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Research Article

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Association between Maternal Antibodies to the External Envelope Glycoprotein and Vertical Transmission of Human T-Lymphotropic Virus Type I

Maternal Anti-env Antibodies Correlate with Protection in Non–Breast-fed Children

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Abstract

Vertical transmission of human T-lymphotropic virus type I (HTLV-I) depends primarily on breast-feeding; substitution of bottle-feeding has reduced the transmission rate from 20% in breast-fed children to 3% among bottle-fed. To determine the correlates of transmission for long breast-feeding (≥ 6 mo), short breast-feeding (< 6 mo), and bottle-feeding mothers, the antibody titers of transmitter (T) mothers and non-transmitter (nT) mothers were analyzed by using synthetic and recombinant epitopes representing the immunodominant epitopes of *gag* (Gag1a, r24), *env* (Env1/5, MTA1, RE3), and *tax* (Tax8/22–24) proteins. Seroreactivity to *gag* and *tax* epitopes was not significantly different except for anti-r24 antibody titer, which was significantly higher among T-mothers (geometric mean 134) when compared with nT-mothers (62) in the long-feeding group ($P < 0.001$). Profiles of antibody titers against *env* epitopes were different. Within the long-feeding group, Env1/5, MTA1, and RE3 titers were significantly higher among T-mothers (258, 1,476, and 738, respectively) when compared with nT-mothers (106, 279, and 320, respectively) ($P < 0.01$ for all three epitopes). In contrast, within the bottle-feeding group, antibody titers to Env1/5 (269) and RE3 (418) among nT-mothers were significantly higher than those among T-mothers (80 and 113, respectively) ($P < 0.01$). These data confirm that high-titered anti–HTLV-I antibodies in the long-feeding group correlate with milk-borne transmission of HTLV-I and, more importantly, imply that maternal anti-*env* antibodies may reduce the risk of non-milkborne infection. (*J. Clin. Invest.* 1995. 95:2920–2925.) Key words: HTLV-I • vertical transmission • passive immunization • antibody titers

Introduction

Human T-lymphotropic virus type I (HTLV-I)[1] is endemic in southwestern Japan, the Caribbean Islands, and certain parts of Africa and is etiologically linked with adult T cell leukemia/lymphoma and HTLV-I–associated myelopathy (1, 2). The major routes of transmission for HTLV-I include sexual contact, blood transfusion, and mother to child (1, 3). Seroepidemiologic studies of pregnant women and their children, as well as oral inoculation of carrier mothers' breast milk into common marmosets, indicates that breast-feeding plays an important role in the transmission of HTLV-I (4, 5). In 1987, the ATL Prevention Program (APP) was initiated in Nagasaki, Japan, to determine whether screening and counseling of pregnant women could prevent transmission of HTLV-I by breast-feeding (6). Follow-up studies have shown that ~ 20% of breast-fed children and only 3% of bottle-fed children born to HTLV-I–carrier mothers become infected (7, 8). The observation that some breast-feeding and non–breast-feeding carrier mothers transmit HTLV-I while others do not implies that there may be mechanisms other than feeding that affect the transmission of HTLV-I from mother to child.

Several maternal risk factors for mother-to-child transmission of HTLV-I have been proposed: these include high levels of HTLV-I–positive lymphocytes in breast milk (9, 10), high proviral load in peripheral blood (11), high titer of anti–HTLV-I antibody (12), and anti-tax antibodies (13, 14). However, these studies on antibody titers have not identified the specific epitopes within the HTLV-I genome that might correlate with mother-to-child transmission. Thus, analysis of maternal HTLV-I antibodies directed against various antigens coded for by the HTLV-I genome may provide clues as to their potential use as markers for mother-to-child transmission, or alternatively, a protective role of these antibodies.

The APP cohort provided us a unique opportunity to assess the relationship between antibody reactivities and HTLV-I transmission. In the search for possible associations between the presence of specific antibodies and mother-to-child transmission of HTLV-I, we analyzed the antibodies in sera of mothers who transmitted HTLV-I (transmitter; T-mothers), and mothers who did not (non-transmitter; nT-mothers), using various peptides and recombinant proteins representing the immunodominant epitopes of HTLV-I. Our results indicate that high-titered maternal *env* antibodies are associated with transmission in long-term (≥ 6 mo) breast-fed children and that high-titered maternal antibodies to certain epitopes of external glycoproteins appear to be protective in bottle-fed children.

