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# Detection of Human T-Lymphotropic Virus (HTLV) *tax* Sequences in New York City Blood Donors Seronegative for HTLV Types 1 and 2

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**A potential public health concern is the reported detection of the human T-lymphotropic virus (HTLV) *tax* gene in the lymphocytes of up to 11% of a low-risk group of New York City blood donors (NYBD). This study aimed to independently confirm the prevalence of HTLV *tax* sequences in 293 NYBD. All NYBD tested negative for antibodies to HTLV types 1 and 2 and HTLV Tax. HTLV *tax* sequences were not detected in the NYBD lymphocytes. These data demonstrate the lack of HTLV-1 *tax* in this group of NYBD at low risk for HTLV infection.**

Human T-lymphotropic virus type 1 (HTLV-1) is etiologically associated with two major diseases, adult T-cell leukemia and HTLV-1-associated myelopathy–tropical spastic paraparesis (HAM-TSP) (3). HTLV-2 is a closely related retrovirus but has not been etiologically associated with any disease. Worldwide, HTLV infects 15 to 20 million people (2, 8) via sexual (primarily male-to-female), vertical (mother-to-child), and parenteral (drug use and blood transfusion) routes. Because of the high risk of transfusion-related transmission, the United States began to screen donated blood for HTLV-1 infection in 1988 and for HTLV-2 infection in 1997 by means of enzyme immunoassays (EIAs). These assays have high specificity and sensitivity for HTLV-1 and -2 and have effectively prevented HTLV transmission through blood and its components. In the United States, the incidence rate of HTLV in blood donors is estimated to be 1.59 per 100,000 person-years (4), and the residual risk of transmitting HTLV infection by transfusing screened blood is estimated to be 1 in 641,000 units (15).

Two papers by Zucker-Franklin et al. (19, 20) have reported HTLV-1 and -2 *tax* gene sequences in the lymphocytes of healthy blood donors, i.e., persons who had no known risk factors for HTLV-1 or -2 and who were serologically HTLV negative according to licensed screening assays. In the first study, 11 of 100 randomly selected blood donors at the New York University Medical Center Blood Bank had the HTLV *tax* sequence. Further, 8 of these 11 had antibodies to the Tax protein as determined by the Western blot assay (20). A subsequent study of 250 healthy persons (210 blood donors and 40 volunteers at New York University Medical Center) reported that 22 persons tested positive for the HTLV *tax* sequence. Plasma samples from these 22 blood donors were Western blot reactive to the HTLV Tax protein (19). These studies led to the speculation that some healthy blood donors might harbor a defective virus containing only HTLV *tax* sequences and that such blood was escaping detection by standard serological assays for HTLV-1 and -2.

To address this issue, a comprehensive, multicenter study was designed to determine the prevalence of HTLV *tax* sequences in 100 randomly selected HTLV-seronegative blood donors from the Baltimore, Md.-Washington, D.C., area (1). The samples were processed and prepared by the American Red Cross and sent without identification of donor to four testing centers. The PCR procedure used by the original investigators was followed, and HTLV *tax* sequences were not detected in any of the donors.

Despite these findings, a question remained about whether the demographics of the blood donors from New York City might differ from those of the donors in the Baltimore-Washington area. Therefore, for this study, we obtained during the fall of 1999 heparinized blood from randomly selected New York City blood donors (NYBD) who were seronegative for HTLV-1 and HTLV-2 and tested the samples for the presence of HTLV *tax* sequences by PCR and for anti-Tax antibodies. To ensure that we could detect with 95% assurance at least one person with HTLV *tax* sequences, in accordance with reported prevalence rates, 300 NYBD specimens were collected. Plasma samples and lymphocyte cell pellets were isolated from whole blood as previously described (11) and stored at −80°C until they were tested. Seven of the 300 NYBD samples were not usable because of sample clotting and were not included in this analysis. Additionally, heparinized blood was obtained from nine HTLV-1-infected persons and processed in a similar manner to serve as PCR-positive controls.

The 293 NYBD specimens were HTLV-1 and -2 serologically negative according to the Vironostika HTLV-I/II Microelisa System (bioMérieux, Durham, N.C.) (Fig. 1). To determine if antibodies to HTLV-1 Tax could be detected, plasma specimens were assayed with a peptide-based HTLV-1 Tax EIA (6). Twenty of the 293 plasma samples were initially re-

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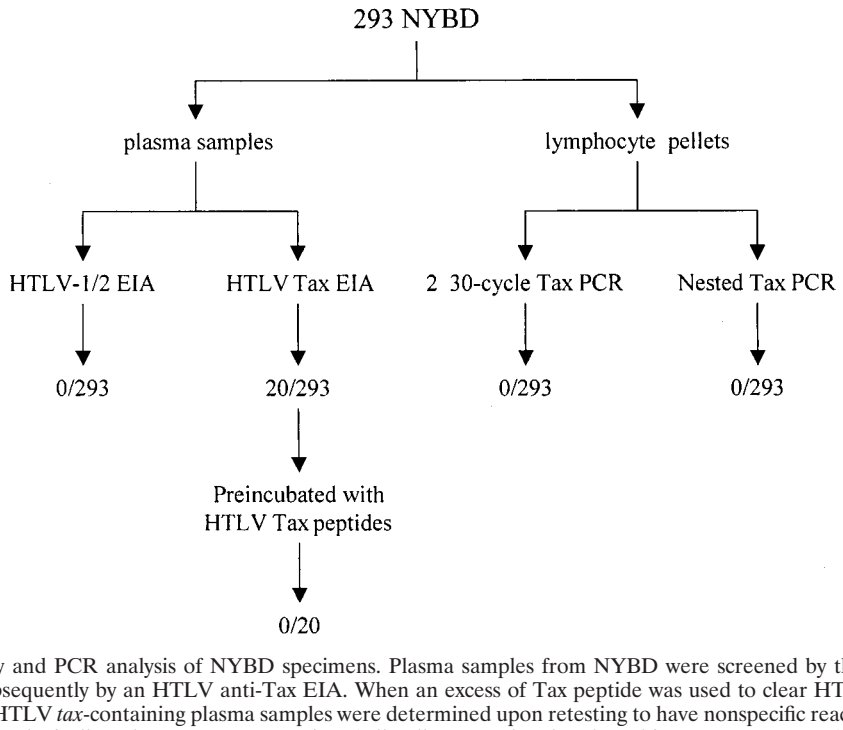


