American Journal of Pathology, Vol. 150, No. 2, February 1997

No Evidence of HTLV-I Proviral Integration in Lymphoproliferative Disorders Associated with Cutaneous T-Cell Lymphoma

Gary S. Wood,* John M. Schaffer,* Roland Boni,† Reinhard Dummer,† Gunter Burg,† Morishige Takeshita,‡ and Masahiro Kikuchi‡

From the Departments of Dermatology and Pathology and the Skin Diseases Research Center, Case Western Reserve University, and the VA Medical Center, Cleveland, Ohio; the Department of Dermatology,† University of Zurich, Zurich, Switzerland; and the Department of Pathology,‡ Fukuoka University, Fukuoka, Japan*

Several recent studies have reported detection of HTLV-I genetic sequences in patients with cutaneous T-cell lymphoma (CTCL) including mycosis fungoides and Sezary syndrome. The purpose of this study was to determine whether HTLV-I was detectable in lesional tissues of patients suffering from diseases known to be associated with CTCL. Thirty-five cases were obtained from diverse geographical locations including Ohio, California, Switzerland, and Japan. Six of them had concurrent CTCL. Cases were analyzed using a combination of genomic polymerase chain reaction (PCR)/Southern blot, dot blot, and Southern blot analyses. All assays were specific for HTLV-I provirus. Sensitivity ranged from approximately 10^{-6} for PCR-based studies to 10^{-2} for unamplified genomic blotting. Lesional DNA from patients with lymphomatoid papulosis (fourteen cases), Hodgkin's disease (twelve cases), and CD30^{+} large-cell lymphoma (nine cases) was tested for the HTLV-I proviral pX region using a genomic PCR assay followed by confirmatory Southern blot analysis with a nested oligonucleotide pX probe. All cases were uniformly negative. All of the Hodgkin's disease cases, eight of the large-cell lymphoma cases, and six of the lymphomatoid papulosis cases were then subjected to dot blot analysis of genomic DNA using a full-length HTLV-I proviral DNA probe that spans all regions of the HTLV-I genome. Again, all cases were negative. Finally, eleven of the Hodgkin's disease cases were also subjected to Southern blot analysis of EcoRI-digested genomic DNA using the same full-length HTLV-I probe. Once again, all cases were negative. These findings indicated that, despite utilization of a variety of sensitive and specific molecular biological methods, HTLV-I genetic sequences were not detectable in patients with CTCL-associated lymphoproliferative disorders. These results strongly suggest that the HTLV-I retrovirus is not involved in the pathogenesis of these diseases. (Am J Pathol 1997, 150:667–673)

Cutaneous T-cell lymphoma (CTCL) is a CD4^{+} T-cell neoplasm consisting of mycosis fungoides and its leukemic variant, the Sezary syndrome.[1] Although the etiology of CTCL remains to be determined, several recent studies have suggested a role for the HTLV-I retrovirus in the pathogenesis of this lymphoproliferative disorder. It has been postulated that portions of the HTLV-I RNA genome, reverse transcribed into proviral DNA, become integrated into the host T-cell genome and result in clonal expansion *via* deregulation of cellular activation and proliferation mechanisms.[2,3] The primary evidence for this hypothesis has come from molecular biological studies in which various portions of the HTLV-I genome have been detected in lesional tissues or cell lines obtained from CTCL patients.[4–13] The full-length HTLV-I genome is approximately 9 kb long and consists of *gag*, *pol*, *env*, and pX regions flanked by long terminal repeat sequences.[2] Of these regions, the one most commonly detected in CTCL specimens has been the pX. This region contains the *tax* and *rex*

Supported by National Institutes of Health grants CA60014, AR39750, and AR40844 and Merit Review funding from the Department of Veterans Affairs (G. S. Wood).

Accepted for publication September 18, 1996.

Address reprint requests to Dr. Gary S. Wood, Dermatology Service, VA Medical Center, 10701 East Blvd., Cleveland, OH 44106.