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1. *Abbreviations used in this paper:* APP, ATL prevention program; HTLV-I, human T-lymphotropic virus type I; nT-mothers, non-transmitter mothers; T-mothers, transmitter mothers.

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Methods

APP. Nagasaki Prefecture, one of 47 prefectures in Japan, is located in southwestern Japan on the island of Kyushu and has a population of ~ 1.5 million. APP was implemented in this prefecture in 1987. Informed consent was obtained from each mother, and the study was approved under the guidelines of Nagasaki University. Pregnant women were offered serologic testing for HTLV-I, and infected women were counseled to refrain from breast-feeding. Since 1987, ~ 15,000 women, representing > 85% of all pregnant women in the prefecture, have been screened annually. The HTLV-I prevalence in this group is ~ 4%. As of December 1992, > 3,000 seropositive mothers were enrolled in the study.

At enrollment and thereafter, blood samples were taken from mothers in the last trimester of each pregnancy. After delivery, mothers were requested to bring their newborns to one of 17 designated pediatric units in the prefecture at 6, 12, 18, 24, and 36 mo of age. Approximately one-half of the mothers showed up for follow-up testing; on these visits information on breast-feeding was collected. Only children with a serum sample taken at ≥ 18 mo were considered eligible for purposes of classifying their mothers as T-mothers or nT-mothers. As of December 1992, records were available for 1,253 such children born to 889 mothers. In addition, 98 children up to 13 yr old, born to the same mothers before APP, had also been studied. Information on their breast-feeding status was also obtained.

Serologic testing for HTLV-I. Serum specimens from all participating subjects (mothers and children) were tested by gelatin particle agglutination assay (Fuji-Rebio, Tokyo, Japan) at commercial laboratories. All positive and indeterminate samples were sent to the Department of Bacteriology, Nagasaki University School of Medicine, where they were tested by particle agglutination and by an indirect immunofluorescence assay that used a mixture of MT-2 and CEM cells. Serum specimens with positive results in both assays were scored positive; those with positive results in one assay, or with indeterminate results in either assay, were tested further by immunoblot (15).

To confirm the specificity of serologic tests, randomly selected specimens from 30 seropositive mothers and 26 seropositive children (≥ 18 mo old) were tested at the Centers for Disease Control and Prevention by a modified Western blot assay incorporating recombinant *env* proteins (HTLV 2.3; Genelabs Diagnostics Inc., Redwood, CA) (16). A serum specimen was designated HTLV-I/II–positive if antibody reactivity was detected to *gag* p24 and *env* r21e or rgp46. All specimens that were confirmed to be HTLV-I/II positive in the APP were also positive at the Centers for Disease Control and Prevention.

Selection of HTLV-I–infected mothers for the study. Mothers were selected based on the presence of infection in one or more of their children (transmitters) or absence of infection in all eligible children (non-transmitters). All T-mothers whose blood samples were available in frozen stock were included in the study. The T-mothers were further classified based on the feeding history of the child: breast-fed for ≥ 6 mo (long-feeding), or for < 6 mo (short-feeding), or bottle-fed. One mother who transmitted HTLV-I to both a long-feeding and a bottle-fed child was deleted from the study.

Many nT-mothers had eligible children in different feeding categories because of the implementation of APP. Mothers with more than one eligible breast-fed child were classified according to the child with the longest breast-feeding history. nT-mothers were selected using computer-generated random numbers so that the ratio of nT- to T-mothers in each feeding category would be ~ 1.5:1.

