Transmission of HTLV-I by Blood Transfusion and Its Prevention by Passive Immunization in Rabbits

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To determine the minimum volume of blood required to transmit human T-cell leukemia virus type I (HTLV-I), heparinized blood was collected from a virus-infected female rabbit and aliquots of 10, 5, 1, 0.5, 0.1, and 0.01 mL were transfused into groups of two male rabbits each. All 10 rabbits transfused with 10 to 0.1 mL and 1 of 2 rabbits transfused with 0.01 mL seroconverted for HTLV-I after 2 to 4 weeks. HTLV-I-producing lymphoid cell lines of recipient origin were established from one seroconverted rabbit of each aliquot group. To determine the ability of passive immunization to protect against HTLV-I infection, two groups of three rabbits were first transfused with 5 mL of blood from the same virus-infected rabbit and then infused after 24 or 48 hours with 10 mL of HTLV-I immune globulin (77 mg/mL of IgG) prepared from seropositive healthy persons. None of the 24-hour immunization group seroconverted for HTLV-I during the observation period of six months; however, all of the 48-hour immunization group became seropositive after 2 to 4 weeks. These results indicate that HTLV-I can be transmitted with as little as 0.01 mL of virus-infected blood, and that passive immunization is effective in preventing cell-to-cell infection of HTLV-I when given within 24 hours of transfusion of virus-infected blood.

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MATERIALS AND METHODS

Animals. Non-inbred Japanese white rabbits weighing approximately 3 kg were used. They were purchased from a commercial breeder and given a pelleted diet and tap water ad libitum.

Blood transfusion. A female rabbit that had been infected with HTLV-I by maternal transmission and having a serum antibody titer of 1:320 was used as a transfusion donor. Heparinized blood obtained from this rabbit was divided into aliquots of 10, 5, 1, 0.5, 0.1, and 0.01 mL. Each aliquot was transfused into two male rabbits. Ten and 5 mL were directly transfused, while 1 to 0.01 mL were diluted to 5 mL with physiological saline before transfusion. The donor rabbit was apparently healthy and had a leukocyte count of 4.8 x 10⁶/μL with 36% lymphocytes. The lymphocyte morphology was normal on smear preparations.

Detection of antibodies to HTLV-I. Blood samples were taken from rabbits at intervals of 1 to 2 weeks, and the sera were titrated for HTLV-I antibodies by indirect immunofluorescence against the MT-2 cell line. Briefly, acetone-fixed MT-2 cells were first reacted with appropriately diluted rabbit sera and then with fluorescein isothiocyanate-conjugated swine antirabbit immunoglobulins (DAKO, Copenhagen) for 30 minutes at 37°C. Anti-HTLV-I positive and negative rabbit sera were included as parallel controls. The presence or absence of IgG antibodies was verified by Western blot using a MT-2 cell lysate as antigen. Some sera were also tested for IgG antibodies by enzyme-linked immunosorbent assay (ELISA) against disrupted HTLV-I virions in accordance with the manufacturer's instructions (Eisai, Tokyo). The specimens were tested at 1:20 and 1:6 dilutions by Western blot and ELISA, respectively.

Neutralizing antibody assay. Neutralizing antibodies were assayed against vesicular stomatitis virus (VSV) bearing envelope antigens of HTLV-I as previously described.

Passive immunization. HTLVIG containing 77 mg/mL of IgG was prepared from pooled plasma from seropositive healthy persons by the method of heat inactivation and polyelectrolyte glycol fractionation. The purity of IgG was 98% when tested by cellulose acetate membrane electrophoresis. The preparation had an anti-HTLV-I titer of 1:5, 120 by immunofluorescence and a neutralizing antibody titer of 1:6, 250 by VSV (HTLV-I) pseudotype inhibition assay. It reacted with HTLV-I gp68, p31, p28, p24, and p19 by Western blot. Two groups of three rabbits were first transfused with 5 mL of blood from the same virus-infected donor rabbit used in the transfusion experiment and then infused intravenously with 10 mL of HTLVIG 24 or 48 hours later.

Lymphocyte culture. Twenty milliliters of blood were collected from rabbits, and lymphocytes were separated by Ficoll-Conray gradient centrifugation. The cells were cultured at 1 x 10⁶/mL in 35-mm Petri dishes containing 3 mL of RPMI 1640 medium supplemented with 20% fetal calf serum, 1 μg/mL of phytohemagglutinin, and antibiotics. The cultures were incubated at 37°C in a humidified 7.5% CO₂ atmosphere and fed twice a week with medium.

