*Proc. Natl. Acad. Sci. USA*
Vol. 88, pp. 1182–1186, February 1991
Medical Sciences

# Sexual transmission of human T-cell leukemia virus type I associated with the presence of anti-Tax antibody

(seroconversion/envelope protein/prospective study)

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*Communicated by Ludwik Gross, November 16, 1990 (received for review August 7, 1990)*

**ABSTRACT The *tax* gene product (Tax protein) of human T-cell leukemia virus type I (HTLV-I) is a specific transcriptional activator of the viral long terminal repeat sequence and is essential for the replication cycle of the virus. To elucidate the relationship between the presence of anti-Tax antibody and the transmission of the viral infection, annual consecutive serum samples from married couples serologically discordant or concordant for HTLV-I were examined. These included 5 individuals whose spouses seroconverted during this 5-year follow-up study period. The samples were tested by a Western blot assay using a recombinant Tax protein as the antigen. The results showed that 24 of 32 (75%) men in the concordant couples (both husband and wife were HTLV-I carriers) had anti-Tax antibody, while only 5 of 18 (27.8%) men in the discordant couples (husband was carrier and wife was seronegative to HTLV-I) were positive for anti-Tax antibody ($P$ = 0.0012). Furthermore, all spouses of the 5 seroconverters (4 women and 1 man) had anti-Tax antibody, while only 23 of 46 (50%) age-matched randomly selected HTLV-I carriers from the discordant-couple group had anti-Tax antibody. When the data were analyzed by gender, all husbands of the female seroconverters had anti-Tax antibodies, which was significantly higher than the prevalence of anti-Tax antibodies in men who did not transmit the virus to their spouses during the follow-up period ($P$ = 0.017). In addition, antibody reactivity to other HTLV-I antigens (including Env gp46, transmembrane protein gp21, and Gag p19 and p24) were examined. The results indicated no significant difference between the prevalence of antibody reactivity to any of the antigens in the spouses of the seroconverters and the reference group. We conclude that the presence of anti-Tax antibody in men may indicate a high risk of viral transmission to their wives via heterosexual routes.**

The identification of serological markers that are associated with transmissibility of human retroviruses is important for understanding the natural history of the viral infection and for planning appropriate preventive strategy. Attempts have been made to define types of sexual behavior or other factors that are associated with sexual transmission of human immunodeficiency virus (1) or human T-cell leukemia virus (HTLV) (2). Among heterosexuals, there appears to be a relation between the acquisition of the viral infection and the number of sexual partners (2–4). However, the inability to detect a consistent association between the seropositivity, the length of relationship, the number of sexual contacts, and specific sexual practices suggests that there may be important biological determinants of viral infection in the carriers.

HTLV type I (HTLV-I) has been etiologically associated with adult T-cell leukemia (5–7) and HTLV-I-associated myelopathy/tropical spastic paraparesis (8–10). Aside from the classic structural genes (*gag*, *pol*, and *env*) shared by all retroviruses, HTLV-I has an additional regulatory gene, *X*, at the 3′ end of its genome (11). The overlapping open reading frames of the *X* gene can encode three proteins: p40, p27, and p21. It is known that protein p40 (Tax) is a potent transactivator of transcription from the viral long terminal repeat (12, 13) and that protein p27 (Rex) can induce the expression of the unspliced messenger RNAs encoding the viral structural proteins (14). The role of p21 is still unknown. In general, antibodies to structural proteins are detected more efficiently than antibodies to regulatory proteins when sera from infected people are analyzed (15).

Since HTLV-I Tax protein can enhance replication of HTLV-I itself and can stimulate proliferation of lymphocytes harboring virus through activation of some cellular genes [e.g., interleukin 2 (IL-2) receptor gene, IL-2 gene] (16–19), we hypothesized that the presence of anti-Tax antibody might be associated with efficiency of viral transmission. HTLV-I can be transmitted through three major routes: sexual contact (20, 21); exposure to contaminated blood products, as through transfusion or needle-sharing (22, 23); or mother-to-child, as through breast-feeding and transplacental or perinatal transmission (24, 25). In a 5-year prospective follow-up study conducted in Miyazaki, Japan, a total of five seroconverters have been identified among married couples who were anti-HTLV-I antibody-discordant (26). Since sexual contact with an infected spouse was the most likely source of exposure, we used a recombinant Tax protein (27) to analyze the relationship between sexual transmission of HTLV-I and the presence of anti-Tax antibody. Consecutive serum samples from spouses of seroconverters and other HTLV-I carriers belonging to serodiscordant or concordant married-couple groups were evaluated.

