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Reexamination of human T cell lymphotropic virus (HTLV-I/II) prevalence

(mycosis fungoides/injection drug users/Tax/HTLV proviral sequence/HTLV antibodies)

DOROTHEA ZUCKER-FRANKLIN*†, BETTE A. PANCAKE*, MICHAEL MARMOR‡, AND PATRICIA M. LEGLER*

*Departments of Medicine and ‡Environmental Medicine and the Kaplan Comprehensive Cancer Center, New York University Medical Center, 550 First Avenue, New York, NY 10016

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ABSTRACT **In the United States, blood donors are being screened for infection with human T cell lymphotropic viruses I and II (HTLV-I/II) by serologic means, which detect antibodies to the structural proteins of these viruses. Because patients with mycosis fungoides (MF) usually do not have such antibodies even though their cells harbor HTLV-I Tax and/or pol proviral sequences, it was questioned whether the prevalence of HTLV infection among healthy blood donors may also be underestimated by current means of testing. To examine this possibility, a study on specimens of relatives of mycosis fungoides patients (MFR) was begun. In addition, to collect data more expeditiously, a cohort of former injection drug users (IDUs) was tested by routine serologic methods, as well as by PCR/Southern blot analysis for Tax, pol, and gag proviral sequences and Western blot analysis for antibodies to the Tax gene product. To date, 6/8 MFRs and 42/81 (51.8%) of HIV-negative IDUs proved to be positive for HTLV, whereas routine serology identified none of the MFR and only 18/81 (22.2%) of the IDUs. Among the latter test subjects, the incidence of HTLV-I also proved to be 10 times higher than expected. Therefore, it is likely that among healthy blood donors infection with HTLV-I/II is more prevalent than is currently assumed. Since Tax is the transforming sequence of HTLV-I/II, testing for Tax sequences and antibodies to its gene product may be desirable in blood transfusion and tissue donor facilities.**

The first human retrovirus, human T cell lymphotropic virus type I (HTLV-I), was discovered more than 15 years ago (1, 2). Since that time numerous investigators have studied its pathogenicity, epidemiology, and molecular biology (for reviews see refs. 3–5). The etiologic role of HTLV-I in adult T cell leukemia/lymphoma and tropical spastic paraparesis/HTLV-I-associated myelopathy has been well established. The vast majority of patients with these diseases have antibodies to the structural proteins of the virus (6). While in the United States the prevalence of infection with HTLV-I appears to be quite low, blood used for transfusion is nevertheless being tested routinely by enzyme-linked immunosorbent (ELISA) and/or Western blot assays (7, 8). On this basis, the prevalence of HTLV-I infection among Americans without obvious risk factors, such as origin from an endemic region or intravenous drug abuse has been estimated to be about 0.016% (9). It may be as high as 0.1% in some locations (10). Our suspicion that infection with HTLV-I among Caucasian Americans may be higher than determined by routinely used methods was aroused by studies on the cutaneous T cell lymphoma mycosis fungoides (MF) (11, 12). MF patients usually do not have antibodies to the structural proteins of the virus, but harbor proviral sequences of HTLV-I in their peripheral blood mononuclear cells (PBMCs) and skin (11–14). A high percentage of these patients were also shown to have antibodies to HTLV-I Tax (15), an antigen not included in commercially available HTLV serologic test kits. This led us to begin a study of healthy relatives of MF patients who were serologically negative for antibodies to HTLV-I when their specimens were tested at a major blood transfusion center. Of the first eight individuals tested, six proved to have Tax sequences in their PBMCs and antibodies to the Tax antigen by Western blot analysis. Since family studies of a relatively rare disease manifested mostly in middle-aged and elderly individuals are time consuming, a more expeditious approach was chosen to determine whether currently used serologic methods are adequate to establish the true prevalence of HTLV infection among blood donors. Accordingly, a cohort of individuals among whom the prevalence of HTLV infection was known to be high, i.e., former injection drug users (IDUs), was selected for study. Matched sera and PBMCs obtained from 81 HIV-negative methadone clinic attendees were tested by routine serologic methods, as well as for Tax, pol and gag proviral sequences by PCR/Southern blot analysis and antibodies to viral structural proteins as well as to the Tax gene product. Routine serology proved 18/81 (22.2%) of these specimens to be positive for antibodies to HTLV, which concurred with results obtained by other investigators. On the other hand, 39/81 (48.1%) were found positive for HTLV proviral sequences by biomolecular means and 42 (51.8%) were positive when both serologic tests and PCR/Southern blot analyses were used. Together, the results of these studies suggest that the prevalence of infection with HTLV, particularly when efforts are made to detect Tax sequences, may be considerably higher than is currently believed.

