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A Maternal Risk Factor for Mother-to-Child HTLV-I Transmission: Viral Antigen-producing Capacities in Culture of Peripheral Blood and Breast Milk Cells

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We examined the relationship between productivity of HTLV-I antigen-positive cells in cultured peripheral blood mononuclear cells (PBMC) and breast milk mononuclear cells (BMMC) and the incidence of mother-to-child transmission of HTLV-I. Among 61 cases of HTLV-I carrier mothers, 17 cases were revealed to produce large numbers of HTLV-I antigen-positive cells (high HTLV-I antigen-producing mothers) whose positive rate was 9.6% in PBMC and 10.2% in BMMC, while the remaining 44 cases produced small numbers of HTLV-I antigen-positive cells (low HTLV-I antigen-producing mothers) whose positive rate was 0.3% in PBMC and 0.5% in BMMC. The HTLV-I transmission rate among children born to the high HTLV-I antigen-producing mothers was 37.5% (6/16 children from 11 mothers), while that of the low HTLV-I antigen-producing mothers was 3.2% (1/31 children from 20 mothers). The transmission rate of HTLV-I was significantly different between high and low HTLV-I antigen-producing mothers ($P<0.05$). However, there was no positive relationship between anti-HTLV-I antibody titers and productivity of HTLV-I antigen-positive cells ($P=0.11$). These results suggested that mother-to-child transmission of HTLV-I might be influenced by a maternally determined factor to produce HTLV-I antigen-positive cells in PBMC and BMMC of HTLV-I carrier mothers.

Key words: HTLV-I — Mother-to-child transmission — HTLV-I antigen-positive cells — Peripheral blood and breast milk

Natural HTLV-I infection is known to occur by mother-to-child transmission via breast milk[1] and husband-to-wife transmission probably via seminal fluid.[2,3] Infectious HTLV-I cells are associated with lymphocytes and are excreted into breast milk[4] and seminal fluid.[5] Thus, HTLV-I infection is more prevalent among breast-fed children than bottle-fed children.[6,7] Milk-borne infection with HTLV-I has been demonstrated by sero-epidemiological studies of breast-fed children born to HTLV-I carrier mothers[8,9] and by laboratory experiments on HTLV-I infection *in vitro*[10] and *in vivo*.[11,12] However, the rate of mother-to-child transmission varies widely among breast-fed children born to HTLV-I carrier mothers, even if they were breast-fed by their mothers for a long time after birth.[10,13] Thus, the risk for mother-to-child transmission of HTLV-I may be influenced by maternal and infant's factors. Although the infant's risk factor for HTLV-I transmission is yet unknown, the maternal risk factors are thought to be related to the production of HTLV-I antigen-positive cells or HTLV-I viral load in the mononuclear cells of peripheral blood[14,15] and breast milk.[16]

The present study was designed to investigate the relationship between productivity of HTLV-I antigen-positive cells in cultured peripheral blood mononuclear cells (PBMC) and breast milk mononuclear cells (BMMC) and the incidence of HTLV-I transmission from mother to child.

MATERIALS AND METHODS

One thousand six hundred and fifty-eight pregnant women from Kagoshima Prefecture, Kyushu, Japan, were screened for anti-HTLV-I antibody by using the particle agglutination (PA) test with Serodia HTLV-I kit (Fijirebio Inc., Tokyo) and the EIA test with Eitest ATL kit (Eisai Co., Ltd., Tokyo). Positivity in the PA test or in EIA was confirmed by the indirect immunofluorescence (IF) test using MT-1 cells.[17] The sera positive in the IF test at 10-fold dilution were designated as HTLV-I seropositive. Serum samples of low PA titers and those showing nonspecific IF staining were subjected to western blotting using the HTLV-I problot kit (Fijirebio Inc.).

