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Intrauterine Transmission of Human T-Cell Leukemia Virus Type I in Rats

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To analyze intrauterine transmission, MT-2 cells, a human T-cell line producing human T-cell leukemia virus type I (HTLV-I), were injected into eight pregnant F344 rats, and cesarean section was performed at day 23 of pregnancy. HTLV-I provirus was detected by PCR in the liver and spleen taken from one of the eight fetuses. Moreover, 71 offspring were delivered by cesarean section from the remaining seven dams and fostered by seven normal rats. HTLV-I provirus was detected in peripheral blood mononuclear cells in 2 of the 71 offspring 4 weeks after cesarean section. These results indicate for the first time the intrauterine transmission of HTLV-I. To confirm the postnatal transmission, MT-2 cells were injected into a dam within 24 h after delivery, and six offspring were fostered by this dam. HTLV-I provirus was detected in peripheral blood mononuclear cells of all six offspring. This animal model may be useful for analysis and prevention of mother-to-child transmission of HTLV-I.

Human T-cell leukemia virus type I (HTLV-I) (22) was the first human oncogenic retrovirus to be identified and isolated. It is claimed to be an etiological agent of adult T-cell leukemia/lymphoma (ATL/L) (33) and HTLV-I-associated myelopathy/tropical spastic paraparesis (8, 21). Typical ATL is an aggressive leukemia, and the prognosis of patients with acute ATL is poor. HTLV-I provirus contains no oncogene. However, the viral genome encodes two regulatory proteins, Tax and Rex, which are involved in the control of gene expression of HTLV-I, growth factors, and proto-oncogenes (7, 27, 34). Tax is reported to have immortalizing activity when an expression vector with it is introduced into cells (9, 31), and transgenic mice that contain the *tax* sequence develop neurofibromatosis (11) and mesenchymal tumors (19). Thus, infection by HTLV-I is the first step in the development of ATL/L or HAM/TSP.

Mother-to-child transmission of retroviruses is generally known (23). For example, the mother-to-child transmission of human immunodeficiency virus type 1 (HIV-1), which is another human retrovirus, is the major route of transmission in the pediatric population (23). However, the prenatal route of mother-to-child transmission of HIV-1 or HTLV-I is still unclear, in part because of the absence of a convenient animal model of HIV-1 or HTLV-I transmission.

We have established a small-animal HTLV-I carrier model (28) and demonstrated mother-to-child transmission in rats (3). In the prenatal period, transmission of HTLV-I could occur either during the intrauterine period or during delivery. Transplacental transmission has been analyzed in human samples (15), but no clear evidence of prenatal transmission has been reported. In this study, to analyze the intrauterine transmission of HTLV-I in rats, we performed cesarean section and analyzed the HTLV-I provirus in the organs of the fetuses and in peripheral blood mononuclear cells (PBMCs) of the offspring 4 weeks after cesarean section. We also analyzed the incidence of postnatal transmission with this new rat model.

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Cell lines and animals. MT-2 cells, a human HTLV-I-infected cell line (17), were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. Cell line ATL-1K, which was established from PBMCs of an ATL patient (12), were cultured in RPMI 1640 supplemented with 20% fetal bovine serum. MT-2 cells in logarithmic growth phase with more than 98% viability were used for injection. The cell concentration was adjusted to 5.0×10^6/ml with 1× Dulbecco's phosphate-buffered saline (0.01 M phosphate buffer, 0.138 M NaCl, 0.0027 M KCl [pH 7.4]) before injection to rats.

F344 rats were purchased from Charles River (Kanagawa, Japan). In the intrauterine transmission study, we injected 2.0×10^6 MT-2 cells into the tail veins of eight dam rats at day 14 of pregnancy under ether anesthesia (Fig. 1A). At day 23 of pregnancy, we removed 71 offspring from 7 pregnant rats by cesarean section under ether anesthesia. To avoid contamination of the fetuses with maternal blood during the cesarean section, we washed uteri containing fetuses with 100 ml of saline (37°C) twice. After bilateral ligation of the ovarian and uterine arteries and veins, the vessels were cut. After washing out maternal blood, we removed the offspring from the uterus. When they started to breathe, these 71 offspring were foster nursed by seven normal rats. The eight fetuses from another dam were sacrificed and frozen at −20°C. To avoid any contamination by the maternal blood, the liver and spleen of each fetus were extracted in a frozen condition.

Figure 1B shows the strategy for the postnatal transmission study. A pregnant 10-week-old F344 rat was purchased. Within 24 h after delivery, we injected 2.0×10^6 MT-2 cells into the dam rat through the tail vein under ether anesthesia. Six offspring were fostered by the same dam rat.

