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Identification of Human T Cell Leukemia Virus Type IIb Infection in the Wayu, an Aboriginal Population of Colombia

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Human T cell leukemia virus type II (HTLV-II) is endemic in a number of native American populations and high rates of infection have also been demonstrated in intravenous drug abusers (IVDAs). Studies of virus isolates in the latter population have shown the existence of two closely related subtypes of the virus, HTLV-IIa and HTLV-IIb. To characterize the viruses present in native Americans, we analyzed by nucleotide sequence analysis the proviruses from the Wayu, an aboriginal population residing in Colombia, South America. The results showed HTLV-IIb infection in this population, and also demonstrated remarkable conservation of sequence when compared to the proviruses in IVDAs.

Key words: HTLV-IIb — Wayu people — Aborigines (Colombia)

The human T cell leukemia viruses, type I (HTLV-I) and type II (HTLV-II) are members of a group of retroviruses having similar biological properties and a tropism for T lymphocytes.[1,2] HTLV-II infection has been shown to be endemic in several native American populations. These include the Navajo and Pueblo Indians in New Mexico, the Seminole in Florida, the Guaymi in Panama, the Wayu who reside on the Caribbean coast of Colombia, and several Brazilian tribes including the Cayapo, the Kraho, and the Paragaminos.[2–4] Importantly, the Cayapo and Kraho have until recently remained relatively isolated, and have had little or no outside contact, even with other native populations. This suggests that HTLV-II infection is old and probably existed either in its present form(s) or possibly evolved from a proto-HTLV during the early migrations of these populations.[3]

Outside of these endemic foci, high rates of HTLV-II infection have also been documented in certain intravenous drug abusers (IVDAs) in urban areas of North America.[5] In our preliminary studies using restriction endonuclease mapping and nucleotide sequence analysis on HTLV-II isolates from IVDAs in New York City, it was clearly demonstrated that there are two closely related, but distinct molecular subtypes of this virus, which we have designated HTLV-IIa and HTLV-IIb.[2,6,7] The finding of two molecular subtypes of HTLV-II has provided a foundation for characterizing the viruses present in native American populations and may eventually help to establish the origin of the viruses present in the IVDA populations. In terms of the former, preliminary nucleotide sequence studies of the *env* gene region encoding the gp21 transmembrane protein have demonstrated the existence of the type IIb molecular subtype in Seminole Indians of Florida[2] and the Pueblo of New Mexico.[8] Importantly, these studies did not identify any new or additional subtypes or variants thereof in these populations.

To characterize the virus subtype in a Southern American population, we have now analyzed the proviruses present in the Wayu, an aboriginal population of Colombia. Two DNA samples (WY018 and WY100) obtained from two individuals, were subjected to nucleotide sequence analysis. The *env* gene region encoding the entire surface glycoprotein, gp46, and the amino terminus of the transmembrane glycoprotein, gp21, (bp 5121–6185) was amplified using a nested polymerase chain reaction (PCR) as previously described.[6,7] In brief, a hot start PCR was carried out using two primers; sense (5′CCCTACAATCCAACCAGCTCAG3′) and antisense (5′GAGATAGGGAGCCTGTTACT3′). After an initial denaturation for 60 s, 30 cycles were carried out in a 50 μl reaction volume. Each cycle consisted of denaturation for 45 s at 94°C, annealing for 30 s at 54°C, and extension for 90 s at 72°C. To ensure complete synthesis, the last cycle at 72°C was extended to 10 min. After amplification, a 2 μl aliquot was amplified using a second sense

