

INFECTION OF RABBITS WITH HUMAN
IMMUNODEFICIENCY VIRUS 1

A Small Animal Model for Acquired Immunodeficiency Syndrome

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Human immunodeficiency virus (HIV-1) has been associated with acquired immunodeficiency syndrome (AIDS) in man (1-4). Development of successful immunization and/or therapeutic regimens for AIDS has been hampered by the lack of easily manipulated animal models for HIV-1 infection (5). Recent reports (6, 7) suggest that rabbits may be useful for the study of HIV-1 infection. In the present study rabbits previously infected with HTLV-1, as well as normal rabbits, were injected with a human T cell line infected with HIV-1. Both groups showed signs of HIV-1 infection, which were substantiated using serologic, biologic, and molecular analyses.

Materials and Methods

Rabbits. Outbred New Zealand white rabbits were maintained under specific pathogen-free conditions in biosafety level 2 (BSL-2) facilities and all manipulations were carried out observing strict BSL-3 practice (8).

Viral Inoculation. Rabbits were infected with HTLV-1 by intravenous injection of 5×10^6 irradiated MT-2 cells (9). For HIV-1 infection, rabbits were given an intravenous injection of 5×10^6 A3.01 cells (10) at near peak HIV-1 infection as determined by reverse transcriptase (RT) activity (11). Control animals were given 5×10^6 uninfected A3.01 cells.

Serologic Testing. Serum samples were tested at 3-wk intervals post-infection for antibodies directed against HTLV-1 and HIV-1 proteins using ELISA kits from DuPont Co. (Wilmington, DE). Western blot analyses were carried out using nitrocellulose strips containing HIV-1 proteins (Epitope Inc., Beaverton, OR) and gold-labeled goat anti-rabbit IgG (Janssen, Piscataway, NJ).

Culture of Rabbit PBL. Blood samples were taken at 3-wk intervals post-infection. PBL, prepared by Ficoll/Hypaque separation (6), were cultured in medium containing human rIL-2 (20 U/ml; Genzyme, Boston, MA); a second aliquot was cocultured with A3.01 cells at a 1:1 ratio. Cultures were monitored for syncytia formation and supernatants for RT activity. Cell-free supernatants from PBL or PBL/A3.01 cocultures were incubated with A3.01 cells in order to amplify the virus; infected cells were pelleted and stored at -70°C .

Gene Amplification by Polymerase Chain Reaction (PCR). PCR (Perkin-Elmer Corp., Norwalk, CT) was performed on genomic DNA extracted from cell pellets using oligonucleotide primers complementary to conserved regions in the HIV-1 gag gene. Aliquots of amplified samples

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were subjected to electrophoresis on 3% NuSieve/1% Sea Kem agarose (FMC BioProducts, Rockland, ME) and to Southern blot analysis (ONCOR Sure blot) using a ³²P-labeled probe specific for the HIV-1 gag region (12, 13).

Necropsy of Infected Animals. At various time points post-infection, heavily sedated rabbits were exsanguinated by cardiac puncture. Spleens were taken under sterile conditions and divided into portions for cell culture and for pathology. Flow cytometry analysis was carried out on spleen cells using antibody reagents for human (Becton Dickinson & Co., Mountain View, CA) and rabbit lymphoid cell populations (14). Organs were fixed in neutral formalin and sent to a veterinary pathology lab (Maryland Medical Inc., Baltimore, MD) for diagnosis.

Results

Four groups of experimental and control rabbits were formed for infections with HIV-1: group 1 consisted of five rabbits infected with HTLV-1; group 2 consisted of three rabbits injected with HIV-1-infected A3.01 cells; group 3 consisted of six rabbits seropositive for HTLV-1 subsequently injected with HIV-1-infected A3.01 cells; and group 4 consisted of two control rabbits given A3.01 cells not infected with HIV-1.

