Brief Definitive Report

In Vivo Cellular Tropism of Human T Cell Leukemia Virus Type II (HTLV-II)

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Summary

To investigate the in vivo cellular tropism of human T cell leukemia virus type II (HTLV-II), subpopulations of fresh peripheral blood mononuclear cells from infected individuals were isolated and analyzed by polymerase chain reaction for the presence of provirus. In eight of nine patients, HTLV-II was detected exclusively in the $CD8^+$ T lymphocyte population. In the remaining patient, provirus was also detected in $CD4^+$ T lymphocytes. Provirus was not detected in B lymphocytes or monocytes of any patient. These results suggest that in vivo HTLV-II has a preferential, and perhaps in some cases, an exclusive tropism for $CD8^+$ T lymphocytes. The findings contrast sharply with those on HTLV-I where there is a preferential tropism for $CD4^+$ T lymphocytes. Although HTLV-II infection has not been consistently associated with any lymphoproliferative disorders, the results suggest that if these occur, they may be different from those known to be associated with HTLV-I.

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 (1–3). HTLV-I is endemic in well-defined geographic areas, and although the majority of infected individuals remain asymptomatic, infection is associated with adult T cell leukemia (ATL), a malignancy of mature $CD4^+$ T lymphocytes (4–6) and a chronic neurologic disorder known as HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) (7, 8). HTLV-II infection has recently been shown to be endemic in certain New World native Indian populations (9–11), and high rates of infection have also been demonstrated in intravenous drug abusers (IVDAs) in North America (12–14). Infection however, has not yet been definitely associated with disease. Whereas the virus was originally isolated from two patients with atypical variants of hairy cell leukemia (15, 16), subsequent studies have failed to reveal an association of HTLV-II infection with this disorder (11, 17). The apparent lack of pathogenicity of HTLV-II remains poorly understood. Although this may only reflect the relatively small number of individuals so far identified with infection, it may also be related to inherent properties of the virus and/or its cellular tropism. Recent studies using the PCR have demonstrated that HTLV-I has a preferential tropism for $CD4^+$ T lymphocytes in both asymptomatic patients and those with neurological disease (18). In the present study, we have used similar methods to determine the in vivo tropism of HTLV-II, and demonstrate, in contrast to HTLV-I, that the virus has a preferential tropism for $CD8^+$ T lymphocytes.

Materials and Methods

Subjects. Nine patients with HTLV-II infection were studied. HTLV-II infection was confirmed by virus isolation and PCR amplification using methods previously described (14). Four of the patients were also infected with HIV (Table 1). All patients, except three (B.V., J.V., and W.F.) had a history of intravenous drug abuse. Written informed consent was obtained before initiation of the studies.

Cell Separation. PBMCs were obtained from samples of heparinized venous blood by Ficoll-Hypaque centrifugation. Individual cell populations were purified using a two-step selection procedure using washed polystyrene magnetic beads coated with mAbs to CD19, CD8, and CD4, respectively (Dynabeads; Dynal, Oslo, Norway). An initial positive selection step was carried out as follows. PBMCs were incubated with anti-CD19 mAb coated beads (20–25 beads/target cell), and the mixture was incubated in PBS containing 2% (vol/vol) FCS and 0.02% sodium azide (PBS/FCS) for 30 min at 4°C with intermittent gentle mixing. Rosetted cells were washed five times with PBS/FCS and isolated using a MPC-6 magnetic particle concentrator (Dynal) according to the manufacturers instructions. The remaining nonrosetted cells were then incubated in separate steps with beads coated with mAb to CD4 and CD8 (five beads/target cell) under identical conditions. To isolate monocytes, cells remaining after the positive selection steps were incubated in RPMI-1640 medium containing 10% FCS for 1 h in polystyrene flasks at 37°C. Adhered cells were washed and removed by cell scraping.

Individually rosetted cell populations were detached from the magnetic beads by incubation with a polyclonal antibody (Detachabead; Dynal) for 45 min at room temperature with gentle mixing. Detached beads were washed twice with RPMI-1640 medium con-

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taining 1% FCS and removed using the MPC-6 concentrator. Released cells were washed three times with PBS/FCS, and the populations further purified by a negative selection procedure as follows. B, $CD4^+$, and $CD8^+$ lymphocytes, and the adherent monocyte populations were incubated with magnetic beads coated with mAbs to CD2, a mixture of CD19 and CD8, a mixture of CD19 and CD4, and a mixture of CD2 and CD19, respectively at a ratio of >50 beads/target cell. Rosetted cells were removed using MPC-6 concentrator and the nonrosetted cells collected and used for analysis by the PCR. Flow cytometric analysis (Epics-C; Coulter Cytometry, Hialeah, FL) was used to determine the homogeneity of the cell populations.