MATERIALS AND METHODS

Study Subjects and Specimens. The eight healthy individuals who were relatives of Tax-positive MF patients consisted of (*i*) the wives of MF patients JH and LDeV, (*ii*) the mother of MF patient AD, (*iii*) the daughter of male MF patient JB, and (*iv*) the husband, two daughters, and one son of MF patient ZA. The MF patients were Caucasian Americans as reported elsewhere (12). Neither they nor their relatives had any recognized risk factors. The cells and sera were processed as described (12).

The IDU specimens consisted of paired sera and PBMCs obtained from 81 former IDUs enrolled in methadone maintenance programs of Bellevue Hospital, Beth Israel, and

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Abbreviations: HTLV-I, human T cell lymphotropic virus type I; MF, mycosis fungoides; MFR, relatives of MF patients; PBMCs, peripheral blood mononuclear cells; IDUs, injection drug users.

†To whom reprint requests should be addressed at: Department of Medicine, New York University Medical Center, Room TH-445, 550 First Avenue, New York, NY 10016.

Veteran Affairs Medical Centers in New York City. The specimens had been stored at −80°C for 2–3 years. The sera had tested negative for antibodies to HIV.

Detection of HTLV Proviral DNA Sequences in PBMCs by PCR/Southern Blot Analysis. The procedures used to prepare whole cell lysates of PBMCs, in addition to the conditions for PCR and Southern blot analysis using digoxigenin-tailed probes, have been reported in detail elsewhere (12, 16). Briefly, 1–2 × 10^5 PBMCs or cultured cells were lysed by boiling for 8–10 min in 55 μl autoclave-sterilized, distilled water, followed by incubation for 1 hr at 56°C in the presence of proteinase K (2 μg per sample) and subsequent boiling to inactivate the protease. Following preparation of lysates, 80 μl final sample reaction volumes containing 40 pmol of each primer, 2.5 mM $MgCl_2$, 1× PCR Buffer II, 200 μM each of dATP, dCTP, dGTP, and dTTP, and 4 units of *Taq* polymerase (Perkin–Elmer) and overlaid with 50 μl of autoclaved mineral oil, were subjected to 30 cycles of PCR amplification consisting of 1′ at 94°C, 1′ at 55°C, and 1.5′ at 72°C per cycle and a final 10′ incubation at 72°C, in a Perkin–Elmer/Applied Biosystems Model 480 Thermal Cycler. PCR samples were resolved through 4% ethidium bromide-stained agarose gels, followed by denaturation in 0.5 M NaOH/1.5 M NaCl, and neutralization in 1.5 M Tris·HCl/1.5 M NaCl (pH 7.5) for 15 min each, and Southern transfer to nylon membranes. Membranes were blocked and exposed to the appropriate digoxigenin-tailed HTLV probe and detection of bound probe was carried out using Fab′ fragments of antibodies to digoxigenin, conjugated with alkaline phosphatase. Reagents and procedures for tailing probes with digoxigenin and detection of bound probe were obtained from Boehringer Mannheim and used by us as reported previously (12, 16). The primers and probes used in this study (Table 1) included the HTLV gag-I primers and probe described by Hall *et al.* (17); pol-I/II primers, SK110 and SK111 and probes, SK112 (pol-I) and SK188 (pol-II) and Tax-I/II primers, SK43 and SK44 and probe, SK45 (18). The sequences and HTLV genome locations of additional gag primers and probes that have not been previously published include gag-I/II, sense and antisense primers: 5′-CCCATCTTACGTTCCCTAGC-3′ (HTLV-I: 1690–1709; HTLV-II: 1713–1732), 5′-GGATCTTGACATAGGGGGCA-3′ (HTLV-I: 1939–1958; HTLV-II: 1962–1981) and probe: 5′-AGGACACTGGAGTCGGGACTGCACCCAGCC-3′ (HTLV-I: 1890–1919; HTLV-II: 1913–1942). The gag-II primers included 5′-TCACGGGTTTCCCAACT-3′ (HTLV-II: 817–833), 5′-GGGCAGATAGGTGTCGGAAC-3′ (HTLV-II: 1107–1126) and probe: 5′-TGTCAAAAATCAAGTCTCCCCTAGCC-3′ (HTLV-II: 1067–1092). The genome sequences and locations reported are those of Seiki *et al.* (19) for HTLV-I and Shimotohno *et al.* (20) for HTLV-II. The conditions and temperatures for PCR amplification and hybridization using these primers and probes were the same as those for HTLV Tax (SK43, SK44, and SK45) and pol (SK110, SK111, and SK112) described previously (12, 16).

