CONCISE REPORT

B-Lymphocytic Hairy Cells Contain no HTLV-II DNA Sequences

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HTLV-II has been found in some cases of the rare T-cell form of hairy-cell leukemia (HCL) and in a leukopenic chronic T-cell leukemia mimicking HCL. We asked whether the virus is implicated in the more frequent B-cell form of HCL. DNA extracted from the mononuclear cells derived from spleen (eight cases) or peripheral blood (eight cases) of 16 patients with the B-cell form of HCL was probed. No viral sequences were detected at levels of sensitivity as low as one viral genome in five cells. Therefore HTLV-II may not be involved in the B-cell form of HCL.

THE HUMAN lymphotropic virus HTLV-II has been isolated from two patients with a variant T-cell form of hairy cell leukemia (HCL).[1,2] However, HCL is seen predominantly as a malignancy of B-cell lineage. Some suggestive evidence for HTLV-II in connection with HCL comes from the observation of cell-surface Tac antigen, which is correlated with T cells and selected T-cell malignancies, in B lymphocytic cells.[3] In adult T-cell leukemias, the expression of cell-surface Tac antigen is strongly correlated with the presence of human T-cell leukemia virus type-I (HTLV-I).[4] Furthermore, both HTLV-I and II are capable of infecting and transforming normal T lymphocytes in vitro.[5-7] HTLV-II, whose host range has not been fully established, also infects B-cells in vitro.[5] These observations led us to ask directly whether HTLV-II is associated with the predominant B-cell form of HCL.

MATERIALS AND METHODS

Cells. Mononuclear cells from 16 patients with HCL were purified by Ficoll-Hypaque gradient centrifugation from either peripheral blood (PB) of patients with high leukemic cell counts (eight cases) or from splenic tissue (eight cases). The number of hairy cells in PB ranged from 70% to 98% as determined by morphologic and immunologic criteria. All 16 HCL cases investigated were of B-cell lineage as determined by reactivity to anti-B1, by lack of reactivity to anti-T4, and by failure to rosette with sheep RBCs.

DNA extraction and molecular hybridization. Total cellular DNA was extracted from cells using proteinase K digestion followed by treatment with phenol, chloroform-isoamyl alcohol, digestion with RNase, and ethanol precipitation.[8] Individual digests of human cellular DNA were made with restriction endonucleases *Bam*H1, *Eco*RI, and *Xho*1 for detection of HTLV-II DNA sequences, or with *Sac*I, *Eco*RI, and *Sma*1 for detection of HTLV-1 DNA sequences; the latter enzymes were also used to detect HTLV-I DNA in infected marmoset cells. The digested DNA (10 to 20 μg) was electrophoresed in 1% agarose gels, transferred by a rapid alkaline method[9] to Genescreen Plus membrane (Dupont), hybridized at 42°C in 1% sodium dodecyl sulfate (SDS)1 mol/L NaCl/10% dextran sulfate, and washed at 65°C with 2 × SSC/1% SDS as recommended by the manufacturer (Dupont catalog no. NEF-976).

HTLV-II probes. The 3.5-kilobase (kb) *Bam*H1 fragment of the HTLV-II sequence[10,11] and a 1.9-kb *Sma*1 fragment of the HTLV-I sequence[12] were obtained from the laboratory of I. Chen (UCLA) and were used as probes. The appropriate fragments were isolated from the plasmids and labeled by ^{32}P-oligolabeling.[13]

Limits of sensitivity. Aliquots of 10 μg human placental DNA were mixed with various amounts of HTLV-II viral DNA in ratios of viral genomes per human cell ranging from 20:1 to 1:5. The mixed DNA was digested with the appropriate restriction enzymes, electrophoresed in agarose gels, Southern blotted to Genescreen Plus membrane, and hybridized with ^{32}P-labeled HTLV-II probe following the exact protocol used for the HC samples.