Sixty-one of the pregnant women were found to be HTLV-I seropositive and were entered into this study as HTLV-I carrier mothers (Table I). They gave birth to children during the period from December 1988 to March 1991. The mean of their ages was 29.5 ± 3.2 years. Fifty-six of the mothers had transvaginal delivery and

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Table I. Subjects of This Study

HTLV-I carrier mothers (case ID/yr)	Delivery History[a]/Mode[b]	Anti-HTLV-I antibody in sera (PA titers)	Mononuclear cells in breast milk ($\times 10^5$/ml)	HTLV-I carrier mothers (case ID/yr)	Delivery History[a]/Mode[b]	Anti-HTLV-I antibody in sera (PA titers)	Mononuclear cells in breast milk ($\times 10^5$/ml)
1. MK/31	2/CS	64	1.52	32. HT/28	0/TV	1024	1.43
2. KN/25	0/TV	128	1.85	33. HK/31	2/TV	1024	1.72
3. AY/29	2/TV	128	1.81	34. MH/28	0/TV	1024	1.96
4. SC/34	2/TV	128	2.58	35. MA/29	1/TV	1024	11.52
5. HK/30	0/CS	128	4.82	36. KR/31	2/TV	1024	3.64
6. KM/29	0/TV	256	10.28	37. NM/24	0/TV	1024	4.22
7. KK/27	1/TV	256	1.20	38. IT/26	0/TV	1024	1.65
8. NS/28	1/TV	256	1.45	39. YO/32	1/TV	2048	1.60
9. KA/36	2/TV	256	2.13	40. TH/28	0/CS	2048	1.82
10. KT/31	1/TV	256	2.84	41. FU/33	2/TV	2048	3.54
11. SM/30	2/TV	512	3.78	42. MR/31	2/TV	2048	3.50
12. KR/29	1/TV	512	6.67	43. HM/27	0/TV	2048	2.25
13. YK/27	0/TV	512	3.56	44. AY/30	1/TV	2048	9.27
14. TT/27	2/TV	512	2.40	45. NN/28	1/TV	2048	1.65
15. NS/23	1/TV	512	1.45	46. SN/33	2/TV	2048	3.29
16. EA/30	2/TV	512	2.25	47. OY/29	1/TV	2048	1.05
17. ST/34	0/CS	512	2.55	48. AN/22	0/CS	2048	1.35
18. SK/30	2/TV	512	5.54	49. KY/30	0/TV	4096	1.52
19. AR/29	2/TV	512	3.20	50. NY/28	1/TV	4096	3.88
20. SI/26	1/TV	512	9.58	51. HY/30	0/TV	4096	1.50
21. TA/36	3/TV	512	3.14	52. SY/29	2/TV	4096	3.88
22. HS/31	1/TV	512	2.64	53. NY/27	1/TV	8192	1.32
23. SM/35	2/TV	512	3.08	54. SM/32	2/TV	8192	10.42
24. HK/29	0/CS	512	2.67	55. TY/25	1/TV	8192	1.30
25. EK/28	0/TV	1024	1.40	56. HM/32	2/TV	8192	10.50
26. YK/34	2/TV	1024	1.75	57. SM/33	2/CS	8192	5.66
27. SH/28	1/TV	1024	3.52	58. NK/26	0/TV	8192	2.52
28. YA/26	1/TV	1024	2.35	59. AM/32	2/TV	16384	1.62
29. TR/30	2/TV	1024	3.60	60. UK/26	1/TV	16384	7.52
30. KM/29	0/TV	1024	4.25	61. NS/33	2/TV	16384	3.20
31. EN/38	2/TV	1024	10.30				

a) Times of delivery.
b) CS, Cesarean section; TV, transvaginal delivery.

the rest had Cesarean section delivery. Isolation and *in vitro* cultivation of PBMC and BMMC were performed as follows. In brief, the PBMC were isolated from 20–30 ml of heparinized venous blood by centrifugation on a Ficoll-Hypaque density gradient (d=1.077) at 400*g* for 20 min at room temperature as described.[18] The BMMC were isolated by centrifugation on a Ficoll-Hypaque density gradient as described above using 50–100 ml of breast milk which was collected aseptically from the mothers on post-delivery day 5. The isolated PBMC and BMMC were cryopreserved in a liquid nitrogen until use, as described elsewhere.[18]

