Endemic Human T Cell Leukemia Virus Type II Infection in Southwestern US Indians Involves Two Prototype Variants of Virus

Brian Hjelle, Shi Wei Zhu, Hidehiro Takahashi, Shinji Ijichi, and William W. Hall

Department of Pathology, University of New Mexico School of Medicine, and United Blood Services, Albuquerque, New Mexico; Department of Medicine, Northshore University Hospital, Cornell Medical College, Manhasset, New York

Human T cell leukemia virus (HTLV) type II is endemic in certain American Indians, and high rates of infection occur in intravenous drug users (IVDUs). North American IVDUs are infected with two distinct variants, HTLV-IIa and -IIb. If IVDUs became infected as a result of interaction with members of an American Indian population, both viral forms should be demonstrable in such populations. Nucleotide sequence analysis of 630 bases of the *env* gene encoding the gp21 protein was done on DNA from 12 New Mexico Indians (8 Pueblo, 4 Navajo). All samples were typical subtype a or b viruses. Seven of the 8 Pueblo and 2 of 4 Navajo had subtype b; the rest had subtype a. The results are compatible with an indigenous New World origin for both subtypes of HTLV-II.

Human T cell leukemia/lymphoma virus (HTLV) types I and II are closely related human retroviruses. HTLV-I is associated with adult T cell leukemia/lymphoma and tropical spastic paraparesis/HTLV-I–associated myelopathy (TSP/HAM). HTLV-I infection is endemic in several regions of the world, where transmission occurs both horizontally (sexually) and vertically (breastfeeding, reviewed in [1, 2]). Newer practices, such as blood transfusion and intravenous drug use, have resulted in its introduction into new populations [1, 2]. In Japan alone, there are an estimated 800,000 persons infected with HTLV-I [3].

HTLV-II was originally isolated (and designated MO and NRA) from T cells of 2 patients with hairy cell leukemia (HCL) [1, 2]. Despite the initial suspicion that HTLV-II may have a role in HCL, most patients with HCL lack antibodies to an HTLV. HTLV-II infection appears to have spread widely in IVDUs in the United States and Europe. The first introduction of HTLV-II into the IVDU population seems to have occurred before the mid-1970s [4].

Virus isolates of HTLV-I obtained from the traditional HTLV-I–endemic regions of Japan and the Caribbean are closely related in sequence [5]. In contrast, HTLV-I isolates obtained from recently discovered remote populations in Papua New Guinea differ from the cosmopolitan (widespread) HTLV-I strains by ~7%–8% of the nucleotide sequence, suggesting ancient divergence between cosmopolitan HTLV-I and the new isolates [6].

Recent studies with HTLV-II isolates from North American IVDUs have suggested that there are at least two distinct subtypes of HTLV-II, designated a and b [7]. Since both types exist in this population, both can be considered to be cosmopolitan. In the portion of the *env* gene encoding the transmembrane protein gp21, HTLV-IIb differs from HTLV-IIa by 4% of nucleotide or 1.5% of amino acid residues. Divergence of 3% and 2% is observed in the *tax* (transactivator) gene and protein [8], and the two subtypes are easily distinguished by sequences in the long terminal repeat, *pol*, and *gag* regions (unpublished data). That HTLV-IIa and -IIb are truly distinct is supported by the consistent bimodal distribution of sequence across multiple viral genes and by the lack of intermediate forms between subtypes a and b. The MO and NRA prototype viruses of HTLV-II are examples of HTLV-IIa and -IIb, respectively [7].

The existence of two distinct subtypes of HTLV-II among seropositive North American IVDUs suggests that HTLV-II infection was introduced into IVDUs from at least two sources. It is unclear when and where this may have occurred, but there is reason to suspect that HTLV-II was introduced into IVDUs years before the human immunodeficiency virus [4], and this may have involved blood donation by or needlesharing with members of a population(s) with endemic infection.

Recently several small and relatively remote populations with endemic HTLV-II infection have been identified, predominantly among New World Indians [9–11]. The extremely isolated Guaymi in Panama and Ge-speaking tribes of Amazonia would seem to be unlikely sources for the introduction of HTLV-II into IVDUs. In contrast, individuals from North American Indian groups, such as the Pueblo and

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Reprints or correspondence: Dr. Brian Hjelle, Dept. of Pathology, University of New Mexico School of Medicine, Albuquerque, NM 87131.