PCR. One milliliter of peripheral blood was withdrawn from the orbital vein of a rat under ether anesthesia; 80 μl of 0.3 M EDTA was added, and PBMCs were separated by Ficoll-Conray gradient (Lymphosepal II; Immuno-Biological Laboratories, Gunma, Japan). PBMCs were washed with 0.9% NaCl, and an aliquot of 10^5 PBMCs was mixed with 12.5 μl of lysis buffer containing 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.45% Nonidet P-40, 0.45% Tween 20, and 300 μg of proteinase K per ml, incubated for 4 h at 60°C, and stored at −20°C.

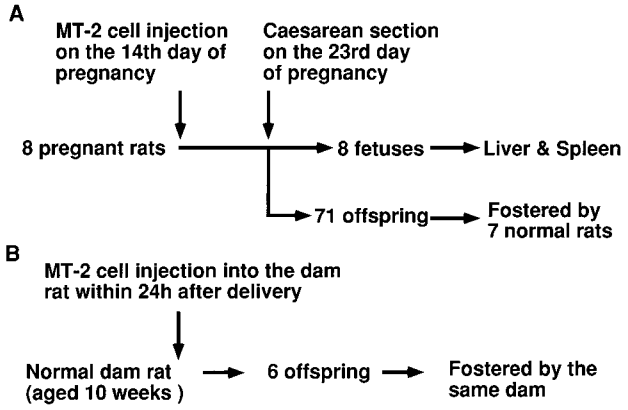

FIG. 1. (A) Intrauterine transmission of HTLV-I in the rat model; (B) postnatal transmission of HTLV-I in the rat model. The procedures are described in the text.

The cell lysate of ATL-1K cells, containing one molecule of HTLV-I provirus per cell (12), was diluted with the lysate of normal rat PBMCs, so that three molecules of HTLV-I provirus per lysate of 10^5 normal rat PBMCs could be used as the positive control for PCR. DNA was isolated from the fetal organs by standard methods (25).

Twenty-five microliters of the reaction mixture for PCR (18) contained lysate corresponding to 10^5 PBMCs, 0.5 μM each primers for the *gag* or *pX* region, 0.2 mM all four deoxyribonucleoside triphosphates, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 1 U of thermostable DNA polymerase (*Taq* DNA polymerase; WAKO, Osaka, Japan). The reaction buffer for DNA contained 0.5 μM each primers for the *gag* or *pX* region, 0.2 mM all four deoxyribonucleoside triphosphates, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.0 mM $MgCl_2$, 0.01% gelatin, and 0.05% Tween 20. We used *gag* and *pX* primers to detect HTLV-I provirus. The primers and probes for HTLV-I used were as previously described, and 50 cycles of amplification reaction were carried out (28). The PCR was performed at least twice with each primer for lysates of PBMCs from all offspring and for the DNA from the liver and the spleen.

The conditions for hybridization were as described by Albretsen et al. (1). The PCR products were separated on a 3% agarose gel, transferred to a positively charged nylon membrane (Hybond-N^+; Amersham International, Amersham, England) in an alkaline condition, and fixed with UV light (6). The filters were prehybridized for 1 h at 37°C in a solution of 5× SSC (1× SSC consists of 0.15 M NaCl in 0.015 M sodium citrate [pH 7.4]), 1% sodium dodecyl sulfate (SDS), 5× Denhardt's solution (1× Denhardt's solution consists of 5 mg of Ficoll [type 400; Pharmacia] per ml, 5 mg of polyvinylpyrrolidone per ml, and 5 mg of bovine serum albumin [fraction V; Sigma] per ml) and 50 μg of denatured salmon testis DNA per ml. The filters were hybridized with ^{32}P-labeled probe overnight at 42°C in the solution used for prehybridization except that 30% formamide was included. The filters were washed with 2× SSC at room temperature for 15 min and with 0.2× SSC–0.1% SDS at 52°C for 30 min. Autoradiograms were generated by a BAS2000 imaging analyzer (FUJI Photo Film, Tokyo, Japan) (2).