Maternal antibodies to synthetic peptides and recombinant proteins. The synthetic peptides derived from the *gag* (Gag-1a$^{102-117}$, PPSSPTHDPPDSDPQI), *env* (Env1$^{191-214}$, LPHSNLDHILEPSIPWKSKLLTLV, and Env5$^{242-257}$, SPNVSVPSSSSTPLLY), and *tax* (Tax8$^{106-125}$, LQAMRKYSPSRNGYMEPTLG; Tax22$^{316-335}$, PISLLFNEKEADDNDHEPQI; Tax23$^{331-350}$, HEPQISPGGLEPPSEKHFRE; and Tax24$^{336-353}$, SPGGLEPPSEKHFRETEV) proteins of HTLV-I were synthesized by 9-fluorenylmethyloxycarbonyl (FMOC) chemistry (see Fig. 1 for approximate location in the HTLV-I genome) (17–19). We have reported previously that a cocktail assay composed of two or more peptides results in enhanced sensitivity of antibody detection as compared with a single peptide. Therefore, we used an equimolar ratio of Env1 and Env5 for *env* detection (18) and Tax8, Tax22, Tax23, and Tax24 for *tax* detection (19). Recombinant proteins included r24, which comprises the entire p24^{gag} protein of HTLV-II (Intracel Corp., Cambridge, MA) and which reacts with anti-p24 antibodies (both HTLV-I and HTLV-II) in human sera (data not shown). Recombinant proteins also included MTA1, which comprises the central region of the external glycoprotein of HTLV-I (amino acids [a.a.] 162–209) (kindly provided by L. Chan, Genelabs Diagnostics Inc.) (16); and RE3, which comprises the COOH-terminal half of the external glycoprotein of HTLV-I (a.a. 165–306) (Repligen Corp., Cambridge, MA) (20). A whole viral lysate spiked with recombinant transmembrane protein (r21e) of HTLV-I was also included to determine the antibodies to whole virus proteins (21). Antibodies to these synthetic peptides and recombinant proteins were determined as described previously (18, 19). Seropositivity was defined as any value greater than the mean of the normal controls + 2 standard deviations. All specimens were coded without knowledge of the mother's category.

Statistical analysis. Analysis proceeded using six categories of mothers, according to transmitter/non-transmitter and feeding status. Each mother was included in only one category. Antibody titers were expressed by reciprocals of the highest dilution that gave positive results in the Env1/5, MTA1, RE3, and r21e-spiked HTLV-I assays after 2-fold dilutions starting from 1:20. Assays of Gag-1a, r24, and Tax8/22–24 assays were performed at a dilution of 1:20. Antibody titers in these assays were computed from a dilution curve of pooled standard positive sera. For calculation, negative sera were scored as one tube earlier than the first dilution (1:10), and overscaled ones were scored as the last dilution. Antibody titers were converted into logarithms for computation, and the geometric mean and 95% confidence intervals (determined by twice the standard deviation) were calculated back into linear scale. Statistical analyses were performed after all the immunological data were obtained by using nonparametrical analysis of variance by the Bonferroni/Dunn method.

Results

Classification of HTLV-I–seropositive mothers. Of 889 mothers whose children were tested at the ages of at least ≥ 18 mo, we selected 169 mothers, including 66 T-mothers and 103 randomly sampled nT-mothers. All mothers were of Japanese descent. These mothers were further categorized based on the feeding history. Of the 66 T-mothers, 32 breast-fed their babies for ≥ 6 mo (long-feeding), 12 breast-fed for < 6 mo (short-feeding), and 22 bottle-fed their babies (Table I). Of the 103 nT-mothers, 56 were in the long-feeding category, 19 were in the short-feeding category, and 28 were in the bottle-feeding category. The intervals between sampling of the mother and birth of the child were greater in breast-feeding than in bottle-feeding mothers because of inclusion of mothers who breast-fed older children. However, within each feeding category, the ages of the mothers and the intervals between sample of the mother and birth of the child in T- and nT-groups were highly comparable (Table I).

Comparison of maternal antibody levels in the long-feeding group. The seroreactivity and antibody titers were compared in T- and nT-mothers using whole virus proteins spiked with r21e protein (HTLV-I) (Table II). Serum specimens from all mothers, except for one nT-mother, reacted in the assay. Comparison of anti–HTLV-I titers demonstrated significantly higher levels among T-mothers (mean: 944; 95% CI: 80–11,092), as compared with nT-mothers (mean: 385; CI: 30–4,966) (P = < 0.001) (see Fig. 2). The antibody reactivities to *gag*

Table I. Demographics and Epidemiological Background of Mothers Tested

Feeding category	Transmission	No. mothers eligible	No. mothers sampled	Age of mothers at sampling	Interval between sampling of mother and birth of child in years*
				mean (95% CI)	*mean (95% CI)*
Breast					
> 6 mo (long)	Yes	39	32	30.6 (22.2–39.1)	−3.1‡ (−9.0–2.7)
	No	186	56	30.5 (22.7–38.3)	−2.6 (−7.3–2.0)
< 6 mo short	Yes	12	12	31.3 (24.1–38.6)	−1.8 (−6.7–3.1)
	No	141	19	30.6 (23.4–37.8)	−2.2 (−5.5–1.1)
Bottle	Yes	25	22	30.2 (23.1–37.3)	0.2 (−1.4–1.8)
	No	486	28	28.1 (19.8–36.4)	1.2 (−2.4–4.7)

* Not significant ($P > 0.05$) by ANOVA (Bonferroni/Dunn) in each feeding category. ‡ The minus sign indicates that child was born before blood sampling of the mother.