Table 1. HTLV-I/II primers and probes used in PCR/Southern blot analysis

HTLV region amplified	Primer (probe) designation	HTLV genome location	Ref.
gag I/II	BP4	1690–1709 (HTLV-I)	19
		1713–1732 (HTLV-II)	20
	BP5	1939–1958 (HTLV-I)	19
		1962–1981 (HTLV-II)	20
	(BP6)	1890–1919 (HTLV-I)	19
		1913–1942 (HTLV-II)	20
gag I	91-311	1402–1422	17
	91-312	1513–1535	17
	(91-320)	1424–1453	17
gag II	BP1	817–833	20
	BP2	1107–1126	20
	(BP3)	1067–1092	20
pol I/II	SK110	4757–4778 (HTLV-I)	18
		4735–4756 (HTLV-II)	18
	SK111	4919–4942 (HTLV-I)	18
		4897–4920 (HTLV-II)	18
	(SK112)	4825–4850 (HTLV-I)	18
	(SK118)	4880–4898 (HTLV-II)	18
tax I/II	SK43	7358–7377 (HTLV-I)	18
		7248–7267 (HTLV-II)	18
	SK44	7496–7516 (HTLV-I)	18
		7386–7406 (HTLV-II)	18
	(SK45)	7447–7486 (HTLV-I)	18
		7337–7376 (HTLV-II)	18

Ref., reference.

Detection of HTLV-I/II Antibodies by ELISA and Western Blot Assays. Sera were diluted 1:100 in tests for antibodies to HTLV-I and -II using the Diagnostic Biotechnology HTLV Blot 2.3 Western blot assay, obtained from Cellular Products. This test permits distinction between individuals seropositive for HTLV-I from those with antibodies to HTLV-II. The majority of IDU sera and all relatives of MF patients (MFR) sera were also tested at the New York Blood Center.