PCR. Cells were prepared for the PCR using an identical method described for similar studies on HTLV-I (18). After washing twice with PBS, cells were suspended in distilled water at a concentration of 10^7 cells/ml, and boiled for 10 min. After brief centrifugation, 10-μl samples, representing 10^5 cells, and serial tenfold dilutions thereof, were analyzed. Primers SK110 (5'CCCTACAATCCAACCAGCTCAG3') and SK111 (5'GTGGTGAAGCTGCCATCGGGTTTT3') were used to amplify a 186-bp region of the *pol* gene, and amplified products were detected by hybridization with an internal oligonucleotide probe, SK188 (5'TCATGAACCCCAGTGGTAA3') (19). Briefly, PCR was performed in a 100-μl reaction mixture, overlaid with mineral oil, containing 0.2 mM of each dNTP, 50 pmol each of primer, 50 mM KCl, 2.5 mM $MgCl_2$, and 10 mM tris-HCl (pH 8.3), and 2 U of Taq polymerase (Perkin-Elmer Cetus Corp., Norwalk, CT). 35 cycles were carried out in a thermocycler (Perkin-Elmer Cetus). Each cycle consisted of denaturation for 40 s at 94°C, annealing for 55 s at 54°C, and extension for 80 s at 72°C. To ensure complete synthesis, the last cycle at 72°C was extended to 10 min. Aliquots (15 μl) of amplified products were separated by electrophoresis on 1.5% agarose gels, and transferred to nitrocellulose membranes (Schleicher & Shuell, Keene, NH). Membranes were hybridized using conditions previously described (14). After hybridization, membranes were washed twice at room temperature with 2× SSPE containing 0.1% SDS for 5 min, once at 42°C for 10 min, and then exposed to X-Omat AR Kodak film (Eastman Kodak Co., Rochester, NY) with intensifying screens at −70°C for 1–2 d.

Results and Discussion

Mononuclear cells from HTLV-II–infected patients were fractionated into enriched populations of B, $CD4^+$, and $CD8^+$ T lymphocytes, and monocytes using an immunomagnetic cell separation technique that was modified to ensure homogeneity of the cell populations, and to reduce the possibility of obtaining false positive PCR results. Analysis of cell populations by flow cytometry demonstrated that the $CD4^+$ and $CD8^+$ T lymphocytes each had purities of >99.5%, and B lymphocytes, >99%. The monocyte fraction contained a maximum of 1.3% contamination by lymphocytes.

PCR analysis performed on isolated cell populations from representative patients is shown in Fig. 1. The results of titration experiments to determine the greatest serial dilution of cells to give a positive signal in all nine patients is shown in Table 1. It can be seen that in four of the five patients infected with HTLV-II alone, provirus was only detected in the $CD8^+$ T lymphocyte population (Fig. 1). In the remaining patient, (B.V.) positive signals were also detected in $CD4^+$ T lymphocytes. However, the number of $CD4^+$ T lymphocytes infected would seem to be considerably smaller, at least tenfold, compared with the $CD8^+$ T lymphocytes. In the four patients infected with both HTLV-II and HIV, provirus was only detected in the $CD8^+$ T lymphocytes (Table 1). At present, it is unclear if the finding of provirus in the $CD4^+$ T lymphocyte population of one patient

Table 1. *Titration by PCR of HTLV-II DNA in Purified Mononuclear Cell Populations*

Patient/age/sex	HIV seropositivity	$CD4^+/CD8^+$ cells/μl‡	Detection of HTLV-II*			
			$CD19^+$	$CD8^+$	$CD4^+$	Monocytes
S.S./42/M	−	1,424/703	−	+ + + +	−	−
B.V./48/F	−	957/522	−	+ + +	+ +	−
J.V./45/M	−	1,004/517	−	+ +	−	−
W.F./43/M	−	865/2,094	−	+ + +	−	−
J.P./42/M	−	1,068/1,640	−	+ + +	−	−
J.G./35/F	+	624/1,086	−	+ +	−	−
D.W./35/F	+	219/1,153	−	+ +	−	−
A.C./32/M	+	277/1,094	−	+	−	−
S.R./37/M	+	127/1,062	−	+ +§	−‖	−

Nine patients with HTLV-II infection, four of whom were also infected with HIV, were studied.

* The cell number in the greatest serial dilution to give a positive signal on PCR analysis is as follows: +, detectable in 10^5 cells; + +, 10^4 cells; + + +, 10^3 cells; + + + +, 10^2 cells; −, not detectable in 10^5 cells, respectively.

