Prophylaxis Against a Melanesian Variant of Human T-Lymphotropic Virus Type I (HTLV-I) in Rabbits Using HTLV-I Immune Globulin From Asymptomatically Infected Japanese Carriers

By Yuji Tanaka, Kazuyoshi Ishii, Takashi Sawada, Yuji Ohtsuki, Hiroo Hoshino, Richard Yanagihara, and Isao Miyoshi

Molecular variants of human T-lymphotropic virus type I (HTLV-I), which diverge significantly from the so-called cosmopolitan prototypes, have been discovered in Melanesia. In this study, HTLV-I IgG (I-lgG) prepared from seropositive healthy Japanese carriers was evaluated for its protective effect against a Melanesian isolate, HTLV-I_MEL, in rabbits. Normal IgG (N-IgG) prepared from seronegative healthy Japanese was used as control. Both preparations contained 50 mg/mL of IgG and I-lgG had a high neutralizing antibody titer, as determined by vesicular stomatitis virus–HTLV-I pseudotype assay. Of four experimental groups (A, B, C, and D), each with three rabbits, groups A and B were infused with 10 mL of N-IgG and I-lgG, respectively, and animals were challenged immediately by transfusion of 5 mL of blood from a rabbit infected with HTLV-I_MEL. Animals in groups C and D were immunized with 10 mL of I-lgG 24 and 48 hours, respectively, after being transfused with 5 mL of blood from the virus-infected rabbit. HTLV-I infection, as determined by seroconversion and verified by polymerase chain reaction, occurred in all rabbits in groups A and D after 2 to 6 weeks, but in none of the animals in groups B and C. These data indicate that I-lgG is protective against HTLV-I_MEL infection when administered before or within 24 hours of transfusion with virus-contaminated blood. Moreover, our study shows that the neutralizing domains of the so-called cosmopolitan and Melanesian strains of HTLV-I are functionally indistinguishable.

Hum T-Lymphotropic virus type I (HTLV-I), the causative agent of adult T-cell leukemia/lymphoma and of tropical spastic paraparesis/HTLV-I-associated myelopathy, is endemic in southwestern Japan, the Caribbean Islands, and parts of Africa. Virus strains isolated from inhabitants of widely separated geographic locales exhibit a high degree (>97%) of sequence homology and are regarded as cosmopolitan prototypes. Genetically distinct variants of HTLV-I have been isolated recently from Melanesians of Papua New Guinea and the Solomon Islands. These Melanesian isolates are highly divergent (~8%) at the nucleotide level from cosmopolitan strains of HTLV-I.

We have established a rabbit model of HTLV-I infection in which HTLV-I immune globulin prevents cell-to-cell virus infection by blood transfusion and by breast feeding. These observations prompted us to evaluate the protective effect of HTLV-I immune globulin against a Melanesian variant of HTLV-I in rabbits. This study provides evidence for cross-neutralization between cosmopolitan and Melanesian strains of HTLV-I.

MATERIALS AND METHODS

Rabbits. Outbred Japanese white rabbits weighing approximately 3 kg were purchased from a commercial source and maintained at biosafety level 3.

Virus. A Melanesian variant, designated HTLV-I_MEL, harbored in the SI-5 cell line derived from a 58-year-old Solomon Islander was used. Umbilical cord blood mononuclear cells from male and female neonates were cocultivated with lethally irradiated SI-5 cells, and two cell lines, SI-cord-1 and SI-cord-2, from the male and female donors, respectively, were established. Both cell lines were positive for CD2, CD4, CD5, CD25, and HLA-DR, but negative for CD8. Electron microscopy showed type C virus particles in both cell lines. A male rabbit was infected with HTLV-I_MEL by intravenous inoculation of 1 × 10^7 SI-cord-2 cells and served as a transfusion donor. Infection with HTLV-I_MEL was confirmed in this donor rabbit by gene amplification using Melanesian variant-specific env gene primers and by sequencing of enzymatically amplified products.

Ig preparations. Normal IgG (N-IgG) and HTLV-I IgG (I-lgG), both containing 50 mg/mL, were prepared from pooled plasma from seronegative and seropositive healthy Japanese, respectively, by the method of heat-inactivation and polyethylene glycol fractionation. I-lgG had an anti-HTLV-I antibody titer of 1:5, 120 by indirect immunofluorescence and a neutralizing antibody titer of 1:3,900 by vesicular stomatitis virus–HTLV-I pseudotype assay.

Serologic testing. Serum samples were tested by enzyme-linked immunosorbent assay (ELISA) at intervals of 1 to 2 weeks for IgG antibodies against disrupted HTLV-I virions according to the manufacturer’s instructions (Eisai, Tokyo, Japan). To differentiate human and rabbit anti-HTLV-I IgG antibodies, alkaline phosphatase-labeled mouse monoclonal antibodies against human IgG (Wako Junyaku, Osaka, Japan) and alkaline phosphatase-labeled goat antibodies against rabbit IgG (Cappel, West Chester, PA) were used. The presence or absence of IgG antibodies was verified by Western blot using lysates of MT-2 cells as the source of antigen.