Antibodies to HTLV Tax were determined by ELISA and Western blot analyses using recombinant full-length tax-I (12, 15). Briefly, recombinant full-length HTLV Tax protein was prepared by cloning PCR-amplified proviral DNA sequences spanning the entire Tax open reading frame from the prototypic HTLV-I-infected cell line, C91PL (21), into the glutathione *S*-transferase (GST) fusion protein expression vector pGEX-2T (22). The recombinant GST-Tax-I fusion protein was expressed in *Escherichia coli* BL21 cells and purified by chromatography using glutathione linked to Sepharose 4B

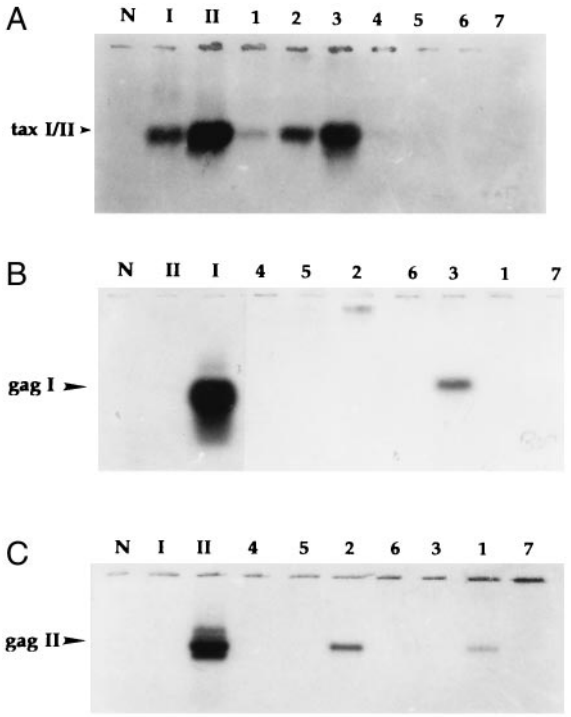

FIG. 1. Representative Southern blots of HTLV Tax and gag proviral sequences amplified by PCR in whole cell lysates of PBMCs from IDUs. (*A*) Detection of 159-bp Tax sequences. (*B*) Detection of 133-bp gag-I sequences. (*C*) Detection of 309-bp gag-II sequences. Lanes N, PBMC lysate from an HTLV PCR-negative healthy volunteer; Lane I, HTLV-I-infected cell line (C91PL); Lane II, HTLV-II-infected cell line (MoT); Lanes 1–7, seven different IDU PBMC lysates.

Table 2. HTLV proviral sequences in lysates of PBMCs from 81 IDUs

Sequences detected	HTLV-I seropositive	HTLV-II seropositive	Indeterminate serotype	HTLV-I/II seronegative
HTLV-I				
gag + pol + tax	1	0	0	1
gag or pol + tax	0	0	1	6
HTLV-II				
gag + pol + tax	0	8	0	0
gag or pol + tax	0	2	2	3
HTLV-I/II				
gag + tax	0	0	0	5
tax	0	1	0	9
PCR negative	0	3	0	39
PCR positive	1/1	11/14	3/3	24/63

HTLV sequences were detected by PCR/Southern blot analysis using the primers and probes listed in Table 1. By standard serology, 63/81 were negative.

(Pharmacia) and subsequent thrombin cleavage. The HTLV Tax antibody ELISA was carried out as described (12, 15). The Tax antibody Western blot assay was performed using purified, recombinant Tax-I protein, which was resolved through 8.5% preparative polyacrylamide gels and electrophoretically transferred to nitrocellulose. Sera from test subjects and controls were diluted 1:10 through 1:100 and Western blots were developed using goat anti-human IgA + IgG + IgM antibodies, conjugated with alkaline phosphatase (Pierce). The recombinant Tax protein was identified by Western blot using a polyclonal antiserum raised to recombinant full-length Tax protein expressed in a baculovirus expression system obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (23).