The decision as to whether the PCR was positive was determined as follows. The PCR was performed twice with each pair of primers. The PCR was considered positive when the *gag* and

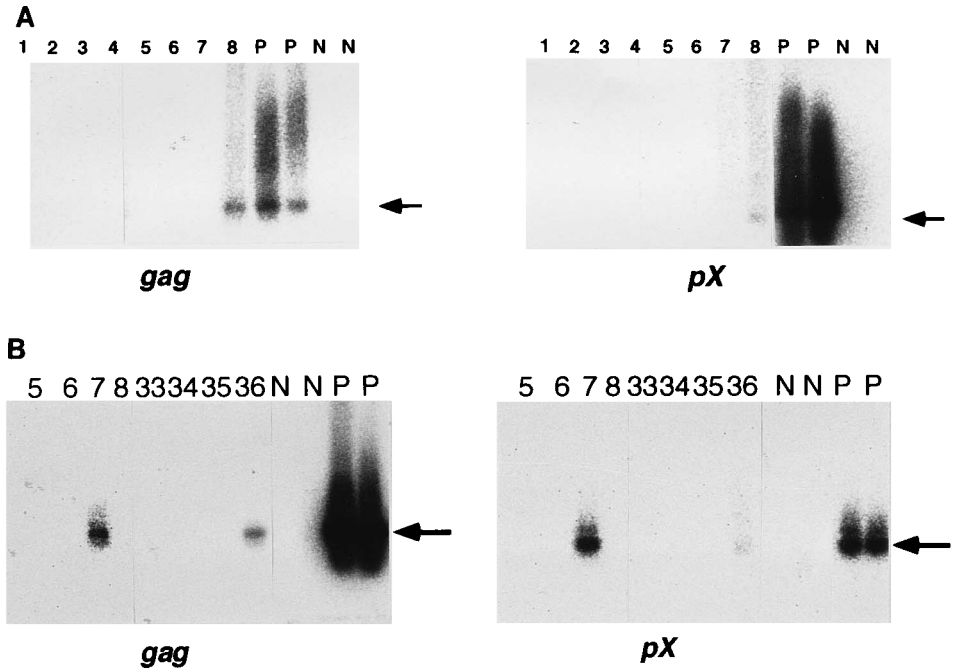

FIG. 2. Intrauterine transmission of HTLV-I as revealed by Southern blot analysis of the PCR products of the *gag* and *pX* regions of HTLV-I provirus. (A) Southern blot analysis of the PCR products, using splenic DNA of eight fetuses as templates. Lanes 1 to 8 correspond to fetus numbers. N, normal rat splenic DNA; P, positive control (DNA containing three molecules of HTLV-I provirus in 10^5 normal rat PBMCs). (B) Southern blot analysis of the PCR products from 8 of the 71 offspring delivered by cesarean section. PBMC lysates were used as templates for PCR. Lanes 5 to 8 and 33 to 36 correspond to offspring numbers. Offspring 5 to 8 belong to the same litter, and offspring 33 to 36 belong to another litter. Offspring 5 to 8 represent 4 of 10 offspring of a litter, and offspring 33 to 36 represent 4 of 10 offspring of another litter. N, normal rat PBMC lysate; P, positive control lysate containing three molecules of HTLV-I provirus in a lysate of 10^5 normal rat PBMCs. The 120-bp PCR products from *gag* and the 159-bp PCR products from *pX* are indicated by arrows.

TABLE 1. Intrauterine transmission of HTLV-I in rats

Group	Dam no.	Offspring no.	No. (%) of HTLV-I-positive offspring
Intrauterine transmission	1	8[a]	1 (12.5)
	7	71[b]	2 (2.8)
Negative control	2	20	0 (0)

[a] DNA was extracted from fetal liver and spleen.
[b] PBMCs were isolated at 4 weeks after delivery.

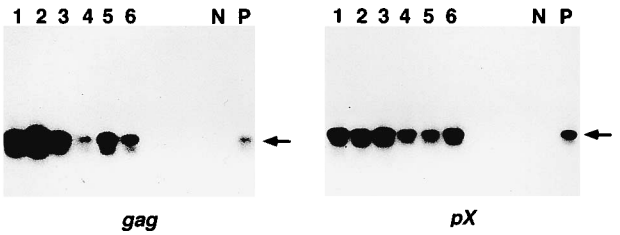

FIG. 3. Postnatal transmission of HTLV-I as revealed by Southern blot analysis of the *gag* and *pX* regions of HTLV-I provirus. Lanes 1 to 6 are lysates from the corresponding six offspring at 4 weeks of age. N, normal rat PBMC lysate; P, positive control lysate containing three molecules of HTLV-I provirus in a lysate of 10^5 normal rat PBMCs. The 120-bp PCR products from *gag* and the 159-bp PCR products from *pX* are indicated by arrows.

pX regions could be detected in the Southern blot analysis at the same time and by both primer pairs. (In total, four determinations of detection were needed.)

Anti-HTLV-I antibody. Anti-HTLV-I antibody of the plasma was titrated by the particle agglutination assay (13), using Serodia-HTLV (Fujirebio, Tokyo, Japan).