Table 1. *CTCL-Associated Lymphoproliferative Disorders: Clinicopathological Features of the 35 Cases Studied*

Diagnosis	Lesional tissue	Number of cases	Geographic origin (n)
Lymphomatoid papulosis*	Skin	14	Ohio (13), Switzerland (1)
Hodgkin's disease†	Lymph node	9	California (11), Japan (1)
	Lung, mediastinum, subcutis	3	
Primary cutaneous $CD30^+$	Skin	8	Switzerland (3), Japan (3)
Large-cell lymphoma‡	Lymph node	1	Ohio (2), California (1)

*Includes three cases of lymphomatoid papulosis/mycosis fungoides and one case each of lymphomatoid papulosis/large-cell lymphoma, lymphomatoid papulosis/large-cell lymphoma/mycosis fungoides, and lymphomatoid papulosis/Hodgkin's disease/mycosis fungoides. HTLV-I serology was negative for all four cases tested.

†Includes seven cases of mixed cellularity subtype and five cases of nodular sclerosing subtype. One case was HTLV-I seronegative Hodgkin's disease from an HTLV-I-endemic region of Japan.

‡Includes one case each of large-cell lymphoma/lymphomatoid papulosis, large-cell lymphoma/mycosis fungoides, and large-cell lymphoma/lymphomatoid papulosis/mycosis fungoides. The latter two cases represent transformation of mycosis fungoides to large-cell lymphoma. All cases were anaplastic large-cell lymphoma except the three Ohio and California cases, which were the immunoblastic subtype. Three cases were HTLV-I seronegative anaplastic large-cell lymphoma from an HTLV-I-edemic region of Japan. One additional Ohio case also known to be HTLV-I seronegative.

genes, which encode regulatory proteins capable of altering T-cell function.[2,3]

In the present study, we employed a variety of molecular biological methods to determine whether HTLV-I proviral DNA sequences were detectable in fresh-frozen lesional tissues obtained from 35 patients suffering from diseases known to be associated with CTCL. These disorders included lymphomatoid papulosis, Hodgkin's disease, and primary cutaneous $CD30^+$ large-cell lymphoma of T-cell lineage. In addition, 6 of these patients also had concurrent CTCL.

Materials and Methods

Tissue Specimens

All tissue specimens were obtained with informed consent and human subjects committee approval. All diagnoses were established using standard clinicopathological criteria.[1] Details concerning diagnosis, tissue source, geographic origin, associated diseases, and HTLV-I serology are included in Table 1. Genomic DNA was extracted from specimens using identical standard techniques.[14]

Polymerase Chain Reaction (PCR) Using pX Primers

PCR reactions were performed according to standard methods.[15] The buffer used for the pX PCR was 50 mmol/L KCl, 10 mmol/L Tris/HCl (pH 8.0), 0.2 mmol/L of each dNTP, 1.0 mmol/L $MgC1_2$ in sterile type I H_2O. Each reaction mixture had a total volume of 50 μl, which contained 35 μl of pX buffer, 20 pmol of each pX primer (described previously by Hall et al[6]), 2.5 U of Amplitaq DNA polymerase, 2 μg of template DNA, and sterile type I H_2O. Before the template DNA and Amplitaq DNA polymerase were added to the reaction mixture, the pX buffer and primers along with the sterile type I H_2O and overlay mineral oil were exposed to 254-nm and 307- to 312-nm ultraviolet light for 20 minutes to reduce PCR contamination. The reactions were performed in a DNA thermal cycler 480 (Perkin-Elmer, Norwalk, CT). Thermal cycling consisted of 30 cycles, with 1 minute of denaturation at 94°C, 1.5 minutes of annealing at 56°C, and 2.0 minutes of extension at 72°C.

Southern Blot Analysis of PCR Products

Aliquots (10 μl) of each reaction were electrophoresed on a 0.8% agarose gel at 100 V and transferred onto a nylon membrane using a Vacugene transfer apparatus. After transfer, the membrane was auto-cross-linked in a Stratlinker and then washed in 4X standard saline citrate (SSC). The membrane was prehybridized and then hybridized using a nested oligonucleotide pX probe (described previously by Hall et al[6]) and incubated at 55°C on a rocker for 24 hours. After hybridization, the membrane was washed with 2X SSC/0.1% sodium dodecyl sulfate (SDS) at room temperature for 5 minutes, 2X SSC/0.1% SDS at room temperature for 15 minutes, and 0.5X SSC/0.1% SDS in a 37°C shaker bath for 30 minutes. The membrane was placed into an x-ray cassette with intensifying screens and film and then placed into an 80°C freezer for 17 hours.