(Gag1a and r24) and *tax* (Tax8/22–24) epitopes were 72, 100, and 22% among T-mothers, and 66, 91, and 16% among nT-mothers, respectively (Table II). While antibody titers to Gag1a and Tax8/22–24 were not significantly different between T- and non-T groups, antibody titers to r24 were significantly higher in the T-group (mean: 134; CI: 21–867) when compared with the nT-group (mean: 62; CI: < 20–531) ($P < 0.001$).

While seroreactivity against *env* epitopes (Env1/5) was not significantly different among T- and nT-groups, antibody titers to this epitope were significantly higher in the T-group (mean:

Table II. Analysis of Seropositivity and Antibody Titers to Immunodominant Epitopes of Structural and Regulatory Proteins of HTLV-I in Transmitter vs Nontransmitter Mothers

Epitope regions	Seroreactivity*		Antibody titer		*P* value‡
	Transmitter	Nontransmitter	Transmitter	Nontransmitter	
	No. pos/No. tested (%)	*No. pos/No. tested (%)*	*mean (95% CI)*	*mean (95% CI)*	
Long-feeding group					
HTLV-I	32/32 (100)	55/56 (98)	944 (80–11092)	385 (30–4966)	< 0.001
Gag1a	23/32 (72)	37/56 (66)	67 (< 20–1734)	44 (< 20–986)	NS
r24	29/29 (100)	51/56 (91)	134 (21–867)	62 (< 20–531)	< 0.001
Tax8/22–24	7/32 (22)	9/56 (16)	< 20 (< 20–78)	< 20 (< 20–79)	NS
Env1/5	32/32 (100)	52/56 (93)	258 (21–3214)	106 (< 20–1875)	0.004
MTA1	29/29 (100)	47/55 (85)	1476 (118–18408)	279 (< 20–13583)	< 0.001
RE3	29/29 (100)	55/55 (100)	738 (106–5129)	320 (26–3899)	0.005
Short-feeding group					
HTLV-I	12/12 (100)	19/19 (100)	285 (29–2773)	514 (65–4064)	NS
Gag1a	8/12 (67)	14/19 (74)	41 (< 20–590)	53 (< 20–841)	NS
r24	11/11 (100)	18/19 (95)	64 (< 20–361)	55 (< 20–251)	0.031
Tax8/22-24	1/12 (8)	6/19 (32)	< 20 (< 20–31)	23 (< 20–312)	NS
Env1/5	12/12 (100)	19/19 (100)	190 (22–1618)	286 (22–3793)	NS
MTA1	11/11 (100)	19/19 (100)	640 (40–10233)	617 (48–7907)	NS
RE3	11/11 (100)	19/19 (100)	193 (< 20–3327)	445 (41–4764)	NS
Bottle-feeding group					
HTLV-I	22/22 (100)	28/28 (100)	374 (32–4355)	640 (97–4227)	NS
Gag1a	10/22 (45)	22/78 (79)	35 (< 20–703)	58 (< 20–867)	NS
r24	20/20 (100)	22/26 (85)	80 (< 20–392)	56 (< 20–481)	NS
Tax8/22-24	4/22 (18)	9/28 (32)	< 20 (< 20–51)	< 20 (< 20–86)	NS
Env1/5	16/22 (73)*	28/28 (100)	80 (< 20–2449)	269 (30–2399)	0.002
MTA1	20/20 (100)	26/26 (100)	453 (< 20–10715)	693 (51–9441)	NS
RE3	20/20 (100)	26/26 (100)	113 (< 20–2767)	418 (33–5309)	< 0.001

* Comparisons made between seroreactivity in transmitters vs nontransmitters using χ^2 test demonstrated differences to be nonsignificant for all comparisons ($P > 0.05$), except Env-1/5 in the bottle-feeding group, where the *P* value was 0.012. ‡ Analysis of variance between antibody titers by Bonferroni-Dunn ($P < 0.05$) method.