RESULTS

Detection of HTLV Proviral DNA Sequences in IDUs. Among HIV-negative methadone clinic attendees, the largest number of infected individuals were identified when HTLV-I/II Tax primers and probes were used in PCR/Southern blot analysis, independent of the serologic status of the donor. By this criterion alone, 39/81 (48.2%) were positive for infection with HTLV without consideration of HTLV-type specificity. Representative Southern blots of HTLV Tax and gag sequences amplified by PCR from lysates of PBMCs obtained from seven different IDUs are illustrated in Fig. 1. As can be seen, Tax and gag-II sequences were detected in the specimens of IDUs 1 and 2, while the lysate of IDU 3 proved positive for Tax and gag-I. Only Tax sequences were present in the samples from IDU 4 (faint band visible in Fig. 1*A*), while no HTLV-related sequences were found in the cell lysates of IDUs 5, 6, and 7. No proviral sequences related to Tax, gag-II, or pol II were found in three cell lysates whose matched sera had antibodies specific for HTLV-II. However, among the 63 individuals who were not expected to be HTLV-infected on the basis of standard serologic tests, there were seven whose cell lysates on PCR/Southern blot analysis revealed gag-I and/or pol-I in addition to Tax sequences (Table 2). Moreover, one of the three individuals who were serologically HTLV-type indeterminate also harbored pol-I sequences in PBMCs, thus bringing the total number found to be infected with HTLV-I to 9/81 (11.1%), which is approximately 10 times higher than expected by routine serology alone. Among the seronegatives, four additional specimens were identified as being infected with HTLV-II by virtue of their having gag-II or pol-II sequences in addition to Tax, thus, also raising the number of HTLV-II-infected individuals beyond that expected by serology alone. Ten former IDUs harbored only Tax sequences, but no specimens were found to have only gag or pol sequences. Five of these 10 individuals had Tax antibodies (Table 3).

Detection of HTLV-I/II Antibodies. Using standard serologic tests, i.e., those employed in the New York Blood Center, which measure antibodies to HTLV-I/II gag and env antigens, a total of 18 of 81 sera (22%) were found to be positive. When type-specific antigens were used, only 1 of the 81 IDUs tested was positive for antibodies to HTLV-I, whereas 14 had detectable antibodies specific for HTLV-II and 3 had to be classified as HTLV-type-indeterminate (Table 2).

When the same 81 sera were tested for antibodies to HTLV Tax, a total of 14 IDUs was found to be positive (Table 3). Among these were nine individuals who had already been identified as seropositive when the viral structural proteins had been used as antigens. Therefore, the Tax antibody tests revealed 5 additional HTLV positive individuals who had been missed by routine serology tests, thus increasing the total number of serologically positive IDUs to 23 (28.4%). Thus, the use of combined serology, including the use of Tax antigens and biomolecular techniques, established that the percentage of HTLV infection among the group of 81 HIV–seronegative IDUs tested was 42/81 (51.9%), which more than doubled the prevalence detected by routine serology. A summary of the combined results obtained on the IDU specimens is shown in Table 4.

Detection of Tax Sequences and Antibodies in Relatives of MF Patients. The freshly isolated lymphocytes of the following healthy relatives of previously reported MF patients (12) were positive for HTLV-I Tax sequences: (*i*) the wives of patients JH and LDeV, (*ii*) the mother of 16-year-old MF patient AD, (*iii*) the daughter of male MF patient JB, (*iv*) the husband and eldest daughter of MF patient ZA, whereas the younger daughter and son of patient ZA were negative. The PCR/Southern blot analysis results are shown in Fig. 2. All Tax sequence-positive healthy individuals also had antibodies to Tax by Western blot assay in the absence of demonstrable antibodies to the structural proteins of the virus (Fig. 3).

Table 3. HTLV Tax antibodies in sera from 81 IDUs

	HTLV-I seropositive	HTLV-II seropositive	Indeterminate serotype	HTLV seronegative
Tax antibody positive	1/1	7/12	1/3	5/63

HTLV Tax antibodies were detected by Western blot analysis. In each column the denominator denotes results obtained by standard serology, whereas the numerator denotes detection of antibodies to Tax specifically, i.e., 63/81 IDUs were HTLV-I/II seronegative by standard serology.