Dot Blot Analysis Using HTLV-I Proviral Clone

A dot blot manifold apparatus from Schleicher & Schuell (Keene, NH) was used for this procedure. Before assembling the manifold and attaching the

vacuum line, the blotter paper and nylon membrane were both moistened with deionized H_2O. Each designated well was loaded with 500 μl of 4X SSC, 10 μg of DNA, 500 μl of denaturation solution, 500 μl of neutralization solution, and 500 μl of 20X SSC. Between each solution and the DNA, the vacuum was applied until transferred through. The membrane was then auto-cross-linked and washed as described above. The membrane was prehybridized and then hybridized with a radiolabeled 8.5-kb HTLV-I fragment, 25 ng of DNA (obtained from Dr. Chou-Zen Giam, Case Western Reserve University) according to the protocol in the Gibco BRL RadPrimer labeling system (18528–011, Gibco BRL, Gaithersburg, MD). After hybridization, the membrane was washed for 5 minutes at room temperature with 2X SSC/0.1% SDS, 10 minutes at room temperature with 2X SSC/0.1% SDS, and 30 minutes at 60°C with 0.1X SSC/0.1% SDS. The membrane was exposed to x-ray film, using intensifying screens, for 7 days in a −80°C freezer.

Genomic Southern Blot Analysis Using HTLV-I Proviral Clone

A 10-μg amount of each *Eco*RI-digested DNA sample in a volume of 15 μl plus 15 μ1 of 2X loading buffer were electrophoresed on a 0.8% agarose gel that ran at 40 V overnight. The electrophoresed DNA was then transferred onto a nylon membrane, auto-cross-linked, and washed as described above. The membrane was prehybridized and then hybridized at 42°C for 24 hours with 25 ng of the same radiolabeled 8.5-kb HTLV-I probe as described above. The membrane was washed and autoradiographed as described in the section immediately above.

Controls

The integrity of all DNA extracts was verified by genomic PCR of the nucleophosmin cellular gene followed by confirmatory Southern blot analysis using a nested oligonucleotide probe as described previously by us.[16] In addition, the majority of cases were also tested by genomic PCR of T-cell receptor-γ gene rearrangements as another control for DNA integrity as well as T-cell DNA content. Primers and methods were identical to those reported previously by us.[17] The MT4 T-cell line was used as a positive control for HTLV-I provirus. The Jurkat T-cell line, reactive lymphoid tissues, and normal skin were used as negative controls. All sensitivity controls were performed using MT4 positive control DNA diluted in human cutaneous and lymphoid tissue DNA to control for any potential PCR inhibitors in DNA preparations. The same DNA the integrity of which was verified as noted above was used for genomic dot blot and Southern blot analyses. DNA integrity was further confirmed by hybridization with a 2.1-kb T-cell receptor-γ probe[1] and, for Southern blots, by visual inspection of DNA in gel lanes after electrophoresis and transfer to nylon membranes, and by detection of a positive signal for HTLV-I in lanes containing DNA from the MT4 positive control.

Results

PCR Analysis of pX Region

The HTLV-I pX region is the one that has been detected most commonly in CTCL specimens using PCR techniques.[4–13] Therefore, we focused our attention on a highly conserved 126-bp portion of the pX region extending from nucleotide 7596 to nucleotide 7722.[6] The HTLV-I-infected MT4 T-cell line was used as a positive control and the HTLV-I-negative Jurkat T-cell line was used as a negative control. The specificity of the PCR assay was confirmed by Southern blot analysis using a nested oligonucleotide pX probe and by nucleotide sequencing of the PCR product amplified from the MT4 positive control.

Using the PCR/Southern blot assay, lesional genomic DNA extracted from the following cases was tested for the 126-bp pX proviral DNA sequence: 14 cases of lymphomatoid papulosis, 12 cases of Hodgkin's disease, and 9 cases of primary cutaneous $CD30^+$ large-cell lymphoma of T-cell lineage. The clinicopathological features and geographical origins of these cases are summarized in Table 1. All cases were uniformly pX negative including four HTLV-I seronegative cases from an HTLV-I-endemic region of Japan and second specimens from 2 of the lymphomatoid papulosis cases. Representative results are illustrated in Figure 1. Figure 2 shows representative controls for DNA integrity involving a PCR product amplified from the nucleophosmin gene.