The cryopreserved PBMC and BMMC were thawed and washed once with 10 ml of RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (10%FCS-RPMI medium) and cultured in 96-well U-bottomed plates with 200 μl of a suspension of 5×10^5 cells/ml of 10%FCS-RPMI medium supplemented with 2 μg/ml phytohemagglutinin-P (Difico, Detroit, MI) and 100 units of rIL-2 (TGP-3, Takeda Chemical Industries, Osaka) in a humidified 5% CO_2 incubator at 37°C. The cultured PBMC and BMMC were harvested every 3 days up to 12 days of cultivation, and were fixed in cold acetone at −20°C to conduct the IF staining for HTLV-I antigens by using mouse anti-HTLV-I p19 monoclonal antibody (GIN-14)[19] and anti-HTLV-I gp-21 monoclonal antibody (F-10)[20] as the first antibodies and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG antibody as the second antibody, as described.[10] Normal mouse IgG was used as a negative control. The percentage of HTLV-I antigen-positive cells was calculated by counting a total of 500 smeared cells under an inverted fluorescence microscope. Statistical significance of the results was examined by using Student's *t* test.

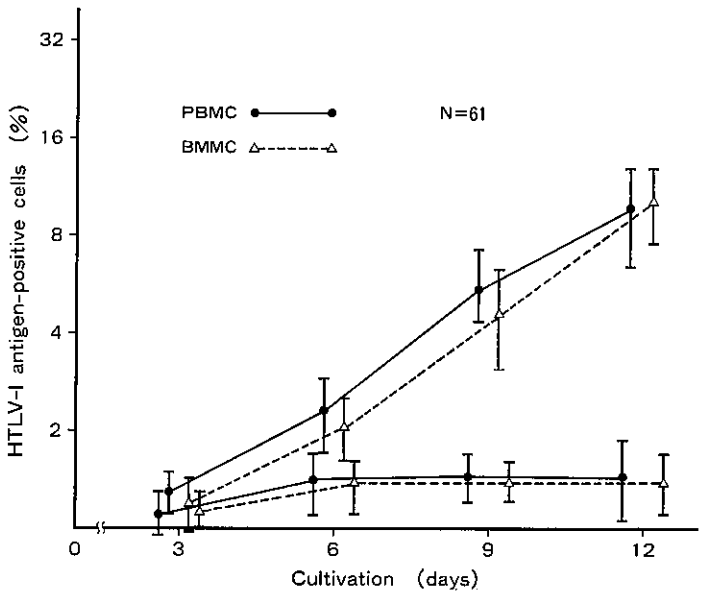

Fig. 1. Production of HTLV-I antigen-positive cells in cultured PBMC and BMMC from HTLV-I carrier mothers. PBMC and BMMC of mother 61 were cultured *in vitro* for up to 12 days and 10^5 cells were taken at the indicated time intervals for measurement of HTLV-I antigen-positive cells by immunofluorescence staining with GIN-14 monoclonal antibody.

RESULTS

Anti-HTLV-I antibody titers in the sera and number of mononuclear cells in breast milk of HTLV-I carrier mothers Sixty-one HTLV-I carrier mothers were investigated for history of delivery, anti-HTLV-I antibody status and number of mononuclear cells in breast milk (Table I). Ages of the mothers ranged from 24 to 38 years. Most of the mothers had a history of one or two deliveries. PA titers of anti-HTLV-I antibody in sera varied from 64 (2^6) to 16384 (2^{14}) fold (mean=$2^{10.1\pm1.9}$). A similar range of antibody titers was found in the cord blood of neonates born to the respective HTLV-I carrier mothers (data not shown).

The number of BMMC varied from 1.20×10^6 to 10.42×10^6 per ml of breast milk (mean=$3.61\pm2.77\times10^6$). There was no significant correlation between number of BMMC and anti-HTLV-I antibody titers of the HTLV-I carrier mothers ($P=0.34$).

Production of HTLV-I antigen-positive cells in cultured PBMC and BMMC from HTLV-I carrier mothers PBMC and BMMC from the HTLV-I carrier mothers were cultured *in vitro* to examine the HTLV-I antigen-positive cells. The time-course experiments revealed that one group of HTLV-I carrier mothers produced a logarithmic increase of HTLV-I antigen-positive cells during cultivation for 6–12 days and the other group produced only a marginal increase of HTLV-I antigen-positive cells throughout the incubation period. Thus, we designated the former group as high HTLV-I antigen-producing mothers and the latter group as low HTLV-I antigen-producing mothers. The kinetics of production of HTLV-I antigen-positive cells in the cultures of PBMC and BMMC was the same in both high and low HTLV-I antigen-producing mothers (Fig. 1). Thus, 27.9% (17/61 cases) of HTLV-I carrier mothers were high HTLV-I antigen-producing mothers and 72.1% (44/61 cases) were low HTLV-I antigen-producing mothers.

