CONCISE REPORT

Mother-to-Offspring Transmission of Human T Cell Leukemia Virus Type I in Rabbits

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We studied the mode of natural transmission of human T-cell leukemia virus type I (HTLV-I) in rabbits. Four virus-infected rabbits (2 males and 2 females) were individually mated with 4 noninfected rabbits. Two virus-infected females mated with noninfected males gave birth to 7 offspring, and 2 noninfected females mated with infected males delivered 5 offspring. Four of the seven offspring born to the virus-infected mothers seroconverted for HTLV-I when aged 6 to 13 weeks with antibody titers of 1:40 to 1:160. None of the five offspring born to the noninfected mothers became sero-positive during the observation period of 6 months, however. Peripheral lymphocytes were cultured with T cell growth factor, and HTLV-I-carrying lymphoid cell lines were established from the four seroconverted rabbits. All four cell lines were of T cells positive for La antigens. In addition, none of five newborn rabbits killed immediately after birth to a virus-infected rabbit was infected with HTLV-I. These findings provide an experimental support for the milkborne transmission of HTLV-I from mother to child in humans and indicate that the virus is tropic for T cells in rabbits as well.

We were surprised to find that HTLV-I was also transmissible orally, and by blood transfusion. Seroserological surveys suggest natural transmission of HTLV-I from mother to child and between spouses. Detection of viral antigens in semen and mothers' milk indicate that the virus was transmitted by sexual contact and by breast feeding. Recently, HTLV-I was shown to be transmissible to marmosets by oral inoculation of virus-infected lymphocytes from ATL patients and by feeding of milk from seropositive women. We showed that rabbits could be infected with HTLV-I intravenously, orally, and by blood transfusion. We now report natural transmission of HTLV-I from mother to offspring in this animal model.

MATERIALS AND METHODS

Animals. Non-inbred Japanese white rabbits weighing ~3 kg were used. They were purchased from a commercial breeder and received pelleted diet and tap water. Two females and two males were infected with HTLV-I by transfusion of 20 mL of blood from virus-infected rabbits, as described previously. All four rabbits seroconverted for HTLV-I after 2 to 4 weeks, and their antibody titers ranged from 1:80 to 1:640 in an immunofluorescence assay. The four virus-infected rabbits were housed individually and were mated with four noninfected rabbits.

Detection of antibodies to HTLV-I. Rabbits born to the virus-infected parents were followed for the development of antibodies to HTLV-I. Blood samples were taken first when the rabbits were aged 4 weeks and then serially at intervals of 1 to 2 weeks for 6 months. Sera were titrated for HTLV-I antibodies by indirect immunofluorescence using the MT-2 cell line. In brief, acetone-fixed MT-2 cells were first reacted with appropriately diluted rabbit sera and then with fluorescein-conjugated FITC-conjugated swine anti-rabbit immunoglobulins (DAKO, Copenhagen) for 30 minutes at 37°C. Anti-HTLV-I positive and negative rabbit reference sera were included as parallel controls.

Cell culture. Lymphocytes were separated from 10 mL of blood by Ficoll-Hypaque gradient centrifugation. The cells were cultured at 1 x 10^6/mL in 35-mm Petri dishes with RPMI 1640 medium supplemented with 20% fetal calf serum (FCS), 10% crude T cell growth factor (TCGF) (Japan Immunoresearch Laboratories, Takasaki), and antibiotics. The cultures were incubated at 37°C in a humidified 7.5% CO2 atmosphere and fed twice a week.

Analysis of surface and antigen markers. Cells were examined for sheep erythrocyte (E) receptors, as described previously. Surface immunoglobulins (SIg) were tested by reacting cells with FITC-conjugated swine anti-rabbit immunoglobulins. They were also tested by membrane immunofluorescence for reactivities with monoclonal antibodies, including Leu-1 (human T cells), OKIal (human la antigens), L11/135 (rabbit T cells), and 2C4 (rabbit la antigens). HTLV-I antigens were detected by indirect immunofluorescence with reference ATL patient's serum and anti-p19 and anti-p24 monoclonal antibodies.

RESULTS

Seroconversion in offspring of HTLV-I-infected rabbits. Four pairs of male and female rabbits each delivered one litter of 1 to 6 newborns. Two virus-infected females mated with noninfected males gave birth to 7 offspring, and 2 noninfected females mated with virus-infected males gave birth to 5 offspring (Fig 1). Four of the 7 rabbits born to the virus-infected mothers seroconverted for HTLV-I at 6 to 13 weeks after birth. Their antibody titers, initially low at 1:10, rose to the highest levels of 1:40 to 1:160 over the following 2 to 8 weeks and remained at those levels thereafter (Fig 2). In contrast, none of the 5 rabbits born to the noninfected...
mothers became seropositive during the observation period of 6 months.

