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Vertical Transmission of HTLV-1 in HTLV-1 Carrier Rat

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A female F344 rat was injected with 2.4×10^6 MT-2 cells intravenously at 3 and 4 weeks old, and was mated with a non-infected male rat at the 17th week after injection, when the dam rat contained HTLV-1 provirus in the peripheral blood mononuclear cells as determined by polymerase chain reaction. HTLV-1 provirus was detected in at least 2 out of 9 offspring. This system should be useful for studies on the routes and prevention of vertical transmission and on elimination of once-transmitted HTLV-1.

Key words: Vertical transmission — HTLV-1 — HTLV-1 carrier — PCR — Rat model

Human T cell leukemia virus type 1 (HTLV-1) is known to increase the risk of adult T-cell leukemia[1-3] and other HTLV-1-related diseases.[4,5] The route of HTLV-1 transmission through blood transfusion was established.[6] Accumulating data also suggest vertical transmission from mother to child through milk[7] and transmission from husband to wife through sexual intercourse. The percentage of vertical transmission in bottle-fed children was recently reported to be 5.7%, while that in breast-fed children was 10.5%.[8] However, nothing is known about routes of vertical transmission other than through milk.

We have demonstrated by polymerase chain reaction (PCR) that rats can be chronically infected with HTLV-1 *in vivo*.[9] As the first step to analyze the mode and routes of vertical transmission and to obtain information which might allow us to prevent HTLV-1 transmission, we examined whether mother-to-child transmission of HTLV-1 could occur in a carrier rat model. A female F344 rat were injected with 2.4×10^6 MT-2 cells, which produce HTLV-1,[10] through the tail vein at 3 and 4 weeks old. We collected the blood at the 10th and the 12th week after MT-2 cell injection and separated the peripheral blood mononuclear cells (PBMC) by Ficoll-Hypaque gradient (Lymphosepal II, Immuno-Biological Laboratories, Gunma). PBMC were hemolyzed with 1% ammonium acetate and 10^5 cells were dissolved in 12.5 μl of lysis buffer (10 m*M* Tris-HCl, pH 8.3, 1.0 m*M* EDTA, 0.45% Tween 20, 0.45% NP-40, 300 μg/ml Proteinase K) and incubated for 4 h at 55°C. PBMC lysate was heated for 10 min at 95°C and was incubated in PCR mixture (20 units/ml *Taq* polymerase, 10 m*M* Tris-HCl, pH 8.3, 0.5 μ*M* each primer, 50 m*M* KCl, 0.2 m*M* dNTP, 1.5 m*M* $MgCl_2$). The PCR reaction was conducted through heating at 93°C for 5 min, followed by 50 cycles of incubation at 93°C for 1.5 min, at 57°C for 2 min and at 72°C for 2 min, and finally at 72°C for 7 min. The amplified regions were *gag* (nt 1301–1420), LTR (nt 37–305) and *pX* (nt 7336–7494) of HTLV-1.[11] Rat c-*myc* sequence (nt 4069–4413) was also amplified as an internal positive control to confirm that the amplification reaction was successful.[9] The amplified products were subjected to electrophoresis in 3% agarose gel and Southern hybridization using ^{32}P-labeled probes. Nt 97–116 of LTR, nt 1341–1360 of *gag* and nt 7363–7373 of *pX* were used as hybridization probes. Anti-HTLV-1 antibody was assayed by the particle agglutination method (Serodia HTLV-1, Fujirebio, Tokyo). Antibody titer in the plasma against HTLV-1 was 1024-fold at the 12th week after MT-2 cell injection.

PBMC of the MT-2 injected rat contained HTLV-1 provirus before mating at the 10th week after MT-2 cell injection. The rat was mated with a non-infected male F344 rat at the 17th week after MT-2 cell injection, and gave birth to 9 offspring, which were fostered by their own dam. At 4 weeks old, blood samples of the offspring were collected for PCR and antibody detection.

Plasma samples from the offspring were diluted 16-fold and checked for anti-HTLV-1 antibody by the particle-agglutination method. The antibody was detected in plasma of all the offspring at 4 weeks old, while it became undetectable at 8 and 20 weeks old (data not shown). Since there is a report of detection of HTLV-1 provirus in seronegative infants born to HTLV-1 seropositive mothers,[12] it seems important to detect HTLV-1 in the offspring.