## METHODS

**Subjects.** The Miyazaki follow-up study (26) was initiated in 1984, in conjunction with a government-sponsored annual physical checkup program, to follow adult residents in two HTLV-I-endemic villages in the southern Miyazaki prefecture. Serum samples were obtained at the time of examination, tested by the particle agglutination method (28), and confirmed by indirect immunofluorescence assay. By the end of 1986, 433 married couples (866 individuals, with a mean age of 55 years) were included in the study population.

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Abbreviations: HTLV, human T-cell leukemia virus; IL-2, interleukin 2; TM, transmembrane.

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Among these, 5 seroconverters prospectively identified in the study, as well as their spouses, were selected for further evaluation. Forty-six couples, randomly selected from 76 age-stratified HTLV-I-serodiscordant married couples whose serological status to HTLV-I remained unchanged, were used as a reference group. The other reference group was composed of 32 seroconcordant couples (H+, W+; both husband and wife were HTLV-I carriers), with the same age distribution as, and randomly selected from, the 88 such couples in the study.

**Particle Agglutination Assay.** A commercially available kit, Serodia ATLA (Fujirebio, Tokyo), was used for this study (28). The viral antigen was purified from an HTLV-I-producing Japanese cell line, TCL-Kan. Serum samples were titrated at serial 2-fold dilutions until reaching a dilution of 1:8192.

**HTLV-I Recombinant Proteins.** Three recombinant proteins (RP-A, -B, and -C) containing different regions (N-terminal, middle, and C-terminal) of HTLV-I exterior Env glycoprotein gp46; a recombinant transmembrane (TM) protein (RP-D; ref. 29); and a recombinant Tax protein (27) were used in Western blot assays.

**Radioimmunoprecipitation.** For analyses, MT2 cells (30) were labeled for 6 hr in cysteine-free RPMI-1640 medium supplemented with 2% heat-inactivated fetal bovine serum, L-glutamine (4 mM), penicillin (50 $\mu$g/ml), streptomycin (50 $\mu$g/ml), and [$^{35}$S]cysteine (New England Nuclear; 100 $\mu$Ci per $10^6$ cells per ml; 1 $\mu$Ci = 37 kBq). Cells were then collected by centrifugation for 10 min at 2000 $\times$ $g$ and extracted with 0.5 ml of RIPA buffer [0.15 M NaCl/0.05 M Tris·HCl, pH 7.2/1% (vol/vol) Triton X-100/1% (wt/vol) sodium deoxycholate/0.1% (wt/vol) SDS]. The supernatant of the cell lysate was collected by ultracentrifugation at 100,000 $\times$ $g$ for 60 min. Ten microliters of the various human serum samples was then added to aliquots of the supernatant (about 50 $\times$ $10^6$ cpm) and the mixture was incubated for 2 hr at 4°C. Next, 100 $\mu$l of 10% pre-swollen protein A-Sepharose (Sigma) was added to the immune complex and incubated overnight at 4°C. After incubation, the mixture was washed five times in washing buffer (RIPA buffer without sodium deoxycholate). The washed immunoprecipitates were added to gel-loading buffer, boiled for 5 min, and then analyzed by SDS/12.5% PAGE. After electrophoresis, the gels were soaked in enhancer solution (New England Nuclear) for 1 hr and cold tap water for 1 hr. The resulting gels were dried and exposed to Kodak X-Omat film at −80°C.

**Western Blot Assay.** For Western blot analysis, 9 $\times$ $10^6$ MT2 cells were lysed in RIPA buffer, and the resulting high-speed supernatant was analyzed by SDS/12.5% PAGE. For analysis of recombinant proteins, partially purified recombinant proteins solubilized in 3 or 8 M urea were used as antigen for SDS/15% PAGE. After electrophoresis, the gels were passively blotted onto two pieces of nitrocellulose membranes for 36 hr. The nitrocellulose membranes were blocked with 3% bovine serum albumin (Sigma) in phosphate-buffered saline and then cut into 3-mm strips to which diluted (1:200) serum samples were added. Details of the immunoblotting assay have been described (31). To determine the titers of anti-Tax antibody, serum samples were titrated at serial 2-fold dilutions from 1:2000 to 1:32,000 for Western blot assay.