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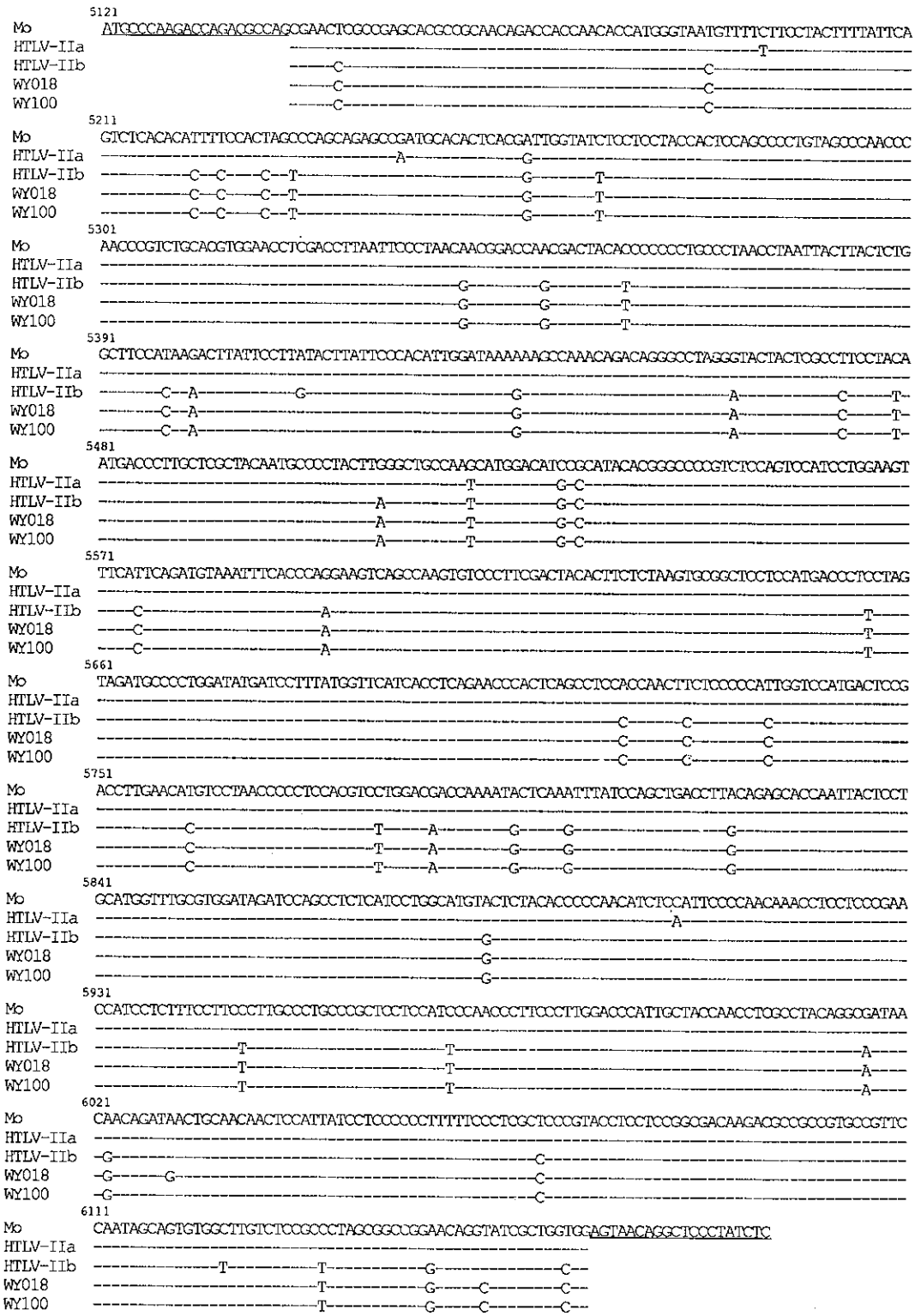

Fig. 1. Nucleotide sequence of the *env* gene region (bp 5121–6185) encoding the entire envelope glycoprotein, gp46 and the amino terminus of the transmembrane glycoprotein gp21. Sequences selected as prototypes of the two molecular subtypes (HTLV-IIa and HTLV-IIb) from two IVDAs from New York City and the sequences found for two Wayu aborigines (WY018 and WY100) are compared to that reported for HTLV-II-Mo.[12] Primers employed in PCR amplification are underlined.