Serologic Analyses of Infected Rabbits. Within ~6 wk all rabbits injected with HIV-1 were seropositive for HIV-1. Rabbits seropositive for HTLV-1 before injection of HIV-1 became seropositive sooner and gave stronger responses than those infected with HIV-1 only. No crossreactivity between HTLV-1 and HIV-1⁺ sera was observed by ELISA, and sera from control rabbits were negative in both assays. Positive samples were shown by Western Blot analyses to have antibodies directed against various HIV-1 proteins.

Isolation and Characterization of HIV-1 from Infected Rabbits. Observation of PBL cultures revealed formation of syncytia in those taken from HIV-1-infected rabbits. Fig. 1 shows PBL from uninfected (A), HIV-1-(B), and HTLV-1/HIV-1-(C) infected rabbits after 2 wk of growth in IL-2-supplemented medium. As shown in Fig. 2, RT activity was observed in cell-free culture (and A3.01 coculture) supernatants of PBL obtained from HIV-1- and HTLV-1/HIV-1-infected rabbits. Supernatants positive for RT activity also showed moderate to high levels of HIV-1 p24 antigen as measured by an antigen capture assay (data not shown). No RT activity or p24 antigen was seen in PBL cultures or cocultures from rabbits infected with HTLV-1 alone or injected with uninfected A3.01 cells.

Confirmation that PBL were infected with HIV-1 was given by analysis of PCR-amplified DNA from A3.01 cells cultured with supernatants from PBL from infected rabbits. The bands (Fig. 3) indicated by ethidium bromide staining were of the predicted size for the intact (574 bp) and Hind III-digested gag gene fragments (448 bp) for the isolate used here (13) and most reported HIV-1 sequences. Further proof that the PCR fragments are HIV-1 derived is given by Southern blot analysis using a probe derived from the gag region of the HIV-1 proviral genome (Fig. 3, right).

Clinical Effects of HIV-1 Infection. Rabbits infected with HTLV-1 or HIV-1 alone showed no consistent signs of illness. Of the six rabbits infected with both HTLV-1 and HIV-1, all had severe diarrhea and two animals suffered losses of ~15% body weight. Another in this group developed a rapidly progressing mammary adenocarcinoma, a rare tumor in rabbits that have never borne offspring. Two of the rabbits displayed transient (~5 d) paralysis of the hind legs.

A rabbit infected with HIV-1 alone was killed at 8 wk post-infection. The spleen was enlarged and hyperplastic, and the lungs of this animal displayed lymphocytic