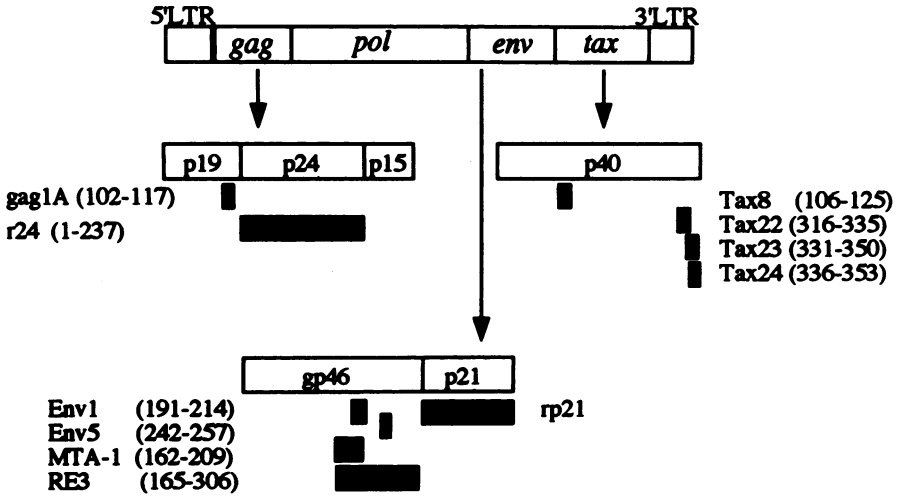

Figure 1. Location of immunodominant B cell epitopes within the HTLV-I genome, as represented by synthetic and recombinant proteins. The filled boxes represent approximate location and size of the epitope studied.

258; CI: 21–3,214) as compared with the nT-group (mean: 106; CI: < 20–1,875) (P = 0.004). Since Env1 and Env5 represent immunodominant epitopes located at the central region and carboxyl terminus of the external glycoprotein of HTLV-I, we next determined whether immune reactivity among T-mothers was restricted to a specific epitope. We used two recombinant constructs—MTA-1, representing a.a. 162–209 which overlaps with synthetic Env-1 (a.a. 191–214); and RE3, representing a.a. 165–306, which overlaps with both Env1 and Env5 (a.a. 242–256) (Fig. 1). Seroreactivity to MTA-1 and RE3 ranged from 85 to 100% in both groups. However, antibody titers to both MTA-1 and RE3 were significantly higher in T-mothers (mean: 1,476 and 738; CI: 118–18,408 and 106–5,129, respectively) than the nT-mothers (mean: 279 and 320; CI: < 20–13,583 and 26–3899, respectively) (P < 0.001 and P = 0.005, respectively) (Table II and Fig. 2).

Comparison of maternal antibody levels in the short-feeding group. Analysis of maternal seroreactivity within the short-feeding group revealed no significant difference between the T- and nT-groups in either the whole virus protein assay or any of the epitope-based assays (Table II). Similarly, analysis of antibody titers in the short-feeding group did not reveal any differences and could be attributed to the fact that T-mothers in this category represent both breast-feeding and non–breast-feeding—related transmission (22).

Comparison of maternal antibody levels in the bottle-feeding group. Overall seroreactivity to HTLV-I, *gag* and *tax* epitopes, and the titers to *gag* epitopes were similar in T- and nT-groups (Table II). Although the titers to HTLV-I were higher in nT-mothers (mean: 640; CI: 97–4,227) than in T-mothers (mean: 374; CI: 32–4,355), this difference did not reach statistical significance.

Seroreactivity to *env* epitopes (MTA1, RE3) was 100% in both groups; however, seroreactivity to Env1/5 was significantly higher among nT-mothers (28/28; 100%) than T-mothers (16/22; 73%) (P = 0.01). Analysis of anti-Env1/5 and RE3 titers demonstrated significantly higher levels among nT-mothers (mean: 269; CI: 30–2,399, and mean: 418; CI: 33–5,309, respectively) than T-mothers (mean: 80; CI: < 20–2,449; and mean: 113; CI: < 20–2,767, respectively) (P = 0.002 and P < 0.001, respectively) (Table II and Fig. 2). Anti-MTA1 titers were also higher in nT-mothers (mean: 693, CI: 51–9,441) than in T-mothers (mean: 453; CI: < 20–10,715), but the difference was not statistically significant.

