

Virus Markers Associated with Vertical Transmission of Human T Lymphotropic Virus Type 1 in Jamaica

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In a prospective study involving 150 mothers and their offspring in Jamaica, we examined maternal viral factors associated with the risk of transmission of human T lymphotropic virus type 1 (HTLV-1). Overall, the incidence of HTLV-1 infection among children was 8.3 occurrences per 1000 person-months. A higher maternal provirus level (odds ratio [OR], 1.9 per quartile) and a higher HTLV-1 antibody titer (OR, 2.2 per quartile) were independently associated with transmission to children, whereas the presence of anti-Tax antibody was not. Higher maternal antibody titers also were associated with older age at infection among children who were breast-fed for ≤ 12 months, which suggests that passively transferred maternal antibodies confer protection against infection while they persist. These data imply that mothers who have high provirus loads should be encouraged not to breast-feed. Alternatively, the successful reduction of maternal provirus loads or maintenance of passive antibody levels in infants during breast-feeding may lower the risk of transmission.

Human T lymphotropic virus type 1 (HTLV-1) is associated with adult T cell leukemia/lymphoma (ATL) and a chronic progressive neuropathy called "HTLV-1-associated myelopathy" or "tropical spastic paraparesis" (HAM/TSP) [1]. The virus is endemic in southwest Japan, the Caribbean, parts of Africa, South America, and the Middle East [2]. Transmission of HTLV-1 occurs through breast-feeding, from mother to child [3, 4]; through sexual contact [5, 6]; or through transfusion of cellular blood components [7, 8]. Vertical

transmission, in particular, is an important public health concern, because infection early in life is associated with subsequent risk of ATL [9].

Serological and molecular markers of HTLV-1 infection, including HTLV-1 antibody titer, presence of antibody to *tax* regulatory gene products (anti-Tax), and provirus load have been instrumental in increasing the understanding of the natural history and disease pathogenesis [10]. It has been shown that higher maternal HTLV-1 antibody titers [4, 11] and the presence of anti-Tax [12] in maternal serum are associated with an increased risk of vertical transmission of HTLV-1 to children. In addition, a recent study reported that a higher HTLV-1 provirus load in peripheral blood mononuclear cells (PBMC) was a significant predictor for the increased risk of mother-to-child transmission [11]. However, the reported associations may have been confounded, because levels of these markers are highly correlated [10]. Thus, an analysis that accounts for all markers simultaneously is needed to assess any inde-

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pendent effect. Furthermore, the absolute risk of vertical transmission in relation to these virus markers is uncertain. We examined the relationships of maternal HTLV-1 antibody titer, provirus load in PBMC, and the presence of anti-Tax antibody to the risk of HTLV-1 infection for breast-fed children enrolled in a prospective study in Jamaica.

SUBJECTS AND METHODS

Study subjects. The subjects of this analysis were participants in the Jamaica Mother Infant Cohort Study. That study initially enrolled 339 pregnant women (212 HTLV-1-positive women and 127 HTLV-1-negative women) through 2 antenatal clinics in Kingston between January 1989 and August 1990, who represented ~60% of the eligible population screened. A total of 181 children born to HTLV-1-positive mothers and 127 children born to HTLV-1-negative mothers had at least 1 postnatal visit [4]. The present analysis includes 150 HTLV-1-positive mothers and their 154 children who had been followed up for at least 18 months.

Blood samples were collected, separated, and frozen at -70°C in a central repository. Peripheral blood samples were obtained from mothers at the time of delivery. Blood samples were obtained from children every 6 weeks for the first 6 months of life, every 3 months from age 6 months to 2 years, and every 6 months thereafter. Twenty-eight children born to HTLV-1-positive mothers became infected. Information on maternal weekly income and breast-feeding practices was collected by questionnaire. Children's gestational age and events during the delivery were abstracted from the medical charts. Informed consent was obtained from all study participants. The study protocol followed the human experimentation guidelines of the US Department of Health and Human Services and the institutional review boards of the National Cancer Institute and University of the West Indies, in accordance with the Helsinki Declaration.