## RESULTS

By the summer of 1989, 4 women and 1 man belonging to a subset of 81 serodiscordant couples from the Miyazaki follow-up study had become seropositive for HTLV-I. The antibody status of these five individuals, aged 50–64 years at seroconversion, was confirmed by both Western blot and radioimmunoprecipitation assays (Table 1). All of the seroconverters were married to HTLV-I carriers for >24 years (mean = 32 years). Although we do not know when the spouses of the seroconverters became HTLV-I carriers, according to the serological data available, they were HTLV-I carriers (confirmed by Western blot and radioimmunoprecipitation; data not shown) at least 1–2 years before their sexual partners seroconverted.

Since none of the seroconverters had a history of blood transfusion, and since they reside in relatively closed, conservative villages, it seems likely that they were infected with HTLV-I through sexual contact with their spouses, although sexual behavior data are not available. In addition to the 5 seroconverted couples mentioned above, we randomly selected 46 couples for comparison, based on village of origin and matched by age, from the group comprising the remaining 76 serodiscordant couples in the study. We then confirmed their antibody status to HTLV-I by both Western blot and radioimmunoprecipitation and studied their seroreactivity to different HTLV-I viral antigens by Western blot assays.

We evaluated the reactivity to a comprehensive set of serological markers for an association with the transmission of HTLV-I infection. These included a recombinant Tax protein; two Gag proteins (p24 and p19); and a panel of recombinant proteins containing N-terminal, middle, or C-terminal regions of the HTLV-I external glycoprotein gp46 (RP-A, -B, and -C) and the entire length of the TM protein gp21 (RP-D). Antibody reactivity to the Gag proteins p24 and p19 was analyzed by Western blot using MT2 cell lysate as antigen. All spouses of the 5 seroconverters had antibody to HTLV-I TM, Tax, and Gag proteins (Table 2). In terms of antibody reactivity to the HTLV-I external glycoprotein gp46, 2 spouses had antibody to the C-terminal regions of gp46 (RP-C) alone, while the other 3 spouses had antibody to both the middle and the C-terminal regions of gp46 (RP-B and -C). None of the spouses had antibody to the N-terminal half of gp46 (RP-A). When we compared the results with the serological profiles of the other 46 HTLV-I carriers whose spouses remained seronegative during the 5-year follow-up period, we found that, between males who presumably transmitted the virus and those who did not (husbands of female seroconverters vs. men from the H+,W− serodiscor-

Table 1. Characteristics of five seroconverters for HTLV-I antibodies by Western blot (WB) and radioimmunoprecipitation (RIP) assays

| Subject | Sex | Age* | Years of marriage† | Antibody status to HTLV-I, WB/RIP | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1984 | 1985 | 1986 | 1987 | 1988 | 1989 |
| 1 | F | 61 | 39 | NT | NT | −/− | +/+ | +/+ | +/+ |
| 2 | F | 58 | 33 | NT | −/− | NT | NT | NT | +/+ |
| 3 | F | 64 | 40 | −/− | −/− | NT | +/+ | +/+ | +/+ |
| 4 | F | 50 | 25 | −/− | −/− | −/− | +/+ | +/+ | +/+ |
| 5 | M | 55 | 25 | NT | −/− | +/+ | +/+ | +/+ | +/+ |

NT, not tested.
*Age when seroconversion was detected.
†Length of marriage when seroconversion was detected.

Table 2. Antibody reactivities of the spouses of HTLV-I seroconverters to HTLV-I-specific antigens in Western blots

| | | | Antibody reactivity | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | gp46† | | | | | |
| Spouse* | Sex | Age | A | B | C | TM‡ | Tax§ | Gag p24/19¶ |
| 1 | M | 60 | – | – | + | + | + | +/+ |
| 2 | M | 61 | – | + | + | + | + | +/+ |
| 3 | M | 56 | – | + | + | + | + | +/+ |
| 4 | M | 50 | – | + | + | + | + | +/+ |
| 5 | F | 46 | – | – | + | + | + | +/+ |

*Matched with subject no. shown in Table 1.
†Recombinant proteins RP-A, -B, and -C contain N-terminal, middle-, and C-terminal regions of HTLV-I gp46, respectively.
‡Recombinant TM protein contains the whole length of gp21.
§Recombinant transactivator protein contains the whole length of Tax.
¶Assay by Western blot using MT2 cell lysate as the antigen.

dant couple group), there was no difference in antibody reactivity to any of those antigens mentioned above, except for the Tax protein.