For each PCR analysis, 2 μg of template DNA was used. Titration experiments determined that the pX PCR product was still readily detectable by Southern blotting at a 10^{-6} dilution of MT4 target DNA into human cutaneous and tonsil DNA. This suggested that any specimens containing more than approximately 1 in 10^6 HTLV-I-infected cells should have been pX positive in this PCR/Southern blot assay.

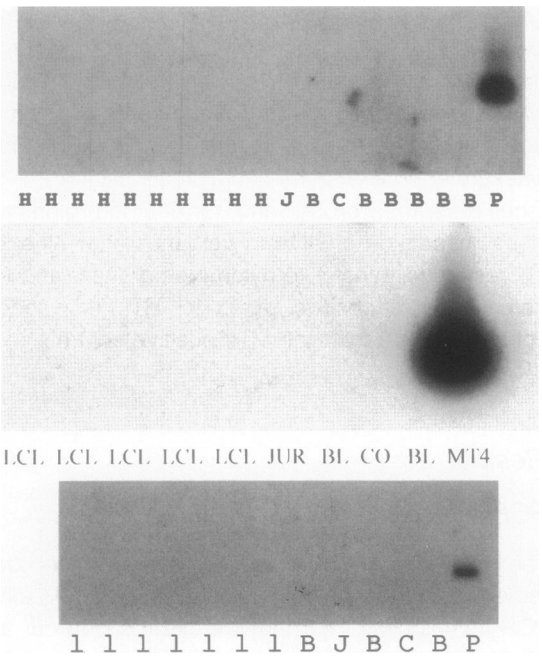

Figure 1. *HTLV-I pX PCR/Southern blot analysis of Hodgkin's disease, CD30+ large-cell lymphoma, and lymphomatoid papulosis. By Southern blot analysis of pX PCR products using a nested oligonucleotide probe, MT4 positive control lanes (MT4 or P) show the expected 126-bp pX PCR product. All other lanes are negative including 10 cases of Hodgkin's disease (H;* **top panel**), *5 cases of large-cell lymphoma (LCL;* **middle panel**), *7 cases of lymphomatoid papulosis (l;* **lower panel**), *and negative controls including the Jurkat T-cell line (JUR or J), carry-over (CO or C), and blanks (BL or B).*

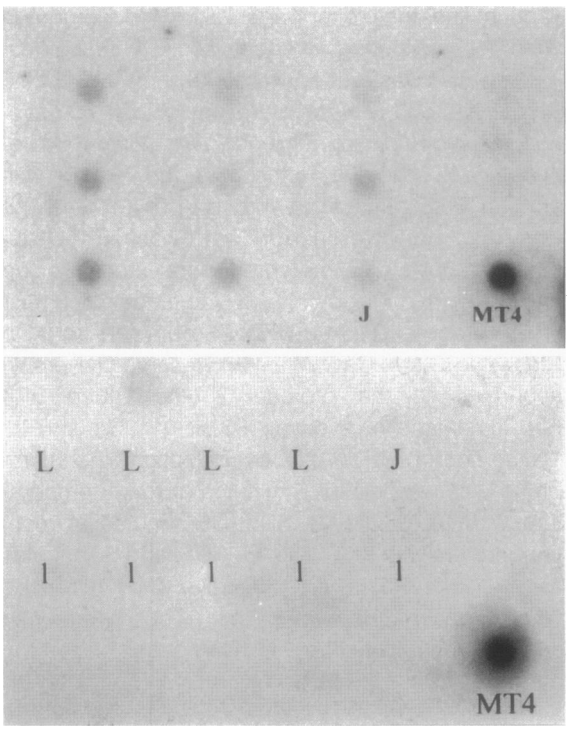

Figure 3. *HTLV-I dot blot analysis of Hodgkin's disease, CD30+ large-cell lymphoma and lymphomatoid papulosis. By dot blot analysis of lesional DNA using an 8.5-kb HTLV-I proviral clone as probe, MT4 positive control dots show the expected strong signal whereas 10 cases of Hodgkin's disease (unlabeled dots in the 3 × 4 array;* **top panel**), *4 cases of large-cell lymphoma (L), 5 cases of lymphomatoid papulosis (l;* **bottom panel**), *and the Jurkat T-cell line negative controls (J) are negative.*