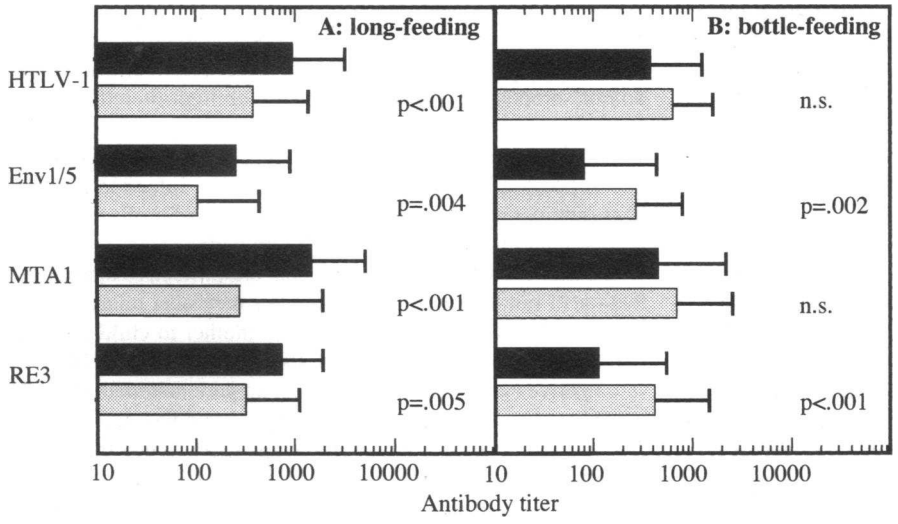

Figure 2. Antibody titers to HTLV-I and *env* epitopes (Env1/5, MTA1, and RE3) among transmitter (*filled bars*) and non-transmitter (*dotted bars*) mothers who breast-fed their babies for ≥ 6 mo (*A*) and those who bottle-fed their babies (*B*). The statistical significance for each comparison is shown at the right side of each panel.

Of the 33 T-mothers originally in the long-feeding group, 20 had children who were bottle-fed and had samples available at ≥ 18 mo. One of these mothers (deleted from the study) also transmitted infection to a bottle-fed child; her HTLV-I, Env1/5, MTA1, and RE3 titers were 160, 40, 320, and 320, respectively. Of the 22 T-mothers included in the bottle-feeding group, only one also had a child who was long-term breast-fed and had a specimen at ≥ 18 mo; this child was uninfected, and the mother's HTLV-I, Env1/5, MTA1, and RE3 titers were 80, 80, 80, and 40, respectively.

Discussion

While mother-to-child transmission is the major route of transmission of HTLV-I in endemic areas (3–5), little is known about the immune responses that might affect such transmission. In this investigation, we have elucidated the serological basis for maternal immunity as it relates to either infectivity or protection from vertical transmission. We have examined this question by measuring specific binding of maternal serum samples to various immunodominant epitopes representing both the structural and regulatory proteins of HTLV-I (18–21). By using the model of mother-to-child transmission of HTLV-I infection, we have demonstrated that high antibody titers to HTLV-I and several epitopes from the envelope protein are associated with transmission to long-term breast-fed babies. We also have established an association between maternal antibodies to selected epitopes of external glycoproteins, in particular Env1/5 and RE3, and absence of infection in bottle-fed children.

There are some limitations in this study regarding classification of mothers. It is possible that the nT categories included mothers who would have been found to transmit HTLV-I if they had more children with serum specimens available at ≥ 18 mo of age. However, such misclassification would decrease the difference between T- and nT-mothers. Another limitation was that nT-mothers who breast-fed an eligible child were so classified even if they bottle-fed younger eligible children. 26 such mothers were included in breast-feeding categories. However, addition of these 26 nT-mothers to the 28 nT-mothers in the bottle-feeding category still resulted in significantly higher *env* antibody titers in nT- versus T-mothers in the bottle-feeding category (data not shown). In the breast-feeding category, we included mothers whose classification was based on children born before the APP began. When such cases were nT-mothers, we cannot confirm that the mothers were carriers during these pregnancies. However, among 852 mothers who had also been tested in an earlier pregnancy, only 4 were seronegative at the earlier date. Thus, it is likely that the vast majority of, if not all, of these mothers were already infected during these earlier pregnancies. We also repeated our analyses, limiting them to mothers whose classification was based on children born during the APP. This resulted in a marked reduction in numbers of long-feeding mothers, and, in this group, T-mothers had higher *env* titers than nT-mothers in only the MTA assay ($P = 0.03$); however, similar trends were also observed in the Env1/5, RE3, and HTLV-I assays (data not shown). The numbers of bottle-feeding mothers and the results in this group were unchanged.

