence that human T-cell lymphotropic virus type II (HTLV-II) as well as HTLV-I may play a role in the disease. *Blood* **80**(6), 1537–1545.