Intrauterine transmission of HTLV-I. Figure 2A shows the Southern blot analysis of PCR products of *gag* and *pX* regions from the fetuses obtained by cesarean section. The *gag* and *pX* regions of HTLV-I provirus were detected by PCR in the DNA of the spleen of one fetus (fetus 8) (Table 1). These regions were also detected in the liver DNA of fetus 8 by PCR (data not shown). Figure 2B shows the Southern blot analysis of PCR products of *gag* and *pX* regions, using as the template the PBMCs of the offspring obtained by cesarean section. The *gag* and *pX* regions of HTLV-I provirus were detected in 2 of the 71 offspring (offspring 7 and 36) at 4 weeks of age (Table 1).

Postnatal transmission of HTLV-I. Figure 3 shows the Southern blot analysis of PCR products of *gag* and *pX* regions of HTLV-I provirus amplified from the lysate of PBMCs from the six 4-week-old offspring of the dam that had been injected with MT-2 cells after delivery. In several of the Southern blots, a doublet appears for the reaction with *pX* primers. We do not know the nature of the doublet, but we detected the doublet when the amount of the virus was high.

The *pX* and *gag* regions of HTLV-I were detected in all six offspring. The antibody against HTLV-I was observed in the plasma of the dam at 4 weeks after delivery. However, we could not detect the antibody in the offspring at 4 and 8 weeks by the particle agglutination method, possibly because of low production of HTLV-I in the offspring during the observation period. The *pX* and *gag* regions were amplified in two of the six offspring at 8 weeks.

HTLV-I is believed to be transmitted via three major routes; mother-to-child transmission (10), husband-to-wife transmission (29), and blood transfusion (20). Breast feeding is considered to be the most important route for mother-to-child transmission of HTLV-I (35), but it is far from clear that this is the only route of mother-to-child transmission (4, 24). The frequency of transmission of HTLV-I by breast feeding has been reported to be from 20 to 30% (10, 14) to 10% (30). Discouraging breast feeding of infants decreased the transmission frequency to 3 to 9% (4, 32). These results show that mother-to-child transmission cannot be completely eliminated by bottle feeding and that we need to determine the other routes in order to eliminate the transmission of HTLV-I and to prevent ATL and HAM/TSP.

The present study is the first demonstration of intrauterine transmission of HTLV-I by using an animal model. Our model using cesarean section can allow a distinction between in utero and intrapartum transmission during delivery, making it possible to determine the true transmission frequency of HTLV-I in utero. Table 1 shows that the incidence of intrauterine transmission of HTLV-I in rats was from 2.8% (2 of 71) to 13% (1 of 8). The incidence of HTLV-I transmission in bottle-fed children (4, 32) is similar to the above value, although evidence for intrauterine transmission in humans is not conclusive (4, 24).

This new rat model also enabled us to examine the oral transmission of HTLV-I through breast feeding and/or through maternal saliva. In our experiment, all six offspring had HTLV-I provirus in PBMCs at 4 weeks of age (Fig. 2). However, similar experiments using more rat samples will need to be performed to determine whether an absence of the maternally transmitted antibody in the offspring is the true cause of this seemingly higher incidence of HTLV-I transmission than in offspring which have maternal antibody (3). The decreased frequency of detection of HTLV-I provirus at week 8 after delivery suggests the presence of unknown defensive mechanisms against HTLV-I in the offspring even in the absence of maternal antibody.

Analysis of prenatal transmission is quite important to prevent mother-to-child transmission. Human HTLV-I carriers have wide variations in the numbers of HTLV-I provirus-carrying cells in the peripheral blood (16, 26). Since none of the dams had been infected prior to pregnancy, we suspect that they had no neutralizing antibodies or cytotoxic anti-HTLV-I T cells during much of their pregnancy. In this regard, our rat model does not really mimic the human in vivo situation. Thus, the frequency of transmission might be modified by several factors, including (i) state of persistent infection, (ii) increased intrauterine transmission because of a bolus inoculum into the tail vein of the pregnant dam, (iii) decreased intrauterine transmission because of no constant production of HTLV-I from the beginning to the end of pregnancy, and (iv) effect of the maternal immune system during pregnancy.

Our rat model will be useful for studying the prenatal conditions which may affect transmission of HTLV-I, by changing the number of the virus-carrying cells and/or the titer and specificity of the antibodies against HTLV-I antigens. We injected MT-2 cells into dam rats at day 14 of pregnancy, when formation of the placenta was complete (5). It will be interesting to determine whether transmission occurs more efficiently before the placenta is formed.

Our rat model may be useful for development of effective measures to prevent the prenatal and postnatal transmission of HTLV-I.

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