Laboratory methods. HTLV-1 seropositivity was determined by whole-virus EIA (Dupont), with confirmation by a whole-virus HTLV-1 Western blot assay (Cambridge-Biotech) that distinguishes HTLV-1 from HTLV type 2 infection. Children who consistently tested seropositive for HTLV-1 after age 12 months were considered to be infected. An end-point dilution method of EIA (Genetic Systems or Cambridge-Biotech) was used to measure the HTLV-1 antibody titer, at 4-fold dilutions. The presence of anti-Tax antibody was detected with EIA by optical density level [12]. Quantitative provirus DNA levels were measured by a real-time automated PCR method; the lower limit of detection was 10 copies/ 10^5 cells. DNA was prepared from frozen PBMC by use of the PureGene DNA Isolation Kit (Gentra Systems). For each test sample, 10 μL of DNA ($\sim 1 \mu\text{g}$) was amplified for 45 cycles with AmpliTaq Gold

polymerase, using an ABI Prism Sequence Detection System and TaqMan PCR Reagent (P/N N808-0230; PE Applied Biosystems), in a 96-well format [13, 14]. The maternal HTLV-1 provirus load was normalized by the number of human endogenous retrovirus type 3 (ERV-3) copies, to adjust for the number of lymphocytes in each sample tested [15].

Statistical analysis. Because all children were breast-fed by their biological mothers, all 154 mother-child pairs (including 4 sets of twins) were included in the statistical analyses. The age at infection for each child was determined by the detection of HTLV-1 provirus load, taking the midpoint between the provirus load in the final PCR-negative sample and the provirus load in the first PCR-positive sample [16]. Undetectable HTLV-1 provirus loads were assigned a value of 5 copies/ 10^5 cells, because the minimum detectable level was 10 copies/ 10^5 cells. The correlation between log-transformed antibody titer and provirus loads was examined by use of Spearman's correlation coefficient.

The distributions of maternal HTLV-1 antibody titer, presence of anti-Tax antibody, and provirus load were compared by children's HTLV-1 status and by anti-Tax positivity, using the Kruskal-Wallis tests. The HTLV-1 titer was categorized at the quartiles, which is consistent with our previously published analysis [4]. Provirus load was also treated as an ordered categorical variable at the quartiles. The presence of anti-Tax antibody was dichotomous. The duration of breast-feeding was categorized as ≤ 6 , 6.1–12, and >12 months. Maternal income was categorized at the tertiles (\leq J\$100, J\$101–200, and $>$ J\$200). The risk of HTLV-1 seropositivity among children per quartile category of maternal provirus load, antibody titer, and the presence of anti-Tax antibody was estimated by odds ratios (ORs) derived from logistic regression models [17]. The associations between child's age at infection and levels of HTLV-1 virus markers were examined by β estimated from linear regression models [18]. Wald-type 95% CIs were calculated. Statistical significance was based on 2-sided tests.

RESULTS

The mean age of the 150 mothers at delivery was 27 years (range, 15–44 years). Seventy-six (49%) of the 154 children were male. Twenty-eight (18%) of the children, all singletons, became infected with HTLV-1, at a mean age of 14 months (range, 4–27 months). The pattern of seroconversion in this group of 28 children has been described elsewhere [4].

Demographic and viral characteristics for the 150 mothers included in the present analysis are described in table 1. The mean antibody titer among the 28 mothers who transmitted HTLV-1 was 18,870, compared with 11,316 among mothers who did not transmit. The mean log-transformed titer was significantly higher among mothers who transmitted the virus

Table 1. Demographic and viral characteristics of 150 human T lymphotropic virus type 1 (HTLV-1)-positive mothers in Jamaica, by the HTLV-1 status of their children.