Analysis of anti–HTLV-I titers provided us a tool to determine the overall antibody status of T- and nT-mothers in all three feeding categories, and analysis of immunodominant regions within *gag, env,* and *tax* allowed us to localize the regions that correlate with transmission or protection. The overall percent reactivities to *gag* and *env* epitopes were similar to those in previous reports (16, 17). However, the seroreactivity to Tax peptides was significantly lower (8–32%) than that previously reported for asymptomatic carriers from the United States (62%) (19). Although the reason for the lower reactivities is not known, it could be related to sequence variations in this immunogenic region of *tax,* such as those found in a Melanesian isolate of HTLV-I (23).

Our finding of higher antibody titers to HTLV-I in T-mothers than nT-mothers in the long-feeding group corroborates previous reports in which high anti–HTLV-I titers were associated with increased frequency of mother-to-child transmission in long-fed babies (12, 24). The high-titered anti–HTLV-I presumably reflects high concentration of infected cells in peripheral blood as evidenced by high proviral load (25). At the epitope level, transmitters had significantly higher levels of antibodies to *env* epitopes, with no difference in *gag* and *tax* epitopes. These results corroborate previous results in which high-titered antibodies to *env* epitope sp4a1, which overlaps with the *env* epitopes tested here, were shown to correlate with a high risk of transmission in breast-fed babies (24). Our results further suggest that the COOH-terminal region of the external glycoprotein correlates with transmission. Taken together, these data imply that high-titered anti–HTLV-I antibodies, particularly those directed against the envelope protein, are associated with mother-to-child transmission, if the mother breast-feeds her child for ≥ 6 mo.

Of greater significance is the finding of high-titered anti-*env* antibodies among nT-mothers as compared with T-mothers in the bottle-feeding group. At the epitope level, antibody titers to both Env1/5 and RE3 were significantly higher among nT-mothers than T-mothers. Anti-MTA1 titers also appeared to be higher in nT-mothers, but the difference was not statistically significant. Thus, levels of high-titered anti-*env* antibodies in the maternal sera may reduce the risk of infection in infants. The antibody pattern to specific *env* epitopes does not appear to reflect a general increase in antibody titers, since antibody titers to other antigens, including anti–HTLV-I and anti-r24, were comparable among T- and nT-mothers. The central region of the external glycoprotein of HTLV-I has been shown to contain a cytotoxic and neutralizing epitope (26–28). Therefore, natural antibodies to these regions should be effective in neutralizing the virus and may provide some measure of natural immunity against HTLV-I. Thus, it is possible that mothers with high-titered neutralizing antibody can passively transfer high enough concentrations of maternal antibodies to protect their children from infection. It is conceivable that immunization of seropositive pregnant women with high-titered anti-*env* antibodies might be an effective means of boosting passively acquired maternal antibodies in infants. This hypothesis is further supported by the evidence that HTLV-I infection of newborn rabbits was prevented by the passive transfer of an immune IgG containing anti–HTLV-I antibody (29).

If high-titered anti-*env* antibodies are protective against infection in bottle-fed children, how are they also related to increased transmission of HTLV-I from mother to child in long-fed babies? Previous study has shown that children born to seropositive carrier mothers passively acquire maternal antibodies prenatally; levels of these maternal antibodies decline over 3–6 mo and disappear at 6–9 mo of age (30). While the babies in the long-feeding group would still be breast-feeding on carrier mothers and ingesting HTLV-I–infected lymphocytes from the

breast milk, the passively acquired high-titered anti-*env* antibodies would gradually disappear from circulation. This continuous ingestion of HTLV-I–infected lymphocytes could lead to HTLV-I infection in the babies (9). Indeed, lymphocyte-facilitated infection of gut-epithelium has been demonstrated in vitro (31). We therefore believe that high-titered anti–HTLV-I in T-mothers reflects a high proviral load that, in long-term breast feeders, results in increased probability of transmission when passively acquired maternal antibodies have disappeared from circulation.

This report is the first to suggest that antibody titers against HTLV-I are associated with vertical transmission of HTLV-I in both milkborne and non-milkborne pathways, but in different directions. Specifically, non-milkborne transmission of HTLV-I was associated with lack of high-titered maternal antibodies to the *env* epitopes. Further study of the epitopes implicated herein may allow prediction of the humoral immune response that protects children from vertical transmission and may lead to immune-based therapy to prevent vertical transmission of HTLV-I.

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