RESULTS

B-type hairy cell DNA was analyzed for HTLV-II. In addition, all samples were probed for HTLV-I sequences to provide a control for possible nonspecific viral signals. The DNAs were digested with various restriction endonucleases to liberate HTLV-II DNA fragments of predictable length if HTLV-II had integrated into the host cell genomic DNA. We were not able to detect any signal homologous to HTLV-II or HTLV-I DNA sequences (Fig 1, lanes c through e) in any of the 16 HCL cases examined. Similar results were found for all 16 samples.

The limit of sensitivity of our Southern blot analysis for 10 μg of DNA/lane was one viral genome in five cells (equivalent to 2.6 pg viral DNA of 9-kb length/10 μg human DNA). At a ratio of one viral genome to each human cell genome, we readily saw a signal after 48 hours on the autoradiogram, as shown in Fig 1, lane a. At a ratio of 1:5, a signal could be seen after 72 hours. Even with exposure to the film for up to two weeks, no HTLV-II–positive signals were obtained in digests of hairy cell DNA.

As a further control on the method, cell lines known to harbor HTLV-I or HTLV-II were probed for the presence of viral sequences. In both cases, the predicted viral DNA fragments were liberated by digestion with restriction enzymes (Fig 1, lane b for HTLV-II). When a similar blot was hybridized with an HTLV-I probe, only the lane containing HTLV-I DNA gave a positive signal. We also probed

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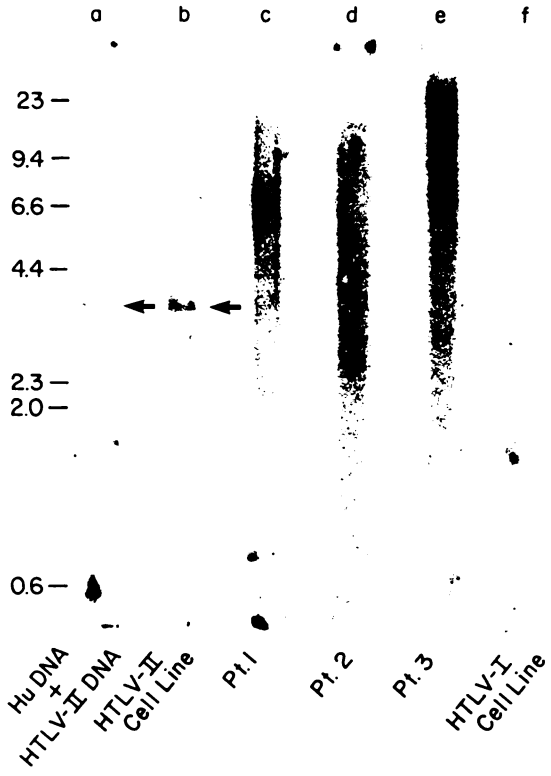

Fig 1. Southern analysis of hairy cell DNA for HTLV-II sequences. For this blot, all samples were digested with *Bam*HI and run on the same agarose gel. Lane a contains human placental DNA to which purified HTLV-II DNA was added at a ratio of one viral genome per human genome; lane b contains DNA from a cell line infected with HTLV-II DNA. Lanes a and b both show the expected 3.5-kb band representing the 3′ end of the HTLV-II genome. Lanes c through e contain *Bam*HI cut DNA samples from three different HCL patients and do not show a positive signal. DNA samples from 13 other HCL patients gave the same result. Lane f is a control containing DNA extracted from a marmoset HTLV-I–infected cell line, and tests for possible cross-hybridization against the HTLV-II probe; it is also negative but gave several bands between 23 and 9.4-kb when the blot was reprobed with HTLV-I DNA.

the hairy cell DNA for immunoglobulin gene rearrangements to show that properly digested DNA was present in the HCL patient DNA lanes. Both rearranged and germline bands were seen. Thus, if the patient DNA samples had contained HTLV-II sequences, positive results would have been expected.

DISCUSSION

Our results are in accordance with the serologic findings of one study performed by Quesada et al[14] and another performed by Rosenblatt et al.[15] In the former study, only 1 of 21 HCL patients tested for the presence of antibodies to HTLV-II was positive; this result might be a false positive. In the latter study, none of 21 HCL patients tested positive for HTLV-II. All these data are consistent with the inference that neither HTLV-II nor HTLV-I is likely to play a role in the B-cell form of HCL.