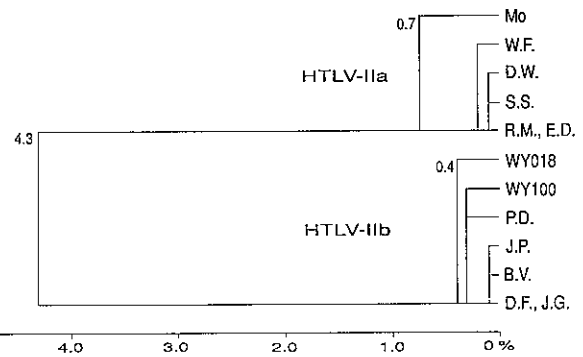

Fig. 2. Dendrogram comparing the nucleotide sequences of 986 bases of the *env* gene region (bp 5180–6165) of the two HTLV-II isolates from the Wayu (WY018 and WY100) and the sequence reported for HTLV-II-Mo. Sequences were aligned to maximize homology, and isolates E.D. and J.G. from two IVDAs as noted in the legend to Fig. 1 were selected as prototypes of the HTLV-IIa and HTLV-IIb subtypes, respectively.

primer (5′ATGCCCAAGACCAGACGCCAG3′) and the same antisense primer noted above in a 100 μl reaction volume. Thirty-five cycles were employed and conditions were as above. Amplified products were electrophoresed on low-melting temperature agarose gels. After ethidium bromide staining, DNA bands were excised, and the agarose was melted by heating at 70°C for 10 min. DNAs were then directly ligated to plasmids (pBluescript II SK, Stratagene) modified as T-vectors[9] and used to transfect *Escherichia coli* (INVαF′, Invitrogen) using transformation and storage solution (TSS).[10] Positive colonies were selected, and grown in LB media. DNA sequencing was carried out on purified plasmids using the dideoxy chain termination method (Sequenase, United States Biochem. Corp.) employing standard M13 primers and an internal primer (5′CGCAAACCATGCAGGAGT3′).

The sequence of the 1064 nucleotides amplified from the *env* gene and including the PCR primers is shown in Fig. 1. This and the dendrogram shown in Fig. 2 revealed that the proviruses had a typical HTLV-IIb sequence. Comparison with HTLV-IIb prototype sequences from IVDAs,[7] demonstrated only 3 and 4 nucleotide differences, respectively, in the two samples. The conservation of nucleotide sequence in this region between these two unrelated populations is quite remarkable, and suggests a marked stability in the virus genome over time. The finding of the HTLV-IIb subtype in two Wayu aborigines does not allow one to conclude that this is the predominant or perhaps the sole subtype in this population. Additional studies with a larger population will be required to establish this.

The Wayu are somewhat unusual in that this population is infected with both HTLV-I and HTLV-II.[11] They are not an isolated population, and have freely interacted with individuals of different ethnic backgrounds in this region of Colombia. It remains unclear how and when these viruses may have been introduced. It is possible that HTLV-I may have originated through interactions with individuals of African ancestry and this may have occurred in relatively recent times. The origin of HTLV-II, and IIb in particular, remains unclear. It has been proposed that HTLV-II has a New World origin,[3] and if this proves to be correct, then the virus may have existed in this population for an extremely long period of time. Alternatively it may have been introduced through recent interactions with other American native populations, or perhaps even through contact either sexually, or via contaminated blood products from non-native groups who are at increased risk of infection, such as IVDAs.

The finding of HTLV-IIb in the Wayu supports the view that either this virus is indigenous to the New World, or alternatively that it may well have been introduced to the New World during the early migrations of the ancestors of currently existing Indian populations. Detailed nucleotide sequence analysis of the viruses in a large number of Indian populations and correlation of the data with the early migrations and interactions of these populations, insofar as they are known, may provide important clues to the origin of HTLV-II.

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