The sensitivity of the PCR was checked by using serial dilutions of HTLV-1 provirus-carrying cell line, ATL 1K, which has 1 molecule of HTLV-1 provirus per cell.[13] Fig. 1 shows that 1 molecule of HTLV-1 provirus was detectable with either *gag* primers or *pX* primers. As shown in Table I, 6 out of 9 offspring revealed HTLV-1 provirus in PBMC by PCR when the *gag* region was analyzed. Since the numbers examined by PCR with *gag* primers were small and the *gag* region was reported to be frequently deleted whereas the *pX* region was conserved in PBMC of ATL patients,[14] we performed 10 assays using *pX* primers with PBMC of offspring nos. 2, 3 and 9, as examples. As shown in Fig. 2 and Table I, the HTLV-1 provirus was revealed in 7/10, 5/10 and 0/10, for offspring nos. 2, 3 and 9, respectively, and it was not detected in 10 assays without template DNA. The positive control tube which contained 3 molecules of HTLV-1 provirus was consistently positive in this PCR assay (Fig. 2D).

The above data gave evidence of the presence of HTLV-1 provirus at least in PBMC of offspring nos. 2 and 3. If the distribution of the ratio of tubes containing one or more molecules of HTLV-1 follows a Poisson distribution, the amount of the provirus in offspring nos. 2 and 3 was expected to be 0.7–1.2 molecules per 10^5 PBMC and that in offspring no. 9 would be less than 0.1 molecule per 10^5 PBMC. The preliminary PCR data using *gag* primers suggest that the incidence of detectable

Fig. 1. Sensitivity of PCR for detection of HTLV-1 provirus. ATL-1K cell lysate was diluted with normal rat PBMC lysate so that the duplicate PCR reaction tubes contain 0 (lanes 1, 2), 0.1 (lanes 3, 4), 1 (lanes 5, 6) and 10 (lanes 7, 8) molecules of HTLV-1 provirus in the 12.5 μl lysate corresponding to 10^5 PBMC. *gag* and *pX* region were amplified with each primer set and probed with the respective probes. A; *gag* fragment. B; *pX* fragment.

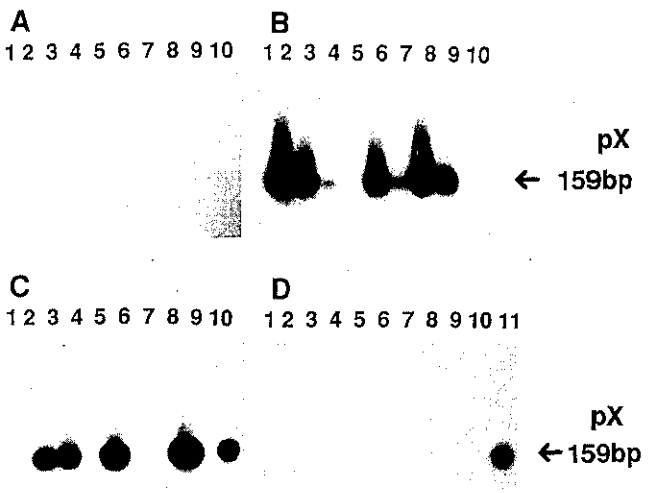

Fig. 2. Detection of HTLV-1 provirus by PCR with *pX* primers in the PBMC of offspring nos. 2, 3 and 9 in 10 assays (lanes 1–10) at the 4th week after birth. Autoradiography was done with *pX* probe, which should detect an amplified *pX* fragment of 159 bp, the position of which is shown by an arrow. A; without template as negative control. B; offspring no. 2. C; offspring no. 3. D; offspring no. 9. Lane 11, positive control which contained 3 molecules of HTLV-1 provirus.

Table I. Detection of HTLV-1 Provirus by PCR

Time at sampling[a]	Analyzed region	Dam	Offspring No. of positive tubes/No. of tubes examined									−DNA[b]
		TF80	1M[c]	2F	3M	4F	5F	6F	7F	8F	9M	
Before[d]	LTR	1/2										
4 weeks	*gag*	1/2	1/2	2/2	2/2	0/2	1/2	1/2	0/2	0/2	1/2	0/4
	pX			7/10	5/10						0/10	0/10

a) The term "before" means before mating, and weeks correspond to the time after delivery or birth.
b) PCR was performed without template DNA.
c) M; male, F; female.
d) The 10th week after MT-2 cell injection.

HTLV-1 provirus decreased during the observation period up to the 20th week. Although two of four other similarly treated dams who gave birth gave similar results (data not shown), it seems that longer-term follow-up study of HTLV-1 provirus in the offspring from other dams with quantitative PCR is required to reach a full understanding of vertical transmission of HTLV-1. It was reported that among breast-fed babies born to seropositive mothers HTLV-1 antigen was not detected before 6 months old, but was found in 77% of them at two years old.[15]

We could demonstrate vertical transmission of HTLV-1 in the rat carrier system by showing the presence of HTLV-1 provirus in the offspring. Since rats give birth to many offspring, this vertical transmission system of HTLV-1 in rats should be useful for analyzing routes of transmission other than through milk, and the factors and conditions for maintenance or elimination of once-transmitted HTLV-1, and also for evaluation of preventive measures against HTLV-1 transmission by active or passive immunization.

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