Dot Blot Analysis of HTLV-I

The PCR/Southern blot analysis provided strong evidence against the presence of pX proviral sequences in lesional tissues of CTCL-associated diseases. However, the possibility remained that portions of the HTLV-I provirus other than the pX region were integrated into host DNA. Therefore, among those specimens with sufficient DNA, we elected to utilize a genomic dot blot technique incorporating a radiolabeled 8.5-kb HTLV-I proviral DNA clone as a probe. Using this assay, we could effectively screen for all major regions of the HTLV-I genome simultaneously. All 12 cases of Hodgkin's disease, 8 cases of CD30+ large-cell lymphoma, and 6 cases of lymphomatoid papulosis were tested using this approach. As with the PCR assay described above, all cases tested by dot blot analysis were uniformly negative. Representative results are illustrated in Figure 3. Figure 4 shows representative controls for DNA integrity using a T-cell receptor Jγ probe.

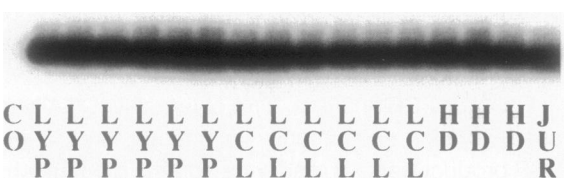

Figure 2. *Nucleophosmin control for the integrity of DNA used in PCR assays. PCR followed by Southern blotting using a nested oligonucleotide probe shows the expected 185-bp band in all tissue samples including six cases of lymphomatoid papulosis (LYP), six cases of large-cell lymphoma (LCL), three cases of Hodgkin's disease (HD), and the Jurkat T-cell line (JUR). Only the carry-over (CO) negative control lane lacks this band.*

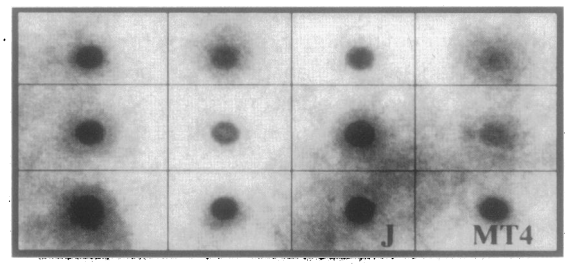

Figure 4. *T-cell receptor Jγ probe control for the integrity of DNA used for genomic dot blotting and Southern blotting. The same membrane shown in the top portion of Figure 3 was stripped and reprobed with the Jγ probe. All samples of Hodgkin's disease (unlabeled dots in the 3 × 4 array), the Jurkat T-cell line (J), and the MT4 T-cell line (MT4) exhibit a positive signal.*

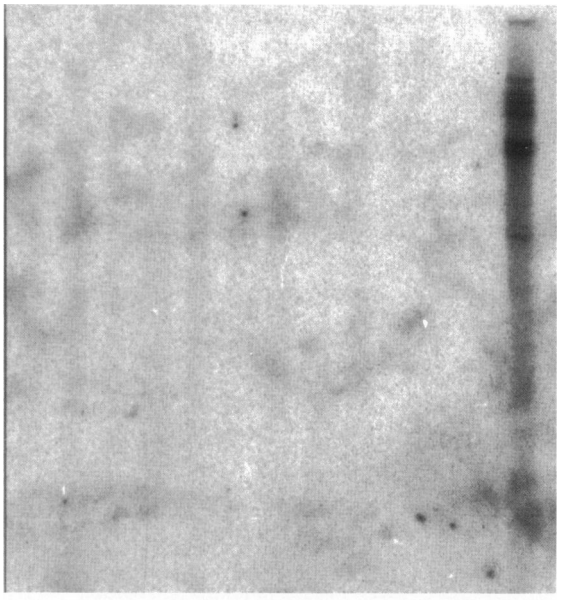

Figure 5. *HTLV-I southern blot analysis of Hodgkin's disease. By Southern blot analysis of* EcoRI*-digested lesional DNA probed with the same 8.5-kb HTLV-I proviral clone used as a probe for dot blot analyses, the MT4 positive control shows a ladder of clonally integrated HTLV-I proviral fragments. All other lanes are negative including 10 cases of Hodgkin's disease (H) and blanks (B).*

