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Cellular Tropism of Human T-Cell Leukemia Virus Type II Is Enlarged to B Lymphocytes in Patients with High Proviral Load

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To establish the *in vivo* cellular tropism of human T-cell leukemia virus type II (HTLV-II) in peripheral blood, subpopulations of mononuclear cells isolated from patients with a history of drug abuse and with high proviral load were analyzed by polymerase chain reaction for the presence of the proviral sequences. After purification of cellular subsets by immunomagnetic fractionation of blood cells of an infected patient, HTLV-II DNA was detected in CD4⁺ and CD8⁺ T-cells as well as in CD19⁺ B-cells. A positive PCR signal was obtained for purified B-cells also at limiting dilutions. This observation was confirmed by purifying the B-cell fraction by a two-step immunomagnetic procedure from the peripheral blood of another patient with very high HTLV-II copy number and quantifying the B-cell proviral load by means of competitive PCR. A proviral copy number of 90/100 B-cells was found, demonstrating that the great majority of these cells were infected by HTLV-II in this subject. The results indicate that HTLV-II has a broad host range in some infected individuals, showing an enlargement of cellular tropism to B lymphocytes and suggesting that this expansion is associated with an increase in proviral load.
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The human T-cell leukemia virus type II (HTLV-II) has been initially isolated from a patient with an atypical case of T-cell malignancy (*1*). However, no definite relationship of this virus with any human disease has yet been established, although there is growing evidence that HTLV-II is associated with neurodegenerative and lymphoproliferative disorders (*2–4*). HTLV-II infection has been demonstrated in a significant proportion of injecting drug users (IDUs) in the United States and in Europe (*5–7*). Endemic HTLV-II infection has been reported among several American Indian tribes (*8, 9*).

HTLV-II is generally considered to be T-lymphotropic and to infect preferentially the T-cell subset defined by surface antigen CD8 (*10*). However, it has been recently demonstrated that HTLV-I, also considered as a T-lymphotropic virus (*11*), can infect non-T-cell populations, including monocytes and B-cells in patients with adult T-cell leukemia and tropical spastic paraparesis (*12*).

By using a new competitive PCR system for the quantitative analysis of HTLV-II DNA copy number (*13*), we have observed that the *in vivo* proviral load among infected Italian IDUs presents a wide distribution, ranging from 0.1 to 15% of total peripheral blood mononuclear cells (PBMCs) (*14*). This study is aimed to verify whether the high proviral load found in some subjects could be associated to an enlargement of cellular tropism of HTLV-II to non-T-cells. To this end, $CD4^+$, $CD8^+$, and $CD19^+$ cell populations were isolated from PBMCs obtained from IDU patients infected by high HTLV-II copy number and each subset was analyzed by PCR for the presence of proviral sequences.

Mononuclear cells were fractionated from the peripheral blood of an HTLV-II infected patient (not coinfected by HIV-1) with 4% proviral load (*14*), using an immunomagnetic cell separation technique. Briefly, PBMCs were initially incubated with anti-CD19 mAb-coated magnetic beads (Dynabeads, Dynal, Oslo, Norway) at the concentration of 20 beads/target cell. Rosetted cells were isolated using a MPC-6 magnetic particle concentrator (Dynal) according to the manufacturer's instructions. Aliquots of nonrosetted cells were then incubated with magnetic beads (Dynal) coated with anti-CD4 or anti-CD8 mAbs (10 beads/target cell) and separated under identical conditions. The analysis of the $CD4^+$ and $CD8^+$ T lymphocytes by flow cytometry demonstrated that these cell populations had purities of >94% and >95%, respectively, and that the B lymphocyte fraction ($CD19^+$) contained a maximum of 1–2% contamination by $CD3^+$ T lymphocytes. The PCR analysis for HTLV-II was performed in all these purified cell fractions and the results obtained are shown in Fig. 1A. It is evident that HTLV-II proviral DNA sequences were present in both $CD4^+$ and $CD8^+$ T-cells and also in $CD19^+$ B-cells. To determine the greatest serial dilution of $CD19^+$ B-cells to give a positive PCR signal, titration experiments were carried out. The B-cell fraction was diluted at the desired concentration and then lysed before the PCR assay. The results of PCR analysis are shown in Fig. 1B. Proviral DNA was clearly detectable after 100-fold dilution. Considering that

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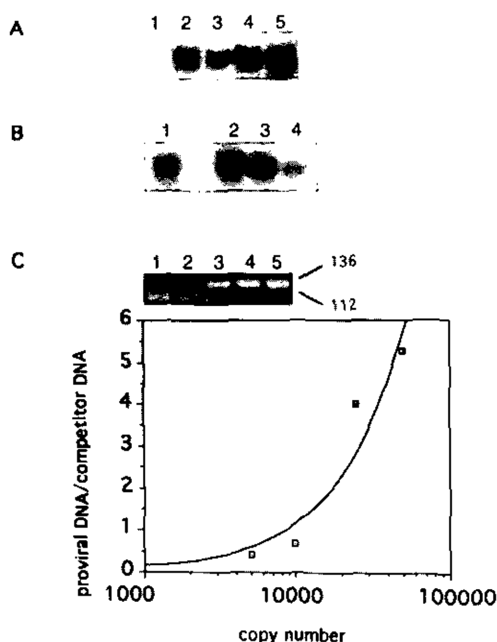