All 5 spouses of the seroconverters had anti-Tax antibody, while only 23 of 46 (50%) HTLV-I carriers from the serodiscordant couple group had anti-Tax antibody ($P = 0.056$) (Table 3). When we further analyzed the data according to gender, we found that the difference in Tax reactivity between males who apparently transmitted the virus and males who did not transmit the virus was more significant. All of the 4 men who presumably transmitted the virus to their wives were positive for the Tax antibody at least 1–2 years before their wives seroconverted, while only 5 of 18 (27.8%) men who did not transmit the virus to their spouses had anti-Tax antibody ($P = 0.017$).

The titers of anti-Tax antibody were determined by serial 2-fold dilutions of serum samples from 1:2,000 to 1:32,000. The result showed that the geometric mean anti-Tax antibody titer (13,454 ± 1414) of the men who presumably transmitted the virus was higher than that (6063 ± 2860) of the men who did not transmit the virus to their spouse. The difference of the titers of anti-Tax antibody between the above two groups was not statistically significant ($P = 0.19$).

When we examined seroreactivity to Tax among 32 concordant couples (H+,W+) randomly selected from the Miyazaki follow-up study, 24 (75%) of the men were found to be positive. This rate of reactivity is significantly higher than that (27.8%) seen in the group of H+,W– discordant couples, where infected men did not transmit the virus to their wives ($P= 0.0012$). Of the women who did not transmit the virus (those in the H–,W+ discordant couple group), 18 of 28 (64.3%) were positive for anti-Tax antibody. A similar rate (62.5%) of anti-Tax antibody was found for women in the group of concordant couples.

Five of the 5 spouses of seroconverters had antibody to the TM protein, compared to only 13 of 18 (72.2%) of the men from the H+,W– discordant couple group. This difference is not statistically significant. However, of the 124 HTLV-I carriers tested in this study, 100 (80.6%) had antibody to TM protein, and 79 (63.7%) had anti-Tax antibody. There were 72 subjects who had both anti-TM and anti-Tax antibodies. The association between seroreactivity to the TM and Tax proteins is over 6-fold and statistically significant ($P < 0.005$).

## DISCUSSION

One curious feature of HTLV-I epidemiology in its endemic areas (that is, southwestern Japan and the Caribbean Islands) is the strong age and sex dependence of its prevalence curve (21, 26, 32, 33). Rates of HTLV-I infection are generally low among children and then slowly increase from adolescence to middle age. For males, prevalence rates plateau after age 50, but they continue to increase for females. A prospective follow-up study of married couples in an HTLV-I endemic area is useful for analyzing the immune response to HTLV-I in seroconverters and their sexual partners during the critical period of viral transmission.

Among standard HTLV-I serological tests, the most sensitive for detecting anti-HTLV antibodies are specific ELISA and Western blot assays using recombinant proteins as antigen (34). In this study we used Western blotting techniques to evaluate the association of antibody reactivity to each HTLV-I-specific antigen and the probability of sexual transmission of HTLV-I infection in married couples that have been followed for up to 5 years. The results indicate that presence of anti-Tax antibody is highly associated with sexual transmission of HTLV-I, especially from male to female.

It is known that HTLV-I Tax protein is a transcriptional transactivator of the HTLV-I long terminal repeat sequence

Table 3. Prevalence of anti-Tax antibody in spouses of HTLV-I seroconverters and in carriers from serodiscordant couples

| Subjects* | Prevalence of anti-Tax antibody,† no. positive/no. tested (%) | P value‡ |
|---|---|---|
| Spouses of seroconverters | 5/5 (100) | 0.056 |
| Carriers among discordant couples | 23/46 (50) | |
| Husbands of female seroconverters | 4/4 (100) | 0.017 |
| Husbands from discordant couples (H+,W–) | 5/18 (27.8) | |
| Wives of male seroconverters | 1/1 (100) | — |
| Wives from discordant couples (H–,W+) | 18/28 (64.3) | |

*H, husband; W, wife; + and –, antibody status to HTLV-I as determined by Western blot and radioimmunoprecipitation tests.
†Tested by Western blot assay using recombinant HTLV-I Tax protein as antigen.
‡Fisher exact test.

and some cellular genes (e.g., IL-2 receptor gene, c-*fos*, and IL-2 gene) (12, 13, 16–19). Since it has been demonstrated using the polymerase chain reaction technique that there is substantial expression of the *tax/rex* gene in fresh peripheral blood mononuclear cells of adult T-cell leukemia patients and HTLV-I carriers (35), HTLV-I carriers who are anti-Tax antibody-positive may have more HTLV-I-infected T-cells in their body fluids than do anti-Tax antibody-negative carriers. This may explain why the rate of anti-Tax antibody of the spouses of the female seroconverters was significantly higher than that of the males who failed to transmit the viral infection to their wives.