Table 4. Prevalence of HTLV-I/II infection in HIV–seronegative IDUs

Category	No. (%)
HTLV seropositive by standard serology	18 (22.2)
Positive for Tax proviral DNA sequences	39 (48.2)
Positive for HTLV antibodies plus DNA sequences	42 (51.8)

Total number of IDUs, 81.

DISCUSSION

In the course of interviewing healthy MFR it was learned that some are frequent blood donors. This led us to initiate a study testing for Tax sequences and antibodies to Tax in such individuals. As of this writing, the cells and sera of eight MFRs have been analyzed. Six of these proved to be Tax sequence-positive, as well as having antibodies to Tax on Western blot assays. Since some relatives of our patients with MF are repeat blood donors whose HTLV carrier state had escaped detection by routinely used serologic tests, it seemed of interest to establish to what extent currently used analyses may underestimate the prevalence of HTLV infection. Such studies were facilitated by the availability of stored sera and matched isolated cells procured from methadone treatment centers affiliated with our institution. The data recorded in Tables 2 and 4 show that about 22% of HIV-negative methadone clinic attendees would have been reported as infected with HTLV-I/II, had they been tested only by standard serologic methods used universally since 1988. This is in good agreement with similar studies performed by others on IDUs in our geographic region (24). If the Tax antigen had been included in such serologic tests, 23 of 81 (28.4%) would have been considered positive. However, the largest number of infected individuals, 39/81 or 48.2% was detected with the use of the primers/probes for HTLV Tax in PCR/Southern blot analysis of PBMC lysates. Therefore, like patients with MF, a significant number of IDUs also harbor defective or variant rather than complete proviral sequences, at least as detected by currently available methods. Only 14 of the 39 individuals who had demonstrable Tax proviral sequences had developed antibodies specific for the Tax protein. Among Tax sequence-positive patients with MF, more than 80% were shown to have Tax antibodies and all had Tax mRNA (15). It is noteworthy that all relatives of MF patients whose lymphocytes harbored Tax sequences had antibodies to this protein as well. It is well recognized that the immune response of IDUs is often impaired which, in some individuals, could account for their inability to develop antibodies to Tax. It should be noted that the antigen used here was specific for Tax-I, whereas the majority of IDUs are infected with HTLV-II (24). Among HTLV carriers in an endemic area in Japan, less than 60% of HTLV-seropositive individuals had demonstrable antibodies to this protein (25). Conversely, in this study, three IDUs were serologically positive without having demonstrable proviral sequences. This could simply be due to accidental mislabeling of archival specimens. However, in a large study of HTLV-seropositive donors that was designed to distinguish HTLV-I from HTLV-II by PCR, proviral sequences also failed to be detected in some donors (25, 26). It is possible that in such individuals the viral footprints are sequestered in lymphoid tissue and not associated with circulating PBMCs subjected to analysis.

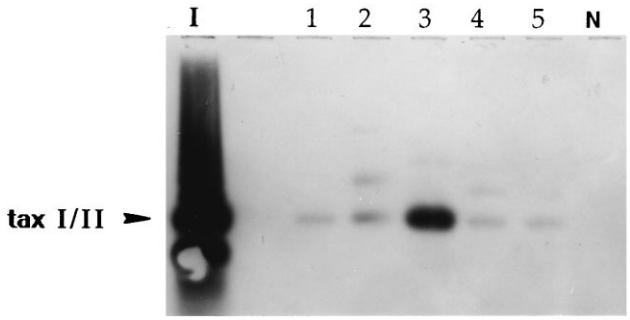

FIG. 2. Southern blot of HTLV Tax proviral sequences amplified by PCR in PBMC lysates from relatives of MF patients. Lane I, HTLV-I-infected cell line (C91PL); Lanes 1–5 and N, PBMC lysates from daughter of HB, daughter of ZA, wife of DeV, wife of JH, mother of AD, and an HTLV proviral sequence and antibody-negative, healthy volunteer, respectively.