Characteristic	No. (%) of mothers			<i>P</i> ^a
	All	Of HTLV-1-positive children (<i>n</i> = 28)	Of HTLV-1-negative children (<i>n</i> = 122)	
HTLV-1 antibody titer				.0001
<1000	36 (24)	1 (3)	35 (29)	
1000–4000	36 (24)	3 (11)	33 (27)	
4001–10,000	38 (25)	10 (36)	28 (23)	
>10,000	40 (27)	14 (50)	26 (21)	
Provirus load, log ₁₀ copies/10 ⁵ cells				.0001
<2.20	39 (26)	3 (11)	36 (29)	
2.20–3.10	39 (26)	2 (7)	37 (30)	
3.11–3.80	39 (26)	10 (36)	29 (24)	
>3.80	33 (22)	13 (46)	20 (17)	
Anti-Tax antibody status ^b				.001
Positive	79 (53)	22 (81)	57 (47)	
Negative	70 (47)	5 (19)	65 (53)	
Duration of breast-feeding, ^b months				.001
>12.0	64 (43)	20 (70)	44 (36)	
6.1–12.0	37 (25)	6 (22)	31 (26)	
≤6.0	48 (32)	2 (8)	46 (38)	
Maternal weekly income, J\$ ^b				.02
≤100	32 (21)	10 (36)	22 (18)	
101–200	55 (37)	12 (43)	43 (36)	
>200	62 (41)	6 (21)	56 (46)	

NOTE. Provirus load in peripheral blood mononuclear cells is normalized by human endogenous retrovirus type 3 and log transformed.

^a *P* values are estimated from χ^2 statistics when expected values in all cells are ≥ 5 and from Fisher's exact test when expected values in ≥ 1 cell are < 5 . Comparisons are of the distribution of a variable among mothers of HTLV-1-positive children and mothers with HTLV-1-negative children.

^b One data point was missing for these variables.

than among those who did not (4.0 vs. 3.4 log₁₀; *P* = .0001). The ERV-3-normalized mean provirus load among the mothers who transmitted the virus was 10,540 copies/10⁵ cells, compared with 4524 copies/10⁵ cells among those who did not. The mean log-transformed provirus load was significantly higher among mothers who transmitted HTLV-1 to their children than among those who did not (3.6 vs. 2.7 log₁₀ copies/10⁵ cells; *P* = .0001). The prevalence of anti-Tax antibody was significantly higher among mothers who transmitted the virus than among those who did not (81% vs. 47%; *P* = .001). Correlations between antibody titer and provirus load were highly significant (*r* = 0.44; *P* = .0001). In addition, mean antibody titer and mean provirus load were both significantly higher among mothers with anti-Tax antibody than among those without, which indicates that there is a strong correlation between these virus markers.

In univariate logistic regression analyses, the risk of perinatal HTLV-1 transmission was associated with a higher antibody titer (OR, 2.5 per quartile; 95% CI, 1.6–4.0), higher provirus

load (OR, 2.2 per quartile; 95% CI, 1.5–3.5), and anti-Tax antibody positivity (OR, 4.8; 95% CI, 1.7–13.6) in the mother (table 2). A longer duration of breast-feeding was significantly associated with risk of HTLV-1 transmission. Compared with children who were breast-fed for ≤ 6 months, the risk of transmission among children who were breast-fed for 6.1–12 months and among those who were breast-fed for >12 months was 4.4-fold and 10.2-fold higher, respectively (*P* = .001 for trend). The risk of transmission for male children was not significantly different from that for female children (for male children, OR, 1.5; 95% CI, 0.64–3.3). In a multivariate logistic regression model that was adjusted for antibody titer and anti-Tax positivity, the provirus load (OR, 1.7 per quartile; 95% CI, 1.0–2.8) was an independent risk factor for perinatal transmission of HTLV-1. In the same model, antibody titer was an independent predictor of risk of transmission among children who were breast-fed for >12 months (OR, 3.4 per quartile; 95% CI, 1.5–7.5) but not among those who were breast-fed for a shorter duration (OR, 0.76 per quartile; 95% CI, 0.31–1.9). The pres-

Table 2. Odds ratios (ORs) and 95% CIs for the risk of human T lymphotropic virus type 1 (HTLV-1) transmission for 154 children born to HTLV-1-positive mothers in Jamaica.