The clinical responsiveness of HCL to treatment with α-interferon (α-IFN) is unique among the malignant disorders of the hematopoietic system.[16] IFNs have potent antiviral activity in addition to antiproliferative and differentiating properties,[17-19] and α-IFN treatment may mediate remission of B-lymphocytic HCL by its classical antiviral effect. If HTLV-II had been found associated with HCL, infection of lymphocytes and/or stem cells with the virus might be hypothesized as the stage susceptible to α-IFN. The involvement of HTLV-II is excluded, but a different virus (eg, HBLV[20]) or an unidentified virus in the etiology of the B-cell form of HCL remains possible. Alternatively, α-IFN action may be attributable to another effect on malignant cells.

HTLV-I and HTLV-II are capable of infecting both T and B lymphocytes in vitro, yet transformation is reported only of T cells.[5-7] HTLV-I is associated exclusively with T-cell malignancies such as adult T-cell leukemia (ATL) and peripheral T-cell lymphoma[21-24]. With regard to known pathology, HTLV-II was detected only in T-cell HCL,[1,2] a leukopenic chronic T-cell leukemia mimicking HCL,[25] and, more recently, HTLV-II has been implicated in other T-cell lymphoproliferative disorders.[26,27] Other reports describe HTLV-II exposure in AIDS patients without a coexisting T-cell malignancy[28] and present serologic evidence of HTLV-II infection in intravenous (IV) drug users[29] with no obvious malignancy. Together with the results obtained in vitro, these observations suggest that both viruses may be capable of transforming only cells of the T lineage in vivo. Therefore, HTLV-I and HTLV-II may show a T-cell limited range of involvement in lymphoproliferative disorders.[25,30]

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REFERENCES

1. Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Blayney D, Golde D, Gallo RC: A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. Science 218:571, 1982

2. Rosenblatt JD, Golde DW, Wachsman W, Giorgi JV, Jacobs A, Schmidt GM, Quan S, Gasson JC, Chen ISY: A second isolate of HTLV-II associated with atypical hairy cell leukemia. N Engl J Med 315:372, 1986

3. Korsmeyer SJ, Greene WC, Cossman J, Hsu SM, Jensen JP, Neckers LM, Marshall SJ, Bakhshi A, Depper JM, Leonard WJ, Jaffe ES, Waldman TA: Rearrangement and expression of immunoglobulin genes and expression of Tac antigen in hairy cell leukemia. Proc Natl Acad Sci USA 80:4522, 1983

4. Waldmann T, Broder S, Greene W, Sarin P, Goldman C, Frost K, Sharrow S, Depper J, Leonard W, Uchiyama T, Gallo R: A comparison of the function and phenotype of Sezary T cells with human T cell leukemia lymphoma virus (HTLV) associated adult T cell leukemia cells. Clin Res 31:547, 1983 (abstr)

5. Chen ISY, Quan SG, Golde DW: Human T-cell leukemia virus type II transforms normal human lymphocytes. Proc Natl Acad Sci USA 80:7006, 1983

6. Popovic M, Lange-Wantzin G, Sarin PS, Mann D, Gallo RC: Transformation of human umbilical cord blood T-cells by human T-cell leukemia/lymphoma virus. Proc Natl Acad Sci USA 80:5402, 1983

7. Rosenblatt JD, Cann AJ, Golde DW, Chen ISY: Structure and function of the human T-cell leukemia virus II genome. Cancer Rev 1:115, 1986

8. Maniatis T, Fritch EF, Sambrook J: Molecular Cloning. Cold Spring Harbor Laboratory, NY, 1982

9. Reed KC, Mann DA: Rapid transfer from agarose gels to nylon membranes. Nucleic Acids Res 13:7207, 1985

10. Chen ISY, McLaughlin J, Gasson JC, Clark SC, Golde DW: Molecular characterisation of genome of a novel T-cell leukemia virus. Nature 305:502, 1983