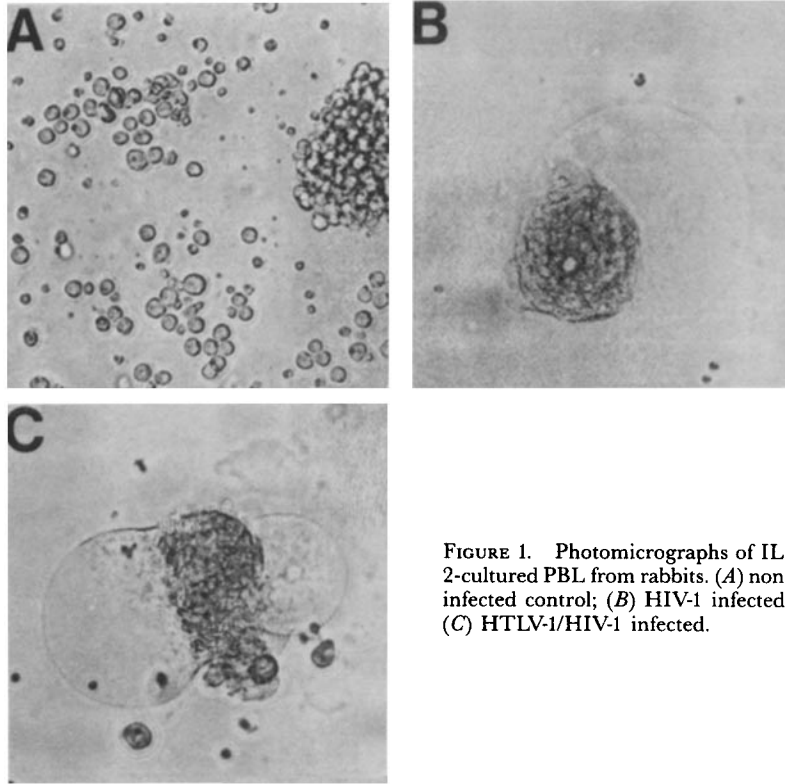


FIGURE 1. Photomicrographs of IL-2-cultured PBL from rabbits. (A) non-infected control; (B) HIV-1 infected; (C) HTLV-1/HIV-1 infected.

infiltration. Flow cytometry of spleen cells using antibodies against human and rabbit lymphoid cell markers (14) revealed predominantly rabbit T cells; no evidence of residual A3.01 cells was noted (data not shown).

Organs from two HTLV/HIV-1-infected rabbits were submitted to pathology 3

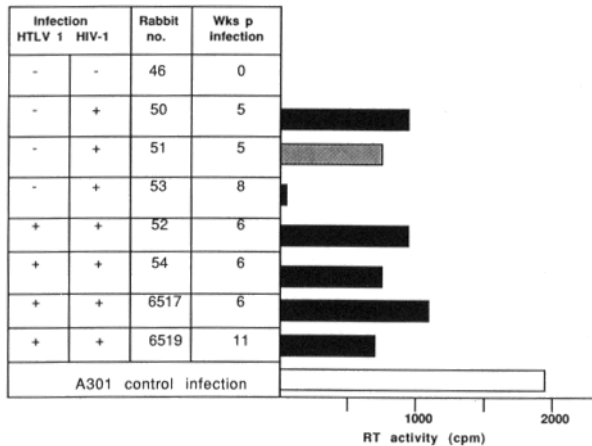


FIGURE 2. Reverse transcriptase (RT) activity in supernatants taken from rabbit PBL cultured with IL-2 (gray bar) or cocultured in the presence of human A3.01 cells (black bars). Infection protocols and times post-infection at which PBL were taken are shown in the figure. The RT assay was performed using 10 μ l of cell-free supernatant, the open bar shows RT activity of control-infected A3.01 culture.