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Navajo of New Mexico and possibly the Seminoles of Florida, have interacted to a greater degree with the majority culture of the Americas. Many members of these groups have been regular blood donors, including some with HTLV-II [9]. Alternatively, some might have engaged in needlesharing during the early phases of the counterculture movement in the 1960s. If this hypothetical indigenous New World origin for cosmopolitan HTLV-II subtypes is correct, it should be possible to demonstrate both subtypes of HTLV-II in members of different tribes of New World Indians. Here we describe DNA sequence–based subtype analysis of the viruses in members of New Mexico Indian tribes, done in an effort to determine whether such tribes could be the original source of introduction of HTLV-II to IVDUs.

Materials and Methods

Identification of HTLV-II–seropositive donors. All but 1 of the seropositive individuals described here were found to be seropositive after blood donation to the United Blood Services (UBS) blood bank in Albuquerque [9]. The remaining individual is patient 2 (isolate MSA-1b) of a previous report describing HTLV-II infection in 2 Pueblo women with a TSP/HAM-like neurologic illness [12].

All blood donors to UBS are screened for HTLV-I seroreactivity with an ELISA using HTLV-I viral lysate (Abbott Laboratories, Abbott Park, IL). ELISA-positive donations are subjected to confirmatory testing by Western blot (Cambridge Biotech, Worcester, MA). All Western blot–positive donors were interviewed for ethnic background and history of sexual or parenteral exposure to HTLV-I or -II [9]. American Indian donors from whom DNA was available or could be obtained (and with no history of transfusion, needlesharing, or needlesharing sex partners) were enrolled. Eight DNA samples were available from Pueblo and 4 from Navajo. Five of the 8 Pueblo studied were female, aged 32–65 years (average, 44). The Navajo donors were 3 women (42, 42, and 41 years old) and a man (39 years). All 12 enrolled subjects had Western blot reactivity to $p24^{gag}$ and recombinant $gp21^{env}$ proteins in a pattern suggestive of HTLV-II infection [1]. Eleven had additional antibodies, including 9 with reactivity to $p19^{gag}$; 7 to $p28^{gag}$, $p32^{gag}$, or $p53^{gag}$; 2 to $p38^{tax}$; and 1 to $gp46^{env}$.

Polymerase chain reaction (PCR) and sequence analysis. PCR analysis was done on peripheral blood mononuclear cell (PBMC) DNA from seropositive Indian donors, using *tax* and *pol* primers to confirm HTLV-II infection as described previously [9]. For sequence analysis a 630-bp amplicon from the *env* region was cloned and sequenced using conditions previously described [7].

Results

The nucleotide sequence of the 630-bp gp21 transmembrane portion of *env* was determined for 8 seropositive Pueblo and 4 seropositive Navajo. PBMC from 2 Navajo and 1 Pueblo contained a provirus that was identical in sequence to that previously reported for MO and HTLV-IIa proviruses. However, the remaining 6 Pueblo and 2 Navajo instead had a provirus identical to the HTLV-IIb seen in IVDUs from New York [7]. HTLV-IIa and -IIb subtypes differed by 25 bp of the 630-bp amplicon. The sequence comparison of HTLV-IIa and -IIb subtypes is shown in figure 1.

The only Pueblo found to be infected with HTLV-IIa was a woman (MSA-1b) identified by her presentation with a TSP/HAM-like neurologic disease [12]. This virus was identical to the prototype MO (IIa) virus in sequence in the transmembrane region.