When we compared the anti-Tax antibody titer between the two groups mentioned above, there was no significant difference. However, one man belonging to the H+/W− group, and still in his fifties, had a very high anti-Tax antibody titer (32,000). Further follow-up is needed on the sexual behavior and serological status of this individual and his wife.

We also found no significant difference between the titers of anti-HTLV-I antibody, determined by particle agglutination, among male carriers who have transmitted or failed to transmit the viral infection to their spouses. Since particle agglutination primarily detects antibodies to HTLV structural proteins, the results indicate that the "virus load" may not be a direct factor of viral transmission.

Although the presence of anti-Tax antibody in male HTLV-I carriers may indicate a high risk of viral transmission to their spouses, the association between anti-Tax antibody and female-to-male transmission of HTLV-I infection cannot be determined from the current work since there was only one male seroconverter. The low rate of female-to-male transmission was reported previously (21, 22), and our study indicates that 4 of 28 (14.3%) H+,W− couples seroconverted within the past 5 years while only 1 of 53 (1.9%) H−,W+ couples did so, which suggests female-to-male transmission of HTLV-I is less likely than male-to-female transmission, and either may be age-related (26).

In a cross-sectional seroprevalence study of HTLV-I in patients from sexually transmitted disease clinics in Jamaica, Murphy *et al.* (2) found that a history of penile sores or ulcers or a current diagnosis of syphilis was an independent risk factor for HTLV-I infection in men. This, along with other reports (30, 36, 37), suggests that cell-to-cell contact is required for HTLV-I transmission. In our study, although >60% of the female carriers in the H−,W+ discordant couple group were positive for anti-Tax antibody, the absence of risk factors mentioned above may affect the viral transmission from females to males in this study.

Concerning the significantly high rate (75%) of anti-Tax antibody in the husbands from the HTLV-I seroconcordant couples, if we assume that the presence of anti-Tax antibody is associated with viral transmission, it is logical to assume that many anti-Tax antibody-positive males might have already transmitted the virus to their wives and shifted from the discordant couple group to the concordant couple group, making the anti-Tax antibody-positivity rate of the second group significantly higher than that (28.8%) of the first.

Kamihira *et al.* (38) used a recombinant Tax ELISA test in a retrospective cohort study to show that the rate of mother-to-child transmission of HTLV-I was significantly higher in anti-Tax antibody-positive mothers than in anti-Tax antibody-negative mothers. This implies that HTLV-I Tax is also important for vertical transmission of viral infection.

In terms of anti-TM antibody and sexual transmission, all five spouses of the seroconverters had anti-TM antibody, and it is known that the N-terminal hydrophobic regions of the transmembrane component of the retroviral envelope complexes may be involved in mediating fusion between the viral envelope and the intracellular endosome membrane (39, 40). Therefore, the presence of TM protein may be necessary for viral transmission. However, the exact role played by the TM protein in the transmission of HTLV-I cannot be determined until further biological studies are performed.

In conclusion, the HTLV-I Tax protein is tumorigenic in transgenic mice (41) and can increase the transcription of certain viral and cellular genes *in vitro* (12, 13, 16–19). The present report traces discordant married couples from a seroepidemiological perspective and shows the association between the presence of anti-Tax antibodies in HTLV-I carriers and the seroconversion of their spouses. Subsequent analysis needs to be performed to follow the high-risk groups identified by this study, especially wives of anti-Tax antibody-positive men. This will allow us to better predict the occurrence and impact of events related to the natural history of HTLV-I infection in order to plan appropriate interventions aimed at preventing new HTLV-I infections. Furthermore, this might provide a model for identifying serological markers to better define transmissibility among human immunodeficiency virus carriers.

We thank Sara Finkelstein and Andrea Wahman for manuscript preparation. We thank Drs. Chung-Cheng Hsieh, Sherri Stuver, Esperanza Gomez-Lucia, and Boris Renjifo and Ms. Lynn Marshall for helpful discussions. This work was supported by Public Health Service Grants CA38450, HL6356, and HL43561 from the National Institutes of Health and Grant 1-P50-HL-33774 from the Center for Blood Research. Y.-M.A.C. is supported by the Fogarty International Center, National Institutes of Health, Program of International Training Grants (D43 TW 00004). N.M. is the recipient of an American Cancer Society Faculty Research Award. M.E. is the recipient of the Outstanding Investigator Award (CA39805) from the National Cancer Institute.

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