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containing 50 units/mL of recombinant human interleukin-2 (IL-2) (Ajinomoto, Tokyo) instead of phytohemagglutinin.

Analysis of surface and viral antigens. Cells were tested by membrane immunofluorescence for reactivities with monoclonal antibodies to rabbit T cells (L11/135) and rabbit La antigens (2C4). HTLV-I antigens were examined by fixed cell indirect immunofluorescence with reference ATL patient’s serum and monoclonal antibodies to HTLV-I p19 and p24.

Transfusion assay. To ascertain the status of HTLV-I infection, 20 mL of blood were obtained from all 18 rabbits inoculated with virus-infected blood and transfused into 18 normal rabbits. Seroconversion of the recipient rabbits indicated a virus carrier state of the donor rabbits.

RESULTS

The minimum amount of blood transfusion capable of transmitting HTLV-I. Aliquots of 10, 5, 1, 0.5, 0.1, and 0.01 mL of blood collected from a virus-infected female rabbit were transfused into each of two normal male rabbits. All 10 rabbits transfused with 10 to 0.1 mL and 1 of 2 rabbits transfused with 0.01 mL seroconverted for HTLV-I after 2 to 4 weeks. Their antibody titers, low at seroconversion, rose to the highest levels of 1:160 to 1:640 within the next four weeks and remained at those levels thereafter (Fig 1). Sera taken from all 11 seroconverted rabbits, but not from the seronegative rabbit, formed bands with HTLV-I proteins including p24 and p19 when analyzed by Western blot 12 weeks after transfusion (Fig 2).

Lymphocytes were separated from blood taken 1.5 to 6 months after transfusion and cultured in the presence of IL-2. Active cell growth occurred after 1 to 2 months and IL-2-dependent lymphoid cell lines were established from one seroconverted rabbit of each aliquot group. The cells cultured from the seronegative rabbit degenerated within one month. All six cell lines possessed a male rabbit karyotype concordant with the sex of the recipient rabbits. These cell lines were reactive with L11/135 and 2C4 and expressed HTLV-I antigens as detected with reference ATL patient’s serum, anti-p19, and anti-p24. Type C virus particles were observed in all the cell lines by electron microscopy (Fig 3).

In transfusion assay carried out 7 to 15 weeks post-transfusion, seroconversion occurred by four weeks in all 11 normal rabbits transfused from the 11 seropositive rabbits but not in one normal rabbit transfused from the seronegative rabbit.

Passive immunization experiment. Two groups of three male rabbits transfused with 5 mL of blood from the virus-infected female rabbit were infused 24 or 48 hours later with 10 mL of HTLVIG. All six sera from these rabbits taken immediately after infusion of HTLVIG showed an immunofluorescence anti-HTLV-I titer of 1:320 and VSV (HTLV-I) pseudotype neutralization titers of 1:250 to 1:1,250. On the basis of plasma volume considerations, the expected dilution of HTLVIG was about 1:15. All three rabbits immunized 24 hours after transfusion remained seronegative for HTLV-I during an observation period of six months, whereas all three rabbits immunized 48 hours after transfusion became seropositive after 2 to 4 weeks with final antibody titers of 1:80 to 1:160. Serial determination of human and rabbit IgG antibodies to HTLV-I indicated that the infused human antibodies were rapidly cleared from the circulation after 2 to 3 weeks to be followed by the production of rabbit antibodies in the 48-hour immunization group but not in the 24-hour immunization group (Fig 4). Western blot analysis done 12 weeks after immunization confirmed the
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Fig 3. Electron micrograph of a lymphoid cell line established from a rabbit transfused with 0.01 mL of virus-infected blood, showing HTLV-I particles in the extracellular space. One particle (arrowhead) has a tail which is reminiscent of recent budding from the cell membrane. (Uranyl acetate and lead citrate, ×61,000.)

lack of rabbit antibodies in the 24-hour immunization group and the presence of rabbit antibodies to HTLV-I p53, gp46, p28, p24, and p19 in the 48-hour immunization group (Fig 5).

Virus isolation was attempted seven weeks after seroconversion from one rabbit of the 48-hour immunization group and an IL-2-dependent lymphoid cell line with a normal male karyotype was established. This cell line, like the six cell lines described above, consisted of Ia antigen-positive T cells and harbored HTLV-I antigens and virus particles.