		Identification Number and Tribe (N=Navajo; P=Pueblo)													
Coordinate	MO	408N	1358N	MSA1bP	HTLVIIb	6040SN	7296SN	130P	3528P	1457P	5258OP	3584P	1214P	6424SP
6070	T	-	-	-	C	-	-	-	-	-	-	-	-	-
6136	C	-	-	-	T	-	-	-	-	-	-	-	-	-
6148	A	-	-	-	G	-	-	-	-	-	-	-	-	-
6163	T	-	-	-	C	-	-	-	-	-	-	-	-	-
6187	G	-	-	-	A	-	-	-	-	-	-	-	-	-
6209	C	-	-	-	T	-	-	-	-	-	-	-	-	-
6226	C	-	-	-	T	-	-	-	-	-	-	-	-	-
6286	G	-	-	-	A	-	-	-	-	-	-	-	-	-
6298	A	-	-	-	G	-	-	-	-	-	-	-	-	-
6349	G	-	-	-	A	-	-	-	-	-	-	-	-	-
6379	C	-	-	-	T	-	-	-	-	-	-	-	-	-
6409	G	-	-	-	A	-	-	-	-	-	-	-	-	-
6430	A	-	-	-	G	-	-	-	-	-	-	-	-	-
6445	C	-	-	-	T	-	-	-	-	-	-	-	-	-
6469	A	-	-	-	T	-	-	-	-	-	-	-	-	-
6478	A	-	-	-	G	-	-	-	-	-	-	-	-	-
6520	T	-	-	-	C	-	-	-	-	-	-	-	-	-
6532	C	-	-	-	T	-	-	-	-	-	-	-	-	-
6553	T	-	-	-	C	-	-	-	-	-	-	-	-	-
6571	G	-	-	-	A	-	-	-	-	-	-	-	-	-
6580	A	-	-	-	G	-	-	-	-	-	-	-	-	-
6603	A	-	-	-	G	-	-	-	-	-	-	-	-	-
6611	T	-	-	-	G	-	-	-	-	-	-	-	-	-
6628	A	-	-	-	G	-	-	-	-	-	-	-	-	-

Figure 1. Nucleotide sequence at sites of known variation in HTLV-II $gp21^{env}$ alleles from 12 southwestern US Indians in comparison with sequence of MO prototype isolate (HTLV-IIa) [13] and consensus HTLV-IIb sequence [7]. Proviral coordinate of each variable nucleotide is listed (according to GenBank accession M10060); base found in HTLV-IIa and -IIb subtypes is indicated. Sequences obtained from each donor or patient are identical to that of sequence of subtype shown to left, as indicated by dashes.

Discussion

Distinctive subtypes of HTLV-II in indigenous populations. The remarkable intrasubtype consistency described for HTLV-IIa and -IIb isolates in the *env* gene (and lack of intermediate forms) obtained from northeastern US IVDUs suggests that the evolution of HTLV-II into two subtypes occurred in antiquity. However, it was not possible to rule out more recent divergence of the two subtypes occurring after the introduction of HTLV-II into the IVDU population. Our observation that HTLV-IIa and -IIb forms indistinguishable from their respective prototypes can be identified among indigenous New World Indians support the idea of an ancient divergence for the two subtypes. One limitation of the present study was that only 4 Navajo who were without risk factors were available for study. The finding that both subtypes of virus could be identified in a small number of seropositive Navajo blood donors makes it difficult to determine whether one, both, or neither HTLV-II subtype was endemic in Navajo before they began to interact with non-Indian cultures in modern times.

Origins of Indian groups and HTLV-II subtypes. The 19 Pueblo groups are believed to be descended from the postulated Paleo-Indian migration 16,000–40,000 years ago. The Navajo and the closely related Apache, by comparison, are descended from a second migration of Na-Dene hunters 12,000–14,000 years ago [14].

Although we are unable to determine whether the Na-Dene ancestors of the Navajo were once host to a single subtype of HTLV-II, the Pueblo groups appear to have predominantly HTLV-IIb. Our operating hypothesis is that HTLV-II sequences can be considered equivalent to genetic markers for tracing lineage among indigenous New World populations. This conclusion should be tempered by the recognition that our sample sizes are small, that viruses may undergo selection and mutation independent of the host population, and that it may not be possible to determine whether an endemic infection is ancient or the result of recent contact between two populations.

It remains unclear if and when the two subtypes of HTLV-II may have arisen from a common prototype virus. Similarly, it is unclear at present whether HTLV-II shares a common ancestor with HTLV-I.

Introduction of HTLV-II into the IVDU population. The rapid mutation of many RNA viruses makes it difficult to determine with any confidence the route by which such viruses spread among populations. However, the HTLVs are remarkable for a high degree of interisolate conservation, and genetic variation among HTLV isolates can be stably maintained between populations that do not admix [6].