FIG. 1. HTLV serology and PCR analysis of NYBD specimens. Plasma samples from NYBD were screened by the Vironostika HTLV-I/II Microelisa System and subsequently by an HTLV anti-Tax EIA. When an excess of Tax peptide was used to clear HTLV Tax-specific reactivity, 20 of the initially reactive HTLV *tax*-containing plasma samples were determined upon retesting to have nonspecific reactivity. These samples were subsequently considered serologically to be HTLV *tax* negative. Cell pellets were lysed and used in two HTLV *tax* PCR methods, both resulting in no HTLV *tax*-positive NYBD specimens. Eight of nine HTLV-positive specimens were positive for *tax* gene sequences by PCR.

active; however, when the plasma samples were preincubated with excess Tax peptides and the EIA was rerun, all samples remained reactive, while HTLV-positive plasma reactivity was removed by the preincubation. These data indicate that the reactivity of the 20 plasma samples was not specific to HTLV-1 Tax (Fig. 1); therefore, these specimens were considered negative for HTLV-1 Tax by EIA.

For PCR analysis, lymphocyte cell pellets were processed in a blind fashion according to the protocol of Pancake et al. (11), with nine HTLV-1-positive cell pellets randomly placed among the 293 NYBD cell pellets. Cell lysates were subjected to two 30-cycle PCR assays for HTLV *tax*. The PCR products were separated through a 2% agarose gel and subjected to Southern blotting. HTLV *tax*-specific bands were detected by hybridization to a digoxigenin-labeled SK45 probe (Genius; Roche Applied Science, Indianapolis, Ind.) and developed. Eight of the nine HTLV-1-positive samples were positive for *tax* by this PCR method. None of the 293 NYBD specimens was positive for HTLV *tax* sequences (Fig. 1), which we confirmed by using a nested-PCR *tax* assay (18). The nested-PCR *tax* assay also confirmed the presence of the *tax* gene in the same eight of nine HTLV-1-positive samples previously tested. Using this nested-PCR method, we were able to detect one HTLV *tax* sequence per 100,000 cells, which was similar to the results of the two 30-cycle PCRs.

The published prevalence rate of 11% for HTLV *tax* sequences in healthy blood donors from the New York City area (19, 20) suggested that our screening of 293 NYBD would detect approximately 32 individuals harboring the HTLV *tax* sequence. However, no individuals tested positive for HTLV *tax* sequences or for antibodies to the HTLV Tax protein. Our work has therefore confirmed the findings of the earlier multicenter study (1). Furthermore, the results indicate that low-risk blood donors, regardless of their geographical locations, do not have HTLV *tax* sequences. The reasons for the discrepancy between our study and the previous studies examining NYBD are not clear. Methods for processing the blood specimens, handling the cell pellets, and performing the PCR were identical in the present and all previous studies (11, 19, 20). Therefore, the inability to detect HTLV *tax* sequences in the lymphocytes in the present study cannot be ascribed to procedural differences. Moreover, we used a highly sensitive, nested-PCR method for *tax* to confirm our original PCR results.

Defective HTLV proviruses that code for the *tax* gene in the absence of other viral genes are readily found in confirmed HTLV-infected adult T-cell leukemia and HAM-TSP patients and asymptomatic carriers (10). It remains unclear how these defective *tax*-only viruses may contribute to viral pathology. Shuh et al. (16) described such a defective virus and showed that expression of *tax* in vitro depends on *trans*-activating factors produced from intact proviruses and that the *tax*-only virus is not expressed alone. Interestingly, two other studies described persons who are seronegative or seroindeterminate for HTLV-1 and HTLV-2 and who have an illness resembling HAM-TSP; in both studies, the HTLV *tax* gene sequences were detected while other gene sequences were not (13, 17). While defective *tax*-only proviruses exist in some high-risk groups, a more recent study shows that lymphocytes of serologically indeterminate blood donors do not harbor defective HTLV proviruses (9). Further, several other studies show that low-risk HTLV-seronegative or -seroindeterminate persons, such as blood donors, with no clinical HTLV symptoms had no

evidence of HTLV infection (5, 7, 14). Collectively, these data suggest that seronegative or seroindeterminate persons with HTLV-like illnesses and/or risk factors for HTLV infection may have defective HTLV proviruses, may harbor low copy numbers of HTLV, or may have a novel retrovirus with partial homology to HTLV and therefore warrant further study. Conversely, low-risk, symptom-free, seronegative or seroindeterminate persons are not HTLV infected.

The results from this study and the earlier multicenter study (1) suggest that the prevalence of HTLV *tax* sequences in low-risk blood donors is not as high as previously reported (19, 20) and provide additional support that the risk of transmission of HTLV by screened blood products is remote (12, 15).

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