FIG. 1. PCR analysis of HTLV-II in cellular subsets obtained by immunomagnetic separation of PBMCs from infected patients. Cells (10^5) from the various subsets were lysed using proteinase K (Boehringer-Mannheim, Germany) and analyzed by PCR for a conserved region of HTLV-II *tax* as previously described (*7*). When used for titration experiments, the samples were first diluted at the desired concentration and then lysed. Quantitative PCR analysis was performed on 10^5 cell aliquots, following the competitive PCR system for HTLV-II previously described (*13*). (A) PCR analysis of different cellular fractions. Lanes: 1, control with no DNA added; 2, $CD4^+$ T cells; 3, $CD8^+$ T-cells; 4, $CD19^+$ B-cells; 5, control with HTLV-II positive cells (isolate Gu, see Ref. 18). (B) PCR analysis of serial dilutions of $CD19^+$ B-cells. Lanes: 1, control with HTLV-II positive cells; 2, 10^5 $CD19^+$ B-cells; 3, 10^4 $CD19^+$ B-cells; 4, 10^3 $CD19^+$ B-cells. (C) Competitive PCR analysis of HTLV-II proviral sequences in purified $CD19^+$ B-cells. The ethidium bromide-stained bands separated on gel electrophoresis correspond to the products of coamplification of constant amounts of B-cell DNA and increasing amounts of competitor DNA (5,000, 10,000, 25,000, 50,000, and 100,000 copies from lanes 1 to 5). The 136- and 112-bp bands represent the amplified products of competitor DNA and B-cell HTLV-II DNA, respectively. The regression curve was calculated by plotting the competitor area/wild-type area ratio against the competitor copy number.

the percentage of $CD4^+$ and $CD8^+$ cells in this sample is not higher than 1–2%, it can be calculated that the number of contaminating infected cells in a sample of 1000 purified B-cells should not be higher than 1–2, which is under the limit of detection of our PCR assay. Since a strong PCR signal was obtained at the lowest dilution rate, this suggests that HTLV-II proviral sequences are present in the B-cell subset of this subject.

To further substantiate this observation, the $CD19^+$ cells were purified from the PBMCs of another IDU subject (coinfected by HIV-1) with an HTLV-II copy number of 16% of total PBMCs (*14*), by following closely a two-step selection procedure with immunomagnetic beads allowing >99% purification of B lymphocyte subset (*10*). The number of proviral copies of HTLV-II in the purified B-cell fraction was determined by means of a quantitative technique based on competitive PCR system recently developed in our laboratory (*13*). The results obtained are shown in Fig. 1C. From the regression curve, the number of copies of proviral DNA was estimated to be 18,000 in a sample of 20,000 cells, demonstrating that about 90% of the B-cells of this patient were infected by HTLV-II.

Concerning the problem of viral antigen positivity of provirus positive cell fractions, it was observed that *in vitro* cultures of PBMCs of HTLV-II infected patients were positive against HTLV-II *gag*, as shown by testing culture supernatants and immunofluorescence of cells.

To possibly understand if the B-cells were also infected *in vivo* by Epstein–Barr virus (EBV) and whether they could grow continuously *in vitro*, the PBMCs of this patient were cultured in conditions known to maximize the growth efficiency of EBV-transformed B-cell lines (*15*). Briefly, RPMI 1640–10% fetal calf serum medium was supplemented with 10% human endothelial culture supernatant (*16*), 20 U/ml of recombinant IL-6 (Sandoz, Basel, Switzerland) and 1 μg/ml of cyclosporin A (Sandoz). Of the 40 minicultures, 20 were inoculated for 48 hr with a cell free supernatant of the EBV-producing cell line B95.8 (*17*) and 20 were maintained as controls. After 24 days in culture, evidence of blast proliferation was observed in 6 of 20 wells infected by EBV, whereas no spontaneous cell growth was noted in any of the 20 control wells. Flow cytometric analysis of the six lymphoblastoid cell lines indicated that they all presented a B phenotype, being 97.8% $CD19^+$. PCR analysis on the same cell lines showed that they were negative for the presence of HTLV-II proviral sequences. These data suggest that the B-cells of this subject were not infected *in vivo* by EBV, otherwise spontaneous proliferation should have occurred. They also suggest that HTLV-II-infected B-cells were not coinfected *in vivo* by EBV and that these cells were not capable of growing continuously *in vitro* in our conditions.

The results indicate that in cases with high HTLV-II proviral load, a broad spectrum of *in vivo* cellular tropism is observed and that the virus can also infect B lymphocytes. This is at variance with what was found by Ijichi *et al.* (*10*), who reported that HTLV-II had a preferential tropism for $CD8^+$ lymphocytes and was not detectable in B lymphocytes nor monocytes of any patient. Different explanations can be put forward for this discrepancy: (i) Italian IDUs are predominantly infected by the IIb subtype of HTLV-II (*18*), whereas in North America the IIa subtype appears to be prevalent among IDUs (*19, 20*); (ii) mutations in the viral genome could induce an enlargement of HTLV-II infection to different lymphocyte lineages; (iii) in some subjects, clonal expansion of infected B-cells could occur and lead to a high proviral load.

Further work is in progress to understand whether

changes in the cellular tropism are associated to the viral copy number and to the control of viral expression and whether tropism expansion is linked to clonal integration of proviral DNA.

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