Any explanation for the introduction of HTLV-II into the IVDU population should account for the existence of both HTLV-IIa and -IIb forms in that population and its presence as early as the mid-1970s. We believe it is likely that these forms were introduced into IVDUs on two or more occasions of blood transfusion or needlesharing that may have involved members of populations with endemic infection by the two forms of HTLV-II. There is also a small possibility that HTLV-II was passed from a population in which the virus was endemic to IVDUs by sexual transmission. Whatever the route, it would seem reasonable that transmission of HTLV-II from populations in which it was endemic to IVDUs would be most likely to occur in areas where there is significant interaction between the majority culture and the HTLV-II–endemic group. Although a number of possible sites of an early introduction of HTLV-II exist, one is the southwestern United States, where populations harboring both subtypes of HTLV-II have been identified. That members of these populations donate blood is documented here and elsewhere [9].

Our finding that both forms of HTLV-II are present in indigenous New World populations is compatible with the theory, but does not prove, that HTLV-II subtypes from such populations are ancestral to the analogous viral forms found in IVDUs. Our ability to determine this is limited by inability to further subdivide HTLV-IIa and -IIb subtypes through examination of the transmembrane domain of *env*. Further investigations will include analysis of other regions of the provirus, where greater variation may occur. Preliminary studies have suggested that the largest variations within a subtype occur in the long terminal repeat (unpublished data). Should such phylogenetic division prove to be possible in regions outside the *env* gene, we will investigate whether the sequence data support Navajo (subtype a) or Pueblo (subtype b) viruses (or both) as precursors of the similar forms observed in IVDUs.

HTLV-IIa in a Pueblo woman with myelopathy. Only 1 of the 8 viral sequences from Pueblo were HTLV-IIa. This patient, unlike the others, was not identified after blood donation but in a search for Pueblo with myelopathy [12]. We consider it most likely that HTLV-IIb has been endemic in the ancestors of the Pueblo since antiquity and that this patient's infection occurred as a result of some more recent interaction with other cultures. However, we are unable to determine at present whether a significant minority of all HTLV-II–seropositive Pueblo might harbor HTLV-IIa or if the selection for patients with myelopathy favored the identification of persons with HTLV-IIa. This question could be resolved when additional patients with putative HTLV-II myelopathy are identified [15].

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Depression of the Immune Response to an Inactivated Hepatitis A Vaccine Administered Concomitantly with Immune Globulin

Manfred S. Green, Dani Cohen, Yehuda Lerman, Maria Sjogren, Leonard N. Binn, Sigalit Zur, Raphael Slepon, Guy Robin, Charles Hoke, William Bancroft, Asaad Safary, Yehuda Danon, and Michael Wiener

Medical Corps, Israel Defense Force, Tel Aviv, Israel; US Army Medical Research Institute of Infectious Diseases, Frederick, Maryland; Walter Reed Army Institute of Research, Washington, DC; SmithKline Beecham Biologicals, Rixensart, Belgium

Inactivated hepatitis A virus (HAV) vaccine is given in a three-dose schedule. When rapid protection is needed, injection of immune globulin (IG) concomitantly with the first dose could provide passive protection until adequate active antibody response has developed. A possible effect of IG on the immune response to the vaccine was studied in healthy volunteers; 28 received vaccine alone, and 34 received the first dose simultaneously with 5 mL of IG. A control group received hepatitis B vaccine, and a fourth group received IG alone. Four weeks after the first vaccine dose, all subjects had detectable ELISA anti-HAV antibodies. Several weeks after each vaccine dose, the geometric mean titer of antibodies was significantly lower in those who received vaccine with IG but higher than in those who received IG alone. Results for neutralizing antibodies yielded a similar trend. If IG is given with HAV vaccine, a further booster vaccine dose may be required to ensure long-lasting immunity.

Inactivated hepatitis A vaccines [1, 2] were found in early volunteer studies to be both safe and immunogenic [3, 4]. A purified, highly immunogenic, formaldehyde-inactivated, alum-adjuvanted hepatitis A vaccine was prepared at SmithKline Beecham Biologicals (SBBio) using the HM-175 strain recovered in certified African green monkey kidney cells and adapted to human diploid MRC-5 cells [5]. The vaccine is administered at 0, 1, and 6 months, and in travelers who will soon enter an endemic area, it may be desirable to administer immune globulin (IG) concomitantly with the first dose to provide protection until adequate antibody response has developed. In general, there are no contraindications for simultaneous administration of IG with inactivated vaccines in the recommendations issued by the Centers for Disease Control and Prevention [6]. In the current study, we examined the effect of concomitant admin-

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Reprints or correspondence: Dr. Manfred Green, Occupational Health Institute, P.O. Box 3, Raanana 43100, Israel.

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