‡ Normal values for $CD4^+$ and $CD8^+$ lymphocytes are 400–1,700, and 240–1,200, respectively. Patient S.R. was persistently lymphopenic, and insufficient cells were available for analysis.

§ Positive signal in 5 × 10^3 cells.

‖ Negative signal in 5 × 10^4 cells on repeat analysis.

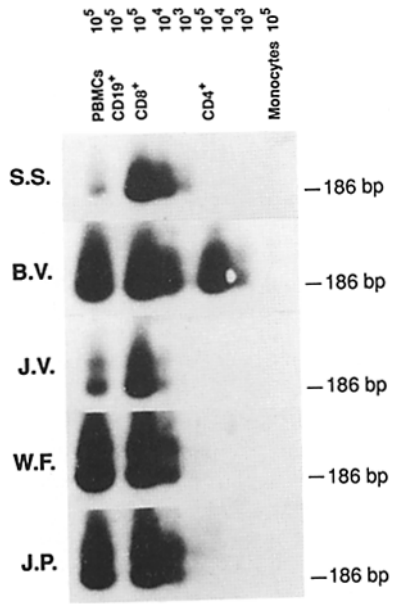

Figure 1. PCR analysis of DNA from fractionated cell populations of five individuals with HTLV-II infection. Southern hybridization analysis of amplified DNA was carried out using an internal oligonucleotide probe as described in Materials and Methods. After washing, membranes were exposed to X-Omat X-ray film with intensifying screens at −70°C for 1–2 d. The number of cell equivalents per amplification is noted above each lane. The autoradiographs of W.F. and J.P. were intentionally overexposed to demonstrate signals only in the $CD8^+$ cells.

represents actual infection, or whether this is due to contamination with $CD8^+$ T lymphocytes. However, in view of the relatively high purity of the populations as determined by flow cytometry and the fact that identical results were obtained on three separate occasions, contamination seems unlikely. No amplification was observed with DNA from B lymphocytes or monocytes of any of the nine patients, suggesting that infection of these cells does not occur in vivo. The observation that HTLV-II preferentially infects $CD8^+$ lymphocytes in vivo contrasts with findings with HTLV-I where there is a preferential tropism for $CD4^+$ T lymphocytes (18).

At present it is difficult to accurately determine the number of $CD8^+$ T lymphocytes infected by HTLV-II, as the PCR assay is not quantitative, and it is unclear if infected cells have single or multiple copies of the provirus. However, if it is assumed that there is one provirus copy per cell, and that the minimum number of cells required to produce a positive signal is ten or less, then in the individuals infected with HTLV-II alone, it can be estimated that at least 1%, and perhaps as many as 10% (patient S.S.) of the $CD8^+$ T lymphocytes are infected (Table 1). In those individuals infected with both HTLV-II and HIV, the number of $CD8^+$ T lymphocytes infected would appear to be at least tenfold less compared with those without HIV infection (Table 1). The reasons for this are unclear, but it might suggest that there is a selective loss of HTLV-II–infected $CD8^+$ T lymphocytes in HIV–infected individuals.

The findings that HTLV-II and HTLV-I infections are preferentially restricted to $CD8^+$ and $CD4^+$ T lymphocytes, respectively, in vivo differs from what is observed in vitro. Under culture conditions, both viruses have been shown to infect not only T and B lymphocytes (20, 21), but numerous nonlymphoid cell types (2, 22). These and studies with vesicular stromatitis virus-HTLV pseudotype viruses suggest that the HTLV-I and -II receptors are widely distributed (23). Importantly, it has also been suggested that the receptor molecule(s) for two viruses may be identical, and this has been mapped to the short arm of chromosome 17 (24). Although the mechanisms involved in the determination of target cell tropism are not fully understood, our findings would suggest that factors other than the receptor molecule(s), perhaps both viral and/or cellular, are important in restricting HTLV-I and -II in vivo to the respective T lymphocyte populations.

The present findings may have important implications for studies on the role of HTLV-II in human disease. As previously noted, no definite disease associations have been observed. HTLV-II was originally isolated from two patients with atypical variants of hairy cell leukemia (15, 16). Importantly, subsequent evaluation of one of these patients revealed that the patient also had a $CD8^+$ lymphoproliferative disorder, and the HTLV-II provirus was detected in this population, but not in the malignant "hairy" cells (25). In retrospect, this was the first indication that HTLV-II has an in vivo tropism for $CD8^+$ T lymphocytes. This observation, together with the results of the present study, would suggest that if HTLV-II plays a significant role in human lymphoproliferative processes, these may be quite different to what has been observed with HTLV-I. Prospective studies on infected individuals should determine if this is correct, and in addition, will show if HTLV-II infection can influence normal $CD8^+$ T lymphocyte function.

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