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Human immunodeficiency virus infection of human lymph nodes in the SCID-hu mouse

(animal model/natural tropism/viremia/drug testing/dose-response)

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ABSTRACT **The SCID-hu mouse is a small animal in which human hematolymphoid organs can be engrafted and maintained *in vivo*. In this study, parameters are described for reproducible infection of SCID-hu mice after i.v. inoculation. Infection was found to be dependent upon the time after inoculation, the virus isolate, the titer of virus, and the human target organ implanted into the mouse. Ten to 14 days after the i.v. administration of HIV isolates derived freshly from patients (e.g., JR-CSF, JR-FL, SM), 100% of engrafted human lymph nodes in SCID-hu mice were infected; >95% of these animals were also viremic. Implants of human thymus or connective tissue, as well as the endogenous murine hematolymphoid organs, were not infected. As demonstrated by a combination of *in situ* hybridization and immunohistochemistry, both T-lymphoid and myelomonocytic lineage cells were infected in this system. HIV isolates that have been adapted to growth *in vitro* (e.g., HTLV-IIIb) were not infectious. When either 3′-azido-3′-deoxythymidine (AZT) or 2′,3′-dideoxyinosine (ddIno) was administered to SCID-hu mice before HIV infection, the animals were protected in dose ranges similar to those used in man. This animal model may now be used as an efficient intermediate step between the lab and the clinic to study the infectious process *in vivo* and to best select efficacious antiviral compounds against HIV.**

Since the discovery that the human immunodeficiency virus (HIV) is the etiologic agent of AIDS (1, 2), tremendous efforts have been devoted towards developing antiviral compounds that might modify the course of infection *in vivo*. Most of this work has been carried out within experimental systems that are of uncertain relevance to infection in man. Recently, for example, it has become apparent that only a subset of isolates derived from patients are able to replicate efficiently in tissue-culture conditions (3). Subsequent selective pressures *in vitro* may then generate viruses that differ significantly from those that are found *in vivo* (4, 5, §).

A relevant animal model for HIV infection might enhance the interpretation of *in vitro* studies and facilitate their extension to the infective process in man. Ideally, this animal would closely reproduce the salient features of HIV infection in man: using the same clinical isolates, within the same human lymphoid organs, and with the same $CD4^+$ cellular tropism.

The severe combined immunodeficient mouse (SCID) transplanted with human organs, known as the SCID-hu mouse (7), may represent such a small animal model for HIV infection. To create this system, human hematolymphoid organs [inclusive of fetal liver, thymus, and lymph node (LN)] are surgically implanted into the immunodeficient C.B-17 *scid/scid* mouse (8). Some of the cell subpopulations in the implants, including $CD4^+$ T and monocytic lineage cells, represent targets for HIV infection in man (9, 10). It has been shown that in a time- and dose-dependent manner, direct injection of virus into the thymus or LN implant resulted in signs of viral replication and spread (11). It was next found that intrathymic HIV infection of the SCID-hu mouse could be suppressed by 3′-azido-3′-deoxythymidine (AZT) (12).

We now have found that simple i.v. inoculation of HIV into the SCID-hu mouse leads to reproducible infection within a short period of time. The course of infection by HIV in this system closely parallels that found in man. Clinical isolates infect human $CD4^+$ T and myeloid cells within the engrafted human LN. Compounds that are known to have an antiviral effect in man [e.g., AZT and dideoxyinosine (ddIno); refs. 13 and 14] are also efficacious in the SCID-hu mouse in doses that are equivalent to those used clinically. In sum, the SCID-hu mouse represents a relevant small animal model for HIV infection, in which questions related to pathogenesis and to drug development can be efficiently explored.

MATERIALS AND METHODS

Construction of SCID-hu Mice. Homozygous C.B-17 *scid/scid* mice (SCID mice) were bred, treated with antibiotics as described (7), and used when 6–8 weeks old. Methoxyflurane anesthesia was applied during all operative procedures. Cells from human fetal thymus or liver (18–23 gestational weeks) were tested for the presence of HIV by DNA polymerase chain reaction (PCR) (see below) in all cases before use. If negative for HIV, thymus or mesenteric LN were carefully dissected and transplanted under the right and/or left kidney capsule separately by using a 18-gauge trocar. In other cases, human LN or connective tissue from mesenterium were transplanted into the subcutaneous region of the fourth mammary fat pad separately.

Virus Preparation and Infection of SCID-hu Mice. The molecularly cloned HIV isolate JR-CSF (15) was provided by I.S.Y. Chen (Univ. of California at Los Angeles). The HTLV-IIIb isolate (2) was obtained from R. C. Gallo (National Institutes of Health). The HIV isolates were expanded in phytohemagglutinin (PHA; GIBCO)-activated peripheral blood mononuclear cells (PBMC) from normal donors. On days 4 through 10 after the initiation of the cultures, virus in the culture supernatant was harvested every 24 hr and kept

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Abbreviations: HIV, human immunodeficiency virus; SCID, severe combined immunodeficient; SCID mouse, C.B-17 *scid/scid* mouse; LN, lymph node(s); AZT, 3′-azido-3′-deoxythymidine; ddIno, 2′,3′-dideoxyinosine; PHA, phytohemagglutinin; PBMC, peripheral blood mononuclear cells; TCID, tissue culture infectious dose; PCR, polymerase chain reaction; ISH, *in situ* hybridization.

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frozen as a standard stock. Each stock was titered on PHA-activated PBMC before use. One tissue-culture infectious dose ($TCID_{50}$) corresponds to the reciprocal of the dilution of virus required to infect 50% of wells, as detected by measurement of cellular HIV p24 with an ELISA (Pharmacia Diagnostics) 7 days after infection. JR-CSF, JR-FL, and SM stocks had titers of 400,000 $TCID_{50}$/ml; HTLV-IIIb stocks had 4,000,000 $TCID_{50}$/ml. For infection of SCID-hu mice, stock vials were quickly thawed and immediately used. A volume of 0.3 ml was injected i.v. into the retro-orbital plexus of anesthetized SCID-hu mice with a blunt-tipped 30-gauge needle.

Detection of HIV by PCR. DNA PCR analysis of the human and murine organs after virus inoculation was performed as described (12). To detect circulating HIV particles by RNA PCR, SCID-hu mice were bled at various times after infection. Whole blood (approximately 100 μl) was mixed with phosphate-buffered saline (100 μl) containing 50 units of heparin per ml and centrifuged at 7000 rpm (Tomy, TMA-2) for 5 min at 4°C. The plasma fraction was transferred to a second tube and centrifuged at 15,000 rpm for 2 hr at 4°C. Viral pellets were incubated in 400 μl of TEN buffer (10 mM Tris, pH 7.5/1 mM EDTA/0.5 M NaCl) containing 1% SDS and 100 μg of proteinase K for 1 hr at 37°C with rotation. Oligo(dT)-cellulose beads (New England Biolabs) were added to each lysate, and the tubes were rotated for 1 hr at 37°C. The beads were washed twice with TEN buffer containing 0.2% SDS and 1 mM phenylmethylsulfonyl fluoride and then once with TEN buffer alone. Purified poly(A)$^+$ RNA was collected in a volume of 100 μl by eluting the beads twice with 10 mM Tris·HCl/1 mM EDTA and then was processed for cDNA synthesis with Moloney murine leukemia virus reverse transcriptase and the manufacturer's recommendations (