Transfusion assay performed ten weeks after immunization caused seroconversion after three weeks in all three normal rabbits transfused from the 48-hour immunization group, whereas all three normal rabbits transfused from the 24-hour immunization group remained seronegative during an observation period of three months.

DISCUSSION

The dose-response transfusion experiment demonstrated that as little as 0.01 mL of virus-infected blood is capable of transmitting HTLV-I in rabbits. The number of lymphocytes contained in this amount blood was calculated to be $1.7 \times 10^4$, although the number of HTLV-I-genome carrying lymphocytes is unknown. If the donor rabbit had been leukemic or lymphocytotic, these figures could have been much smaller. This is not surprising in view of the transmission of bovine leukemia virus, another type C retrovirus to calves with $2.5 \times 10^3$ lymphocytes (0.5 μL of blood) and in agreement with the transmission of HTLV-I to rabbits with $5 \times 10^6$ or less milk or semen lymphocytes from seropositive persons. These findings make rabbits useful as a sensitive assay system for the detection and isolation of HTLV-I.

The present observation raises questions regarding the possible risk of HTLV-I infection by accidental inoculation of virus-infected blood among health care workers. A physician seroconverted for HTLV-I after sustaining a needle-stick injury on his foot when he dropped a syringe containing blood from an ATL patient (personal communication from Dr K Okochi, Kyusyu University Hospital). The doctor’s serum sample that had been stored before this accident was antibody-negative, as were serum samples from his mother and wife, and he had no history of receiving blood transfu-
In our previous experiment, active immunization of rabbits with heat-inactivated HTLV-I or a synthetic env peptide was not protective against HTLV-I infection, when challenged by blood transfusion from virus-infected rabbits, although all immunized rabbits had developed antibodies to viral proteins including gp68 and gp46; however, passive immunization of rabbits with rabbit HTLVIG prevented challenge infection of HTLV-I by blood transfusion. VSV (HTLV-I) pseudotype neutralization assays showed that the actively immunized rabbits had no detectable neutralizing antibody, while the passively immunized rabbits had high neutralizing antibody titers. Therefore, neutralizing antibodies are considered to be essential for protection.

The present experiment was undertaken to determine whether rabbits could be similarly protected from HTLV-I infection when human HTLVIG with a high neutralizing antibody titer was administered after transfusion of virus-infected blood. The interval between transfusion of virus-infected blood and passive immunization was found to be an important factor; all three rabbits immunized 24 hours after transfusion were protected against HTLV-I infection, whereas all three rabbits immunized 48 hours after transfusion were not. The infused human antibodies became undetectable in both groups after 2 to 3 weeks, when all three rabbits of the 48-hour immunization group began to produce antibodies to HTLV-I, but all three rabbits of the 24-hour immunization group remained seronegative during the three-month follow-up period. Because of the heterologous system, the human immunoglobulin was metabolized with a half-life of about 4 days in contrast to a half-life of about 18 days for the rabbit immunoglobulin infused into rabbits. It is postulated that the transfused lymphocytes including those carrying the HTLV-I genome were rejected within two weeks before the clearance of most of the infused human antibodies. Transmission assay from the 48-hour immunization group, but not from the 24-hour immunization group, caused seroconversion in all three recipient rabbits. These results indicate that HTLVIG prevented transfusion-related HTLV-I infection in the 24-hour immunization group. In future studies, polymerase chain reaction would be also useful for validating the presence of absence of HTLV-I infection in rabbits.

It is clear that HTLVIG is effective in preventing cell-to-cell infection of HTLV-I when given within 24 hours of transfusion of virus-infected blood and that HTLV-I genome-carrying lymphocytes transmit HTLV-I to host lymphocytes sometime between 24 and 48 hours after transfusion. This incubation period probably reflects the time required for a sequence of events from the viral gene activation to the formation of infectious HTLV-I virions. A possibility of virus transmission by cell fusion is unlikely, since this precludes the protective effect of passive immunization. All these findings suggest clinical application of HTLVIG for post-exposure prophylaxis among health care workers. It may also be useful for the prevention of milk-borne transmission of HTLV-I from mother to child.

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Fig 5. Western blot analysis of sera from transfused and immunized rabbits, showing the lack of antibodies to HTLV-I in all three rabbits of the 24-hour immunization group (lanes 1 to 3) and the presence of antibodies to HTLV-I p53, gp46, p28, p24, and p19 in all three rabbits of the 48-hour immunization group (lanes 4 to 6).
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