Table II. Comparison of HTLV-I-positive Cell Production Rates in the Cultured PBMC and BMMC between High and Low HTLV-I Antigen-producing Mothers

HTLV-I carrier mothers (N=61)	% of HTLV-I antigen-positive cells[a]		Anti-HTLV-I antibody in sera (PA titers)
	PBMC	BMMC	
High HTLV-I antigen-producing mothers (N=17)	9.6 ± 1.7	10.2 ± 2.8	$2^{10.6\pm1.9}$
	$P<0.005$	$P<0.005$	$P=0.11$
Low HTLV-I antigen-producing mothers (N=44)	0.6 ± 0.6	0.3 ± 0.8	$2^{9.8\pm1.9}$

a) Immunostained with anti-HTLV-I p19 (GIN-14) after 12 days of cultivation.

The rate of HTLV-I antigen-positive cells was 9.6% in PBMC and 10.2% in BMMC of the high HTLV-I antigen-producing mothers, while that of the low HTLV-I antigen-producing mothers was 0.6% in PBMC and 0.3% in BMMC (Table II). The production rate of HTLV-I antigen-positive cells was significantly different

between high and low HTLV-I antigen-producing mothers ($P<0.005$). However, the difference in anti-HTLV-I antibody titers between the two groups was insignificant ($P=0.11$).

Mother-to-child transmission of HTLV-I in high and low HTLV-I antigen-producing mothers Sixteen children born to 11 of the high HTLV-I antigen-producing mothers were available for retrospective study on the relationship between duration of breast feeding and HTLV-I transmission after birth. The duration of breast feeding among children born to the high HTLV-I antigen-producing mothers ranged from 5 months to 24 months (mean $=10.4\pm4.9$ months). The HTLV-I transmission rate was 37.5% (6/16 cases) of the children, whose HTLV-I infection was serologically confirmed at age 2–6 years (Table III, high HTLV-I antigen-producing mothers).

Thirty-one children born to 20 of the low HTLV-I antigen-producing mothers were similarly followed up. The duration of breast feeding of these children ranged from 2 months to 24 months (mean $=8.6\pm4.7$ months). The HTLV-I transmission rate was 3.2% (1/31 cases) of the children whose HTLV-I infection was serologically confirmed at age 2 years. The remaining 30 children were HTLV-I seronegative, as confirmed at age 2–7 years (Table III, low HTLV-I antigen-producing mothers). The difference of the HTLV-I transmission rate between high and low HTLV-I antigen-producing mothers was statistically significant ($P<0.01$).

DISCUSSION

The present study was designed to analyze a maternal factor influencing the risk of mother-to-child transmission of HTLV-I by using PBMC and BMMC obtained from HTLV-I carrier mothers who differed in the titers of anti-HTLV-I antibodies (Table I).

The individual differences in anti-HTLV-I antibody titers among the HTLV-I carrier mothers suggested that variable immune responses to HTLV-I infection might be

Table III. HTLV-I Infection among Children Born to High or Low HTLV-I Antigen-producing Mothers