Variable	Univariate OR (95% CI)	Multivariate OR (95% CI)	
		Primary analysis ^a	Secondary analysis ^b
Antibody titer, per quartile	2.5 (1.6–4.0)	2.0 (1.2–3.3)	2.2 (1.0–3.3)
Provirus load, per quartile	2.2 (1.5–3.5)	1.7 (1.0–2.8)	1.9 (1.1–3.4)
Anti-Tax antibody positivity	4.8 (1.7–13.6)	1.8 (0.57–5.8)	1.7 (0.46–6.3)
Male sex	1.5 (0.64–3.3)	—	1.2 (0.42–3.6)
Duration of breast-feeding, months			
>12	1.0	—	1.0
6.1–12	4.4 (0.83–23.2)	—	2.4 (0.39–14.9)
≤6	10.2 (2.3–46.2) ^c	—	10.8 (2.0–57.8) ^c
Lower income, per tertile	2.1 (1.2–3.6)	—	3.0 (1.4–6.3)

NOTE. ORs are for HTLV-1-positive compared with HTLV-1-negative children. ORs for antibody titer and provirus load are per quartile category increase in level.

^a Mutually adjusted for HTLV-1 provirus load, antibody titer, and anti-Tax antibody positivity.

^b Adjusted for HTLV-1 provirus load, antibody titer, and anti-Tax antibody positivity, as well as the child's sex, duration of breast-feeding, and maternal weekly income level (lower [≤J\$100], medium [J\$101–200], and higher [>J\$200]).

^c *P* = .001 for trend.

ence of anti-Tax antibody was not an independent risk factor for transmission (OR, 1.8; 95% CI, 0.57–5.8) in a multivariate analysis. These associations were essentially unchanged after additional adjustments were made for sex, duration of breast-feeding, and maternal income level (table 2), all of which have been reported to be independently associated with the risk of transmission in previous studies [4, 11].

To explain differences in the ORs for the association of risk of transmission with maternal antibody titer by duration of breast-feeding, we examined the relationship between levels of maternal antibody titer and age at infection among 28 infected children. A linear regression model suggested that a high maternal HTLV-1 antibody titer was a predictor of older age at infection among children who were breast-fed for ≤12 months (table 3). Every quartile increase in antibody titer was associated with ~2.3 months' delay in infection. However, this association was not apparent among children who were breast-fed for >12 months. Provirus load and the presence of anti-Tax antibody were not significantly associated with age at infection.

Overall, the incidence of infection among the 154 children was 8.3 occurrences per 1000 person-months. The risk of transmission was negligible when the mother's provirus load was <2.0 log₁₀ copies/10⁵ cells (i.e., 100 copies/10⁵ cells or 0.1%); however, the risk increased exponentially when the mother's provirus load was ≥3.0 log₁₀ copies/10⁵ cells (i.e., 1000 copies/10⁵ cells or 1%) (figure 1). Similarly, the risk of transmission was negligible when the mother's HTLV-1 antibody titer was <2.0 log₁₀ but increased exponentially when the mother's antibody titer was ≥2.0 log₁₀ (figure 1).

DISCUSSION

With recent advances in technology, molecular markers have become widely used to evaluate the risk of transmission and disease pathogenesis associated with HTLV-1. When they are used in conjunction with serological markers, molecular markers provide us with powerful tools to define the host-virus relationship and determine the highest risk group so that intervention strategies can be initiated.

We have reported elsewhere that the titer of antibody to HTLV-1 in the mother was an independent risk factor for mother-to-child transmission [4]. In the present study, we evaluated the association between additional virus markers and risk

Table 3. Associations between maternal human T lymphotropic virus type 1 (HTLV-1) virus markers with age at infection in 28 Jamaican children, by duration of breast-feeding.