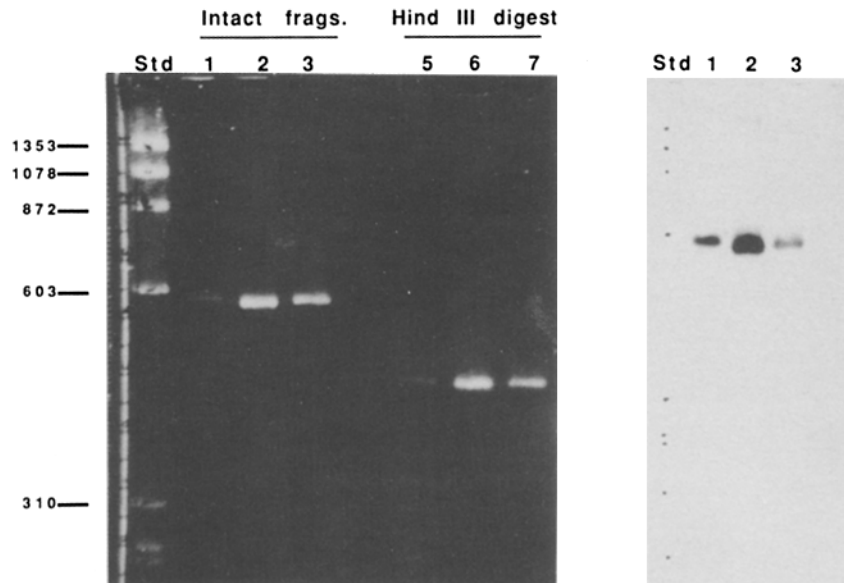


FIGURE 3. (*Left panel*) Agarose gel electrophoresis of the amplification products from PCR using HIV-1 gag gene primers based on the sequence of the BRU isolate of HIV-1 (13) and DNA isolated from A3.01 cells incubated with supernatants from cultured rabbit PBL (see Fig. 2 for rabbit identification). The lane marked Std contains ϕ X 174 DNA digested with Hae III as size standards; the numbers to the left indicate fragment sizes in bp; lane 1 contains products from PCR using input DNA from A3.01 cells infected with supernatants from rabbit 6517 PBL; lane 2, rabbit 50; lane 3, rabbit 53. Lanes 5, 6, and 7 contain the PCR products from lanes 1, 2, and 3, respectively, after digestion with Hind III. (*Right panel*) Southern blot analysis of lanes 1, 2, and 3 of the gel shown at the left using an HIV-1 gag probe (12). Dots in the lane marked Std indicate positions of ϕ X 174 DNA fragments used as size standards.

mo after HIV-1 infection. Splenic hyperplasia and lymphocytic lung infiltration were diagnosed in both and moderate kidney and liver damage was seen. Coculture of the spleens with A3.01 cells yielded RT⁺ supernatants.

Discussion

The *in vivo* infections described here were based on results (6) showing replication of HIV-1 in transformed rabbit T cell and macrophage lines but not in PBL from normal rabbits. It was reasoned from reports utilizing HTLV-1 to infect rabbits (9, 15, 16) and from reported negative results using purified HIV-1 (17) that rabbits could not be infected without some manipulation to render them susceptible. Accordingly, animals were first infected with HTLV-1, and then injected with a human cell line infected with HIV-1. As shown by the present results, preinfection is not necessary to achieve infection although antibody concentrations were higher and disease symptoms more pronounced in the HTLV-1/HIV-1-infected group.

Several key questions remain concerning the infection of rabbits with HIV-1. The most effective means of infection and of transmission are yet to be established. The precise mechanism of infection is obscure; it is not known whether it is similar to that determined for primates. Circumstances under which disease is consistently

produced in this species need to be elucidated. A critical issue relates to determination of the effects of HIV-1 on immunologic status in the rabbit; it is not known whether immune deficiency results. A complete answer to this question will require acquisition of new basic information concerning the immune system of the rabbit. There is also the possibility that predisposition to HIV-1-induced disease involves genetic factors. Recently it was shown that certain inbred rabbits are prone to leukemia-like disease when exposed to HTLV-1 (15, 16) whereas normal rabbits show no signs of HTLV-1-induced disease.

The experiments described here, as well as those recently reported by others (7), indicate that the rabbit may serve as a model for infection with HIV-1, the causative agent in the disease AIDS. Advantages over existing models include availability of animals, relatively lower maintenance costs, and the use of HIV-1 rather than related viruses. Clearly, further information is required for a complete comprehension of the HIV-1 infection process in rabbits, but the model described here offers an immediate opportunity to investigate prophylactic and therapeutic agents directed against HIV-1 infections.

Summary

Injection of rabbits with a human T cell line infected with HIV-1 caused seroconversion within 6 wk, and HIV-1 could be isolated from PBL cultures of infected rabbits. Identity of the isolated virus with HIV-1 was shown by analysis of products amplified by the polymerase chain reaction. HIV-1 infection was seen in rabbits injected with HIV-1-infected cells alone as well as in those that were first infected with HTLV-1 and subsequently with HIV-1. There were no consistent signs of disease in the rabbits infected with HIV-1 alone but HTLV-1/HIV-1-infected rabbits showed signs of illness including diarrhea and weight loss, transient neurologic impairment and, in one animal, a rapidly progressing mammary adenocarcinoma. Examination of organs taken at necropsy from both HIV-1- and HTLV-1/HIV-1-infected animals showed splenic hyperplasia and lymphocyte infiltration of the lungs, as well as moderate damage to liver and kidney in some cases.

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