Children born to	Duration of breast feeding (months)	Anti-HTLV-I tested at (years)	Antibody PA titers	HTLV-I infection
High HTLV-I antigen-producing mothers[a]				
3-1[b] Male	12	5	512	+
3-2[c] Male	10	3	<16	−
11-1 Female	24	5	2048	+
11-2 Female	5	1	<16	−
20-1 Female	7	2	<16	−
23-1 Male	10	5	<16	−
23-2 Female	6	2	<16	−[d]
31-1 Female	12	6	512	+ 6/16(37.5%)
31-2 Male	12	4	256	+
35-1 Male	5	3	<16	−
44-1 Male	6	3	<16	−
50-1 Female	10	4	1024	+
55-1 Female	18	2	<16	−
57-1 Male	8	5	<16	−
57-2 Female	7	2	512	+
60-1 Female	12	2	<16	−
Low HTLV-I antigen-producing mothers[a]				
1-1 Male	12	5	<16	−
1-2 Male	10	3	<16	−
4-1 Female	8	7	<16	−
4-2 Male	6	4	<16	−
7-1 Female	5	4	<16	−
8-1 Female	7	2	<16	−
14-1 Male	5	6	<16	−
14-2 Male	6	4	<16	−[d]
16-1 Female	15	3	<16	− 1/31(3.2%)
19-1 Female	12	5	<16	−
21-1 Female	4	2	<16	−
26-1 Female	3	4	<16	−
26-2 Male	2	3	<16	−
29-1 Male	10	5	<16	−
29-2 Female	6	2	<16	−
33-1 Male	24	2	<16	−
39-1 Male	12	3	<16	−
42-1 Male	10	6	<16	−
42-2 Female	6	4	<16	−
46-1 Male	6	4	<16	−
46-2 Female	6	2	<16	−
47-1 Female	18	2	128	+
52-1 Male	6	3	<16	−
52-2 Male	9	1	<16	−
53-1 Female	15	3	<16	−
54-1 Female	6	5	<16	−
54-2 Female	6	2	<16	−
59-1 Female	6	4	<16	−
59-2 Male	5	2	<16	−
61-1 Male	10	5	<16	−
61-2 Female	12	2	<16	−

a) HTLV-I carrier mothers listed in Table I.
b) First son born to case 3 mother.
c) Second son born to case 3 mother.
d) $P<0.01$.

produced depending upon the genetic background of HTLV-I carrier mothers, whose T-cells varied in the level of replication[21] and in the magnitude of immunological effector functions, as seen among patients with adult T-cell leukemia and HTLV-I-associated myelopathy/tropical spastic paraparesis.[22, 23]

When PBMC and BMMC of HTLV-I carrier mothers were cultured *in vitro*, they produced HTLV-I antigen-positive cells. Their productive responses were categorized into two groups; high and low HTLV-I antigen-producing mothers (Fig. 1). The high HTLV-I antigen-producing mothers were a minority of the HTLV-I carrier mothers, while the low HTLV-I antigen-producing mothers were a majority (Table II). The high and low productions of HTLV-I antigen-positive cells were reproducible in the cultures of PBMC and BMMC from the high and low HTLV-I antigen-producing mothers, but they were not in parallel with anti-HTLV-I antibody titers (PA titers in Table II). These results suggested that production of HTLV-I antigen-positive cells might not be correlated with humoral immune response to HTLV-I.

The transmission rate of HTLV-I among high HTLV-I antigen-producing mothers was 10 times more than that of low HTLV-I antigen-producing mothers (37.5% vs. 3.2% in Table III). This different transmission rate of HTLV-I could not be ascribed to the duration of breast feeding of the children, but rather was due to the individual differences in the ability of mothers to produce HTLV-I antigen-positive cells in PBMC and BMMC, since all of the HTLV-I-infected children had been breast-fed for more than 6 months, as shown in Table III (duration of breast feeding of HTLV-I-infected children). It was thus suggested that the low HTLV-I antigen-producing mothers might be high immune responders in terms of anti-HTLV-I cytotoxic T-cells, while the high HTLV-I antigen-producing mothers might be low immune responders, as postulated elsewhere.[24–26] Therefore, the children born to high HTLV-I antigen-producing mothers might be at high risk for HTLV-I infection when breast-fed for more than 6 months.

The rate of mother-to-child transmission of HTLV-I is decreasing in Japan[27] in accordance with a mathematical model for vertical transmission of HTLV-I.[28] The lowered incidence of HTLV-I transmission among the low HTLV-I antigen-producing mothers appeared to be correlated with decreased production of HTLV-I antigen-positive cells. Indeed, 72.1% of HTLV-I carrier mothers were low HTLV-I antigen-producing mothers and 27.9% of them were high HTLV-I antigen-producing mothers. The majority of the HTLV-I carrier mothers in this study had low risk of transmitting HTLV-I to their children.

In conclusion, the high HTLV-I antigen-producing mothers had a greater risk of transmitting HTLV-I to their breast-fed children than the low HTLV-I antigen-producing mothers. Further studies are necessary to correlate the number of HTLV-I antigen-positive cells with the HTLV-I proviral load in PBMC and BMMC and to clarify the immunological effectors that control the level of HTLV-I antigen positive cells *in vivo*.

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