For each dot blot analysis, 10 μg of lesional DNA was used. Titration experiments determined that this assay was sensitive down to a level of 0.05 μg of MT4 DNA diluted in tonsil DNA. This suggested that any specimen containing more than 0.5% HTLV-I-infected cells should have been clearly HTLV-I positive in this dot blot assay.

Southern Blot Analysis of HTLV-I

In 11 of our cases of Hodgkin's disease, we had sufficient DNA for additional tests. Therefore, we decided to confirm our negative dot blot results using Southern blot analysis of *Eco*RI-digested genomic DNA. The HTLV-I genome has no internal *Eco*RI sites; therefore, each clonally integrated HTLV-I provirus gives rise to a single band in this type of assay. We used the same 8.5-kb proviral probe used previously for the dot blots. As expected, the MT4 positive control showed a ladder of clonally integrated HTLV-I proviral inserts; however, the Hodgkin's disease cases were uniformly negative. Representative findings are illustrated in Figure 5.

Discussion

It is well established that patients with CTCL are at increased risk for a variety of other lymphoproliferative disorders including lymphomatoid papulosis, Hodgkin's disease, and $CD30^+$ large-cell lymphoma.[1] Although still controversial, several recent studies have shown evidence of HTLV-I proviral integration in lesional tissues and cell lines established from CTCL patients.[4–13] Therefore, it was plausible that CTCL-associated diseases might also involve HTLV-I infection. This possibility was strengthened by recent studies that showed that, when more than one of these diseases occurred in an individual patient, they all shared the same clonal origin.[17–20] Furthermore, our patient group included several who suffered from more than one of these disorders or concurrent CTCL itself (see Table 1).

Nevertheless, our findings were consistently negative. This argues strongly against any direct role for HTLV-I in the pathogenesis of CTCL-associated lymphoproliferative disorders. The variety of molecular biological methods that we employed were both sensitive and specific. Sensitivity studies showed that our genomic PCR/Southern blot assay can detect approximately 1 in 10^6 cells containing HTLV-I provirus. Furthermore, the absence of HTLV-I was substantiated not only by PCR but also by unamplified dot and Southern blotting using a full-length proviral probe spanning all regions of the HTLV-I genome. Although the 0.5% sensitivity limit of the genomic blotting does not exclude the possibility of HTLV-I provirus in less than this proportion of cells, it does exclude greater densities of HTLV-I, corroborates the highly sensitive PCR findings, and makes it unlikely that the PCR data represent false negatives due to selective deletion of pX primer template sequences in truncated forms of HTLV-I provirus. In aggregate, these molecular biological studies screened for all major regions of the HTLV-I genome, including the pX region, which is the portion of HTLV-I that has been detected most commonly in previous studies of CTCL.[4–13] The pX region includes the *tax* gene, which encodes a protein that complexes with the host cyclic-AMP-responsive element-binding protein, CREB, to form transacting transcriptional activators of HTLV-I genes and other genes such as interleukin-2, interleukin-2 receptor, adenovirus E3, granulocyte/macrophage colony-stimulating factor, c-*fos*, c-*sis*, and the SV40 enhancer.[2,3] The pX region also includes the *rex* gene. Like *tax* proteins, *rex* proteins are essential for HTLV-I replication, but unlike *tax*, *rex* products appear to act chiefly at the post-transcriptional level to regulate viral gene expression.[2,3]

Our results are in agreement with a 1991 study of childhood lymphomas from Taiwan in which all twenty-three cases of non-Hodgkin's lymphoma tested

were negative for HTLV-I provirus as assessed by Southern blot analysis of *Eco*RI-digested DNA extracted from frozen lesional specimens.[21] Six of these twenty-three cases were peripheral T-cell lymphomas including two $CD30^+$ anaplastic large-cell lymphomas, three $CD30^-$ large-cell immunoblastic lymphomas, and one $CD30^-$ diffuse medium-sized-cell lymphoma. Our findings are also in agreement with a 1995 study of $CD30^+$ anaplastic large-cell lymphoma from an HTLV-I-endemic region of Japan.[22] Among the HTLV-I-seronegative cases, none of seven tested using Southern blot analysis showed any evidence of HTLV-I proviral integration.

Our results differ from those of a 1990 German study in which six cases of $CD30^+$ large-cell lymphoma showed evidence of HTLV-I proviral integration by genomic Southern blot analysis using a full-length HTLV-I probe and/or by PCR analysis using *pol* region primers.[23] Interestingly, this same study found no evidence of HTLV-I in four cases of CTCL analyzed concurrently. There are several potential explanations for the discrepancy between our current findings and those of this former study. First, there may have been technical difficulties with the specificity of the assays used in the 1990 study. For example, the identity of *pol* PCR products was not confirmed by nested Southern blotting. Second, the cases in the former study might have been unrecognized variants of ATL, which is well known to be HTLV-I associated,[2] to contain $CD30^+$ anaplastic large cells in some cases,[22,23] and to have a wide variety of clinicopathological presentations.[22,24] In this context, it is interesting that, although only one of the large-cell lymphoma patients in the 1990 study was tested for anti-HTLV-I antibodies, he was in fact HTLV-I seropositive as would be expected for ATL presenting as $CD30^+$ anaplastic large-cell lymphoma.[22] Third, there may be geographical differences regarding the association of HTLV-I with $CD30^+$ large-cell lymphoma analogous to the geographical differences in the association of Epstein-Barr virus with endemic *versus* nonendemic forms of Burkitt's lymphoma.[11] This latter possibility is made less likely by the relatively diverse geographical origin of our cases, which included Switzerland, Ohio, California, and an HTLV-I-endemic region of Japan (see Table 1).

It will be important to confirm our findings in additional cases of CTCL-associated diseases obtained from an even greater variety of global locations. At present, however, our results do not support any role for the integration of HTLV-I proviral DNA in the pathogenesis of lymphomatoid papulosis, primary cutaneous $CD30^+$ large-cell lymphomas of T-cell lineage, or the nodular sclerosis and mixed cellularity subtypes of Hodgkin's disease.

Acknowledgments

We are grateful to Dr. Roger A. Warnke for providing some of the tissues used in this study.

References

1. Wood GS: The benign and malignant cutaneous lymphoproliferative disorders including mycosis fungoides. Neoplastic Hematopathology, ed 1. Edited by DM Knowles. Baltimore, Williams and Wilkins, 1992, pp 917–952
2. Cann AJ, Chen ISY: Human T-cell leukemia virus types I and II. Virology, ed 2. Edited by BN Fields, DM Knipe. New York, Raven Press, 1990, pp 1501–1527
3. Zhao L-J, Giam C-Z: Interaction of the human T-cell lymphotrophic virus type I (HTLV-I) transcriptional activator Tax with cellular factors that bind specifically to the 21-base-pair repeats in the HTLV-I enhancer. Proc Natl Acad Sci USA 1991, 88:11445–11449
4. Manzari V, Gismondi A, Baarillari G, Morrone S, Modesti A, Albonici L, De Marchis L, Fazio V, Gradilone A, Zani M, Frati L, Santoni A: HTLV-V: a new human retrovirus isolated in a Tac-negative T cell lymphoma/leukemia. Science 1987, 238:1581–1583
5. Detmar M, Pauli G, Anagnostopoulos I, Wunderlich U, Herbst H, Garbe C, Stein H, Orfanos CE: A case of classical mycosis fungoides associated with human T-cell lymphotrophic virus type I. Br J Dermatol 1991, 124:198–202
6. Hall WW, Liu CR, Schneewind O, Takahashi H, Kaplan MH, Roupe G, Vahlne A: Deleted HTLV-I provirus in blood and cutaneous lesions of patients with mycosis fungoides. Science 1991, 253:317–320
7. Zucker-Franklin D, Coutavas EE, Rush MG, Zouzias DC: Detection of human T-lymphotrophic virus-like particles in cultures of peripheral blood lymphocytes from patients with mycosis fungoides. Proc Natl Acad Sci USA 1991, 88:7630–7634
8. Goldman B, Fujihara K, Bisaccia E, Pincus S, Greenberg SJ, Oseroff AR: HTLV retroviral homologous sequences in mycosis fungoides/Sezary syndrome. J Invest Dermatol 1992, 98:660
9. Sahai Srivastava BI, Banki K, Perl A: Human T-cell leukemia virus type I or a related retrovirus in patients with mycosis fungoides/Sezary syndrome and Kaposi's sarcoma. Cancer Res 1992, 52:4391–4395
10. Whittaker SJ, Luzzatto L: HTLV-I provirus and mycosis fungoides. Science 1993, 259:1470
11. Hall WW: HTLV-I provirus and mycosis fungoides. Science 1993, 259:1471
12. Ghosh SK, Abrams JT, Terunuma H, Vonderheid EC, DeFreitas E: Human T-cell leukemia virus type I tax/rex

DNA and RNA in cutaneous T-cell lymphoma. Blood 1994, 84:2663–2671
13. Pancake BA, Zucker-Franklin D, Coutavas EE: The cutaneous T cell lymphoma, mycosis fungoides, is a human T cell lymphotrophic virus-associated disease. J Clin Invest 1995, 95:547–554
14. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual, ed 2. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1989
15. Erlich HA (Editor): PCR Technology: Principles and Applications for DNA Amplification. New York, Stockton Press, 1989
16. Wood GS, Hardman DL, Boni R, Dummer R, Kim YH, Smoller BR, Takeshita M, Kikuchi M, Burg G: Lack of the t(2;5) or other mutations resulting in expression of anaplastic lymphoma kinase (ALK) catalytic domain in $CD30^+$ primary cutaneous lymphoproliferative disorders and Hodgkin's disease. Blood 1996, 88:1765–1770
17. Wood GS, Bahler DW, Hoppe RT, Warnke RA, Sklar JL, Levy R: Transformation of mycosis fungoides: T-cell receptor β gene analysis demonstrates a common clonal origin for plaque type mycosis fungoides and $CD30^+$ large cell lymphoma. J Invest Dermatol 1993, 101:296–300
18. Kaudewitz P, Herbst H, Anagnostopoulos I, Eckert F, Braun-Falco O, Stein H: Lymphomatoid papulosis followed by large cell lymphoma. Br J Dermatol 1991, 124:465–469
19. Davis TH, Morton CC, Miller-Cassman R, Balk SP, Kadin ME: Hodgkin's disease, lymphomatoid papulosis, and cutaneous T-cell lymphoma derived from a common T-cell clone. N Engl J Med 1992, 326:1115–1122
20. Wood GS, Crooks CF, Uluer AZ: Lymphomatoid papulosis and associated cutaneous lymphoproliferative disorders exhibit a common clonal origin. J Invest Dermatol 1995, 105:51–55
21. Lee SH, Su IJ, Chen RL, Lin KS, Lin DT, Chuu WM, Lin KS: A pathologic study of childhood lymphoma in Taiwan with special reference to peripheral T-cell lymphoma and the association with Epstein-Barr viral infection. Cancer 1991, 68:1954–1962
22. Takeshita M, Ohshima K, Akamatsu M, Ohgami A, Kikuchi M, Suzumiya J, Uike N, Okamura T, Nakayama J: CD30-positive anaplastic large cell lymphoma in a human T-cell lymphotropic virus-I endemic area. Hum Pathol 1995, 26:614–619
23. Anagnostopoulos I, Hummel M, Kaudewitz P, Herbst H, Braun-Falco O, Stein H: Detection of HTLV-I proviral sequences in $CD30^+$ large cell cutaneous T-cell lymphomas. Am J Pathol 1990, 137:1317–1322
24. Wantanabe S, Mukai K, Shimoyama M: Adult T-cell leukemia/lymphoma. Neoplastic Hematopathology, ed 1. Edited by DM Knowles. Baltimore, Williams and Wilkins, 1992, pp 1281–1294