Virus marker	Change in age at infection by duration of breast-feeding, β (95% CI)	
	Duration of ≤12 months	Duration of >12 months
HTLV-1 antibody titer, per quartile	2.3 (0.78–3.8)	1.4 (–2.6 to 5.5)
Provirus load, per quartile	0.23 (–1.1 to 1.5)	0.45 (–2.7 to 3.6)
Anti-Tax antibody positivity	1.9 (–1.0 to 4.9)	4.4 (–3.5 to 12.3)

NOTE. Models are mutually adjusted for HTLV-1 provirus load, antibody titer, and anti-Tax antibody positivity. Associations were estimated from linear regression models. Positive values indicate increment (in months) in age at infection. β values for antibody titer and provirus load are per quartile increase in level.

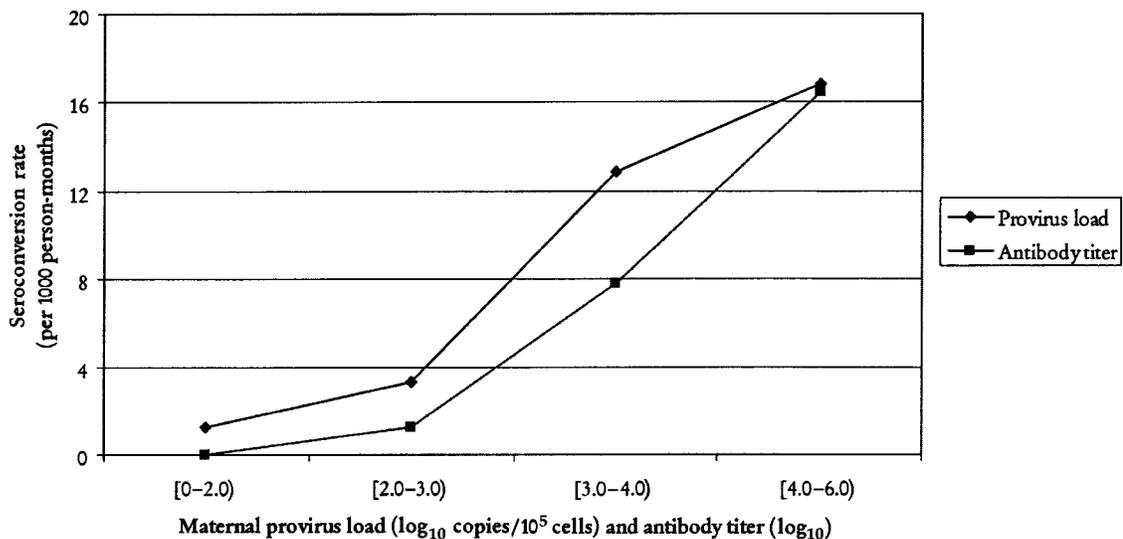
of infection among children. Although a higher provirus load, a higher antibody titer, and the presence of anti-Tax antibody each have been shown to be associated with an increased risk of mother-to-child transmission, no studies have examined the effect of all markers simultaneously in a prospective study.

As would be expected from previously published reports [10, 11, 13], we found a strong correlation between HTLV-1 antibody titer and provirus load among HTLV-1-infected mothers in Jamaica. A comparable and statistically significant level of correlation ($r = 0.38$) between antibody titer and provirus load has been reported for Japanese patients with HAM/TSP [13]. In addition, mean HTLV-1 antibody titers and provirus loads both were much higher among mothers with anti-Tax antibody than among those without. Strong correlations among these virus markers prompted us to account for the markers simultaneously in the logistic regression models.

Our multivariate logistic regression models demonstrated the overall risk of transmission to be 11-fold and 7-fold higher among mothers with HTLV-1 antibody titers and provirus loads, respectively, in the highest quartile, than among those with values in the lowest quartile for each marker. Conse-

quently, mothers with values in the highest quartile for both provirus load and antibody titer were >77-fold more likely to transmit HTLV-1 to children that they breast-fed. Although comparable estimates of an increase in risk of 2.2-fold and 2.6-fold per 1-log_{10} increase in provirus load and 1-log_2 increase in antibody titer, respectively, have been reported from a French Guyana study in which a similar analysis was made [11], the joint effect of these 2 markers had not been examined elsewhere. That higher levels of maternal HTLV-1 antibody and provirus load are independently associated with HTLV-1 transmission to the child, after mutual adjustment for each value, suggests the importance of these markers as overall predictors of risk of transmission. In contrast, the reported association between the presence of anti-Tax antibody and risk of perinatal transmission [12] appears to have resulted from its correlation with provirus load and antibody titer.