Isolation of HTLV-I from seroconverted offspring. When the rabbits were aged 3 to 4.5 months, blood samples were taken from the seven offspring born to the virus-infected mothers; their lymphocytes were cultured in the presence of TCGF. Active cell growth occurred after 2 to 4 weeks and a TCGF-dependent lymphoid cell line was established from each of the four seroconverted offspring, whereas the cells from the three seronegative offspring did not grow for >2 months. These four cell lines, designated Ra-7 to Ra-10, grew in suspension with clumps of cells and consisted of primitive lymphoid cells (Fig 3). Two of the four cell lines became TCGF-independent after 2 months. The four cell lines had a normal female rabbit karyotype corresponding to the sex of the donors. All four cell lines were reactive with L11/135 and 2C4 but were completely negative for E receptors, Slg, Leu-1, and OKIa1. HTLV-I antigens were fully expressed in all the cell lines as detected with reference ATL patient’s serum, anti-p19, and anti-p24. Type C virus particles were demonstrated in these cell lines by electron microscopy (Fig 4). These characteristics are shown in Table 1.

Examination of newborn rabbits for HTLV-I infection. A litter of five newborn rabbits was killed immediately after birth to a virus-infected rabbit, and their splenic lymphocytes were cultured in the presence of TCGF. All five cultures were negative for HTLV-I antigens when examined by indirect immunofluorescence after 3 to 4 weeks and eventually failed to be established as lymphoid cell lines.

DISCUSSION

The concept of mother-to-child transmission of HTLV-I has been previously supported on seroepidemiologic grounds\(^5^,6\) by the detection of viral antigens in milk from seropositive women,\(^5^,6\) and by experimental oral transmission of HTLV-I into marmosets.\(^7^,8\) A rabbit model of HTLV-I infection provided a unique opportunity to investigate the mode of transmission of this virus.\(^9^,11\) In the present experiment, virus-infected female rabbits were mated with noninfected male rabbits and vice versa, and their offspring were followed for evidence of virus infection. Virus transmission occurred in offspring born to virus-infected mothers but not in offspring born to noninfected mothers. These findings give experimental support for the natural transmission of HTLV-I from mother to child in humans.

Two routes of virus transmission, transplacental or milkborne, may be considered. To clarify this point, we attempted...
a foster-nursing experiment by exchanging newborn rabbits between virus-infected and noninfected females. Two litters from virus-infected rabbits and one litter from a noninfected rabbit were all cannibalized after foster-nursing, however. We then examined whether newborn rabbits born to a virus-infected rabbit were already infected at birth with HTLV-I, but the virus could not be detected or isolated from cultures of their splenic lymphocytes. This is consistent with the absence of HTLV-I antigens in cord lymphocytes from neonates born to seropositive women. Thus, milkborne rather than transplacental transmission of HTLV-I appears more likely. Previously, we demonstrated that only one of four adult rabbits could be infected with HTLV-I by twice-weekly oral inoculation of a large number of virus-infected cells. Those results, compared with the present data, indicate that suckling rabbits may be more susceptible to oral transmission of HTLV-I.

HTLV-I is known to preferentially transform T cells from monkeys, cats, and rats, as well as from humans. Therefore, we wished to determine the lineage of the rabbit lymphoid cell lines transformed by this virus. Recently, monoclonal antibodies to rabbit T cells (L1/135) and rabbit la antigens (2C4) became available, and all four cell lines (Ra-7 to Ra-10) were reactive with both monoclonals in the absence of B cell markers. Thus, T cell tropism of HTLV-I appears to be a distinct feature common to a wide range of animal species.

**ACKNOWLEDGMENT**

We thank Dr J. M. Wilkinson for kindly providing L1/135 and 2C4.

**REFERENCES**


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| Table 1. Characteristics of HTLV-I-Carrying Rabbit Lymphoid Cell Lines |
|--------------------------|----------------|----------------|----------------|----------------|
| Markers                  | Ra-7 | Ra-8 | Ra-9 | Ra-10 |
| E receptors              | 0    | 0    | 0    | 0    |
| Sig                     | 0    | 0    | 0    | 0    |
| Lel-1                   | 0    | 0    | 0    | 0    |
| OKta                    | 0    | 0    | 0    | 0    |
| L11/135                 | 73   | 80   | 82   | 89   |
| 2C4                     | 93   | 90   | 100  | 87   |
| HTLV-I antigens         | 75   | 80   | 90   | 90   |
| HTLV-I particles        | +    | +    | +    | +    |
| Karyotype               | 44, XX | 44, XX | 44, XX | 44, XX |

Numbers are percentage of positive cells.

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