11. Gelmann EP, Franchini G, Manzari V, Wong-Staal F, Gallo RC: Molecular cloning of a unique human T-cell leukemia virus (HTLV-IIMo). Proc Natl Acad Sci USA 81:993, 1984

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

13. Feinberg AP, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6, 1983

14. Quesada JR, Reuben J, Hopfer RL, Mundon FK, Hersh EM: Serologic studies in hairy cell leukemia: High prevalence of Epstein-Barr and cytomegalovirus antibodies and absence of human T-cell lymphotropic virus antibodies. Leukemia Res 10:1169, 1986

15. Rosenblatt JD, Gasson JC, Glaspy J, Bhuta S, Aboud M, Chen I, Golde D: Relationship between T cell leukemia virus-II and atypical hairy cell leukemia: A serologic study of hairy cell leukemia patient. Leukemia 1:397, 1987

16. Quesada JR, Reuben J, Manning JT, Hersh EM, Gutterman JU: Alpha-interferon for induction of remission in hairy cell leukemia. N Engl J Med 310:15, 1984

17. Lengyel P: Biochemistry of interferons and their actions. Annu Rev Biochem 51:251, 1982

18. Farrell PJ, Sen GC, DuBois MF, Ratner L, Slattery E, Lengyel P: Interferon action: Two distinct pathways for inhibition of protein synthesis by double stranded RNA. Proc Natl Acad Sci USA 75:5893, 1978

19. Borden EC, Hogan TF, Voelkel JG: Comparative antiproliferative activity in vitro of natural interferons alpha and beta for diploid and transformed human cells. Cancer Res 42:4948, 1982

20. Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Kramarsky B, Gallo RC: Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science 234:596, 1986

21. Poiesz BJ, Ruscetti FW, Reitz MS, Kalyanaraman VS, Gallo RC: Isolation of a new type C retrovirus (HTLV) in primary uncultured cells of a patient with Sezary T-cell leukemia. Nature 294:268, 1981

22. Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto T, Kinoshita KI, Shirakawa S, Miyoshi I: Adult T-cell leukemia: Antigen in an ATL cell line and detection of the antibodies to the antigen in human sera. Proc Natl Acad Sci USA 78:6476, 1981

23. Yoshida M, Myioshi I, Hinuma Y: Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. Proc Natl Acad Sci USA 79:2031, 1982

24. Blayney DW, Jaffe ES, Blattner WA, Cossman J, Robert-Guroff M, Longo DL, Bunn PA Jr, Gallo RC: The human T-cell leukemia/lymphoma virus associated with American adult T-cell leukemia lymphoma. Blood 62:401, 1983

25. Sohn CC, Blayney DW, Misset JL, Mathé G, Flandrin G, Moran EM, Jensen FC, Winberg CD, Rappaport H: Leukopenic chronic T-cell leukemia mimicking hairy cell leukemia: Association with human retroviruses: Blood 67:949, 1986

26. Cervantes J, Hussain S, Jensen F, Schwartz JM: T-prolymphocytic leukemia associated with human T-cell lymphotropic virus II. Clin Res 34:454, 1986 (abstr)

27. Markham PD, Salahuddin SZ: In vitro cultivation of human leukocytes: Methods for the expression and isolation of human viruses. Biotechniques 5:432, 1987

28. Hahn BH, Popovic M, Kalyanaraman VS, Shaw GM, LoMonica A, Weiss SH, Wong-Staal F, Gallo RC: Detection and characterization of an HTLV-II provirus in a patient with AIDS, in Gottlieb MS, Groopman JE (eds): Acquired Immune Deficiency Syndrome. Liss, New York 1984, p 73

29. Tedder RS, Shanson DC, Jeffries DJ, Cheingsong-Popov R, Clapham P, Dalgleish A, Nagy K, Weiss RA: Low prevalence in the UK of HTLV-I and HTLV-II infection in subjects with AIDS, with extended lymphadenopathy, and at risk of AIDS. Lancet 2:125, 1984

30. Koeffler HP, Chen ISY, Golde DW: Characterization of a novel HTLV-II infected cell line. Blood 64:482, 1984