Gene amplification by polymerase chain reaction (PCR). PCR was performed on genomic DNA extracted from rabbit peripheral blood mononuclear cells using oligonucleotide primers at positions 7341–7360 and 7460–7441 corresponding to the pX region of HTLV-I, as described previously. The amplified products were electrophoresed on 6% polyacrylamide gels, transferred to nylon membranes, and hybridized with a 32P-labeled internal oligonucleotide probe (bases 7364–7383).
IMMUNOPROPHYLAXIS AGAINST MELANESIAN HTLV-I

Fig 1. Serial determination of human (●) and rabbit (○) IgG antibodies to HTLV-I by ELISA. Production and persistence of antivirus-specific rabbit antibodies were seen in groups A and D but not in groups B and C. Rabbits in group A receiving N-IgG showed no antivirus-specific human antibodies, whereas animals in groups B, C, and D receiving I-IgG showed a rapid decline of human antibodies in 3 weeks.

RESULTS

Four groups (designated A, B, C, and D), each composed of three female rabbits, were studied. Rabbits in groups A and B were initially inoculated intravenously with 10 mL of N-IgG and I-IgG, respectively, and were challenged immediately by transfusion of 5 mL of blood from the HTLV-I-infected rabbit donor. Rabbits in groups C and D were first transfused with 5 mL of blood from the HTLV-I-infected rabbit and then immunized with 10 mL of I-IgG 24 and 48 hours later, respectively.

All rabbits in groups A and D seroconverted for HTLV-I after 2 to 6 weeks, whereas all animals in groups B and C remained seronegative during the observation period of 7 to 9 months. Serial determinations of human and rabbit IgG antibodies to HTLV-I by ELISA indicated that the group A rabbits, inoculated with N-IgG and lacking human anti-HTLV-I antibodies, began to produce rabbit anti-HTLV-I antibodies after 2 to 3 weeks that were maintained thereafter. By contrast, rabbits in groups B, C and D, which were inoculated with I-IgG, rapidly cleared the infused human anti-HTLV-I antibodies from their circulation in 3 weeks. This was followed by the production and persistence of rabbit anti-HTLV-I antibodies in group D rabbits but not in group B and C rabbits. The antibody response curves in four rabbits representing each of the four groups are shown in Fig 1.

Western blot analysis of sera confirmed the presence of rabbit IgG antibodies to HTLV-I p28, p24, and p19 in group A and D rabbits and their absence in group B and C rabbits (Fig 2, upper panel). HTLV-I proviral sequences
were detected by PCR at 10 weeks in all of the six seroconverted rabbits but in none of the six seronegative rabbits (Fig 2, lower panel).

Rabbits in group C that had been protected from HTLV-I\textsubscript{MEL} infection by immunization with I-IgG were challenged 8 months later by transfusion of 5 mL of blood from the same virus-infected rabbit donor. Seroconversion for HTLV-I occurred after 3 to 4 weeks and HTLV-I pX sequences were detected by PCR at 10 weeks in all of these rabbits, verifying the short-term protection afforded by passive immunization.

**DISCUSSION**

In the present study, we showed that IgG from Japanese asymptomatically infected with cosmopolitan strains of HTLV-I was effective in preventing cell-to-cell infection with a Melanesian variant of HTLV-I. In short, our data indicate that cosmopolitan and Melanesian strains of HTLV-I share common neutralizing epitopes that are functionally indistinguishable. This is not unexpected because comparative analysis of env gene sequences of Melanesian and cosmopolitan strains of HTLV-I\textsuperscript{16} indicates total conservation of the amino acid sequences of the putative neutralizing epitopes that have been mapped to residues 88-98\textsuperscript{24} and residues 191-196.\textsuperscript{25} Collectively, these findings will weigh heavily in strategies aimed at developing vaccines and immunoprophylactic measures against HTLV-I infection worldwide.

It should be noted that rabbits could be protected from infection with either cosmopolitan or Melanesian strains of HTLV-I by passive immunization administered before or 24 hours after transfusion of virus-contaminated blood. Immunization administered 48 hours after transfusion was not effective. These observations suggest that transmission of virus from viral genome-carrying lymphocytes to host lymphocytes takes place sometime between 24 and 48 hours after transfusion. Due to the heterologous system, the infused human anti-HTLV-I antibodies declined with a half-life of approximately 7 days, becoming undetectable in 3 weeks, and the transfused allogeneic lymphocytes, including those carrying the viral genome, were postulated to have been rejected within 1 to 2 weeks by the host. As expected, group C rabbits that had been once protected from HTLV-I\textsubscript{MEL} infection by I-IgG could be readily infected by challenge with virus-infected blood after the disappearance of the passively acquired antibodies.

Finally, we have previously shown that milk-borne transmission of HTLV-I can also be prevented by passive immunization of suckling rabbits.\textsuperscript{20} In some HTLV-I-endemic regions of Japan, seropositive pregnant women are being advised to feed their newborn infants with formula rather than breast milk. This recommendation may be impractical in countries such as Papua New Guinea and the Solomon Islands. Thus, prophylactic immunization of babies against milk-borne transmission of cosmopolitan and Melanesian strains of HTLV-I may be an interim measure until an effective HTLV-I vaccine is developed.

**REFERENCES**


Prophylaxis against a Melanesian variant of human T-lymphotropic virus type I (HTLV-I) in rabbits using HTLV-I immune globulin from asymptomatically infected Japanese carriers

Y Tanaka, K Ishii, T Sawada, Y Ohtsuki, H Hoshino, R Yanagihara and I Miyoshi