A logistic regression model that included all virus markers; low income, which is a correlate of lower socioeconomic status; and duration of breast-feeding revealed that the 2 major predictors of HTLV-1 transmission from our previous study [4] remained significant predictors of HTLV-1 transmission. This



	Maternal provirus load (\log_{10} copies/ 10^5 cells) and antibody titer (\log_{10})			
	[0-2.0)	[2.0-3.0)	[3.0-4.0)	[4.0-6.0)
Provirus load				
No. with seroconversion	1	3	14	10
No. of mothers	34	40	51	29
No. of person-months	781	910	1088	593
Antibody titer				
No. with seroconversion	0	1	13	14
No. of mothers	2	35	77	40
No. of person-months	45	811	1667	849

Figure 1. Incidence rate of human T lymphotropic virus type 1 (HTLV-1) infection among Jamaican children, by maternal provirus load and HTLV-1 antibody titer. The seroconversion rates in each maternal provirus load and antibody titer category were calculated as the ratio of the no. of children who seroconverted to the no. of observed person-months in the category (no. with seroconversion/no. of person-months). A total of 154 mother-infant pairs were included. The risk of transmission increased with provirus load and HTLV-1 antibody titer. The lower limit of detection for provirus load was 10 provirus copies/ 10^5 cells.

finding indicates that social environment and its correlates also are important predictors of virus transmission.

Most infections in our series occurred after passively transferred maternal HTLV-1 antibodies waned [4, 19]. In rabbit studies, passive immunization by HTLV-1 immunoglobulin has been shown to prevent infection in offspring that are breast-fed by HTLV-1-positive mothers [19, 20]. We have shown elsewhere that, in humans, passively transferred maternal antibody levels waned at 11 months, on average [4]. Thus, a breast-feeding duration of >12 months likely reflects an increased risk of transmission from breast milk after maternal antibodies have been lost. These observations suggest the possibility that passively transferred maternal antibody, while it persists, protects against infection. To reconcile this paradoxical effect of maternal antibody titer on risk of transmission, we examined data stratified by duration of breast-feeding. We found that every quartile increase in maternal antibody titer was associated with a significant delay in HTLV-1 infection among children who were breast-fed for ≤ 12 months, but not among those who were breast-fed for >12 months (table 3). This observation provides further support for the contention that passively transferred maternal antibody protects children against HTLV-1 infection. In a Japanese study, the risk of transmission was significantly lower when mothers breast-fed for <6 months [21], which suggests that exposure to HTLV-1 provirus is less likely to result in infection in children during the time when passively transferred maternal antibodies are present. The independent association of maternal antibody titer with risk of transmission in our multivariate analysis can be explained, therefore, by the strong correlation of HTLV-1 antibody titer with provirus load and duration of breast-feeding. Thus, provirus load may be a more direct predictor of virus transmission overall.

We found that the risk of transmission was similar for male and female children. This finding is consistent with the similarity in seroprevalence for male and female children that was seen in Japanese and Jamaican children [1]. However, our observation strongly contrasts with the findings from a retrospective study that reported a seroprevalence among female children that was 3–4 times higher than that among male children [11].

In summary, the risk of infection for breast-fed children born to HTLV-1-positive mothers appears to be primarily determined by the provirus load to which they are exposed in the absence of passively transferred maternal antibody. Early weaning and bottle-feeding are the primary options for reducing the risk of HTLV-1 transmission by infected mothers. The decision to initiate these strategies, however, cannot be made easily when the immediate benefits of breast-feeding clearly outweigh the risk of HTLV-1 infection and the resulting diseases. Our data enable us to identify mothers with a high risk of transmission

and to strongly recommend that they bottle-feed their children. Our data also suggest that reduction of maternal provirus load or maintenance of passive HTLV-1 antibody levels in the infant may reduce the risk of vertical transmission during breast-feeding.

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