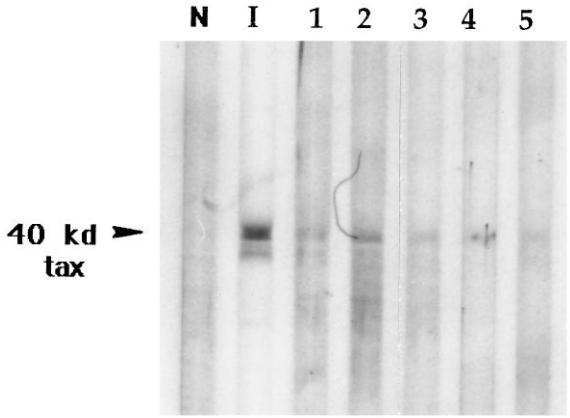

FIG. 3. Western blot of HTLV Tax antibodies in plasmas from relatives of MF patients. Lane N, serum from an HTLV antibody and proviral sequence-negative healthy volunteer; Lane I, rabbit antiserum to HTLV-Tax-I; Lanes 1–5, sera from daughter of HB, daughter of ZA, wife of JH, mother of AD, and wife of DeV, respectively.

Of further interest was the finding that specimens of 9 of the 81 IDUs tested (11%) were characterized as being HTLV-I-infected. Previous studies using type-specific serologic tests on specimens obtained from IDUs have underscored the preponderance of infection with HTLV-II and the relative rarity of infection with HTLV-I in such cohorts. Although the predominance of infection with HTLV-II in IDUs held true also for the observations reported here, the number of specimens positive for HTLV-I was markedly higher than anticipated. Thus, this study clearly reflects the inadequacy of currently used serologic tests to identify all individuals infected with HTLV-I/II. Transmission of these viruses by transfusion has been well documented. Whether the transfusion of cells harboring incomplete proviral sequences, such as Tax, is hazardous for their recipients is not known. It is disconcerting, however, that it is the Tax sequence/protein that appears to be retained preferentially and that Tax is the transforming/transactivating gene product of HTLV-I/II (reviewed in refs. 4 and 27).

A few years ago, when it was realized that infection with HTLV-II was not always detected by serologic tests then in use, type II-specific antigens were included in test kits. The time has come once again to reevaluate currently used methods for the detection of these viruses. Either the inclusion of Tax in serologic test kits or more sensitive techniques incorporating simultaneous amplification of several relevant nucleic acid sequences by an automated system as has been suggested elsewhere (28) could be introduced to screen our blood supply for HTLV more reliably.

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1. Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D. & Gallo, R. C. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7415–7419.
2. Yoshida, M., Miyoshi, I. & Hinuma, Y. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2031–2035.
3. Takatsuki, K., Hinuma, Y. & Yoshida, M., eds. (1992) *Gann Monograph on Cancer Res. No. 39* (CRC, Boca Raton, FL).
4. Gallo, R. C. & Wong-Staal, F., eds. (1990) *Retrovirus Biology and Human Disease* (Dekker, New York).
5. Franchini, G. (1995) *Blood* **86,** 3619–3639.
6. Hinuma, Y., Komoda, H., Chosa, T., Kondo, T., Kohakura, M., Takenaka, T., Kikuchi, M., Ichimaru, M., Yunoki, K., Sato, I., Matsuo, R., Takiuchi, Y., Uchino, H. & Hanaoka, M., (1982) *Int. J. Cancer.* **29,** 631–635.
7. Centers for Disease Control and Prevention (1988) *Morbid. Mortal. Wkly. Rep.* **37,** 736–740, 745–747.
8. Williams, A. E., Fang, C. T., Sullivan, M. T., Starkey, J., Chernoff, A., Epstein, J. S. & Gross, T. P. (1989) *Morbid. Mortal Wkly. Rep.* **39,** 915–924.
9. Lee, H. H., Swanson, P., Rosenblatt, J. D., Chen, I. S., Sherwood, W. C., Smith, D. E., Tegtmeier, G. E., Fernando, L. P., Fang, C. T., Osame, M. & Kleinman, S. H. (1991) *Lancet* **337,** 1435–1439.
10. Williams, A. E., Fang, C. T., Slamon, D. J., Poiesz, B. J., Sandler, S. G., Darr, W. F., Shulman, G., McGowan, E. I., Douglas, D. K., Bowman, R. J., Peetoom, F., Kleinman, S. H., Lenes, B. & Dodd, R. Y. (1988) *Science* **240,** 643–646.
11. Zucker-Franklin, D., Coutavas, E. E., Rush, M. G. & Zouzias, D. C. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7630–7634.
12. Pancake, B. A., Zucker-Franklin, D. & Coutavas, E. E. (1995) *J. Clin. Invest.* **95,** 545–554.
13. Zucker-Franklin, D., Pancake, B. A. & Friedman-Kien, A. E. (1994) *AIDS Res. Human Retroviruses.* **10,** 1173–1177.
14. Khan, Z., Sebenik, M. & Zucker-Franklin, D. (1996) *J. Invest. Dermatol.* **106,** 667–672.
15. Pancake, B. A., Wassef, E. H. & Zucker-Franklin, D. (1996) *Blood* **88,** 3004–3009.
16. Pancake, B. A. & Zucker-Franklin, D. (1996) *J. Acquired Immune Defic Syndr. Hum. Retroviruses* **13,** 314–319.
17. Hall, W. W., Liu, C. R., Schneewind, O., Takahashi, H., Kaplan, M. H., Roupe, G. & Vahlne, A. (1991) *Science* **253,** 317–320.
18. Kwok, S., Kellogg, D., Ehrlich, G., Poiesz, B., Bhagavati, S. & Sninsky, J. J. (1988) *J. Infect. Dis.* **158,** 1193–1197.
19. Seiki, M., Hattori, S., Hirayama, Y. & Yoshida, M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3618–3622.
20. Shimotohno, K., Takahashi, Y., Shimizu, N., Gojobori, T., Golde, D. W., Chen, I. S., Miwa, M. & Sugimura, T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3101–3105.
21. Popovic, M., Sarin, P. S., Robert-Guroff, M., Kalyanaraman, V. S., Mann, D., Minowada, J. & Gallo R. C. (1983) *Science* **219,** 856–859.
22. Smith, D. B. & Johnson, K. S. (1988) *Gene* **67,** 31–40.
23. Jeang, K.-T., Giam, C.-Z., Nerenberg, M. & Khoury, G. (1987) *J. Virol.* **61,** 708–713.
24. Palumbo, P. E., Weiss, S. H., McCreedy, B. J., Alexander, S. S., Denny, T. N., Klein, C. W. & Altman, R. (1992) *J. Infect. Dis.* **166,** 896–899.
25. Okayama, A., Korber, B., Chen, Y., Allan, J., Lee, T. H., Shioiri, S., Tachibana, N., Tsuda, K., Mueller, N., McLane, M. F., Maayan, S., Orgado, S., Ernst, J., Marlink, R. & Essex, M. (1991) *Blood* **78,** 3323–3329.
26. Shioiri, S. T., Tachibana, N., Okayama, A., Ishihara, S., Tsuda, K. & Essex, M. (1993) *Int. J. Cancer.* **53,** 1–4.
27. Yoshida, M. (1994) in *The Molecular Basis of Blood Diseases*, eds. Stamatoyannopoulos, G., Nienhuis, A., Majeris, P. W. & Varmus, H. (Saunders, Philadelphia), pp. 929–941.
28. Sunzeri, F. J., Lee, T. H., Brownlee, R. G. & Busch, M. P. (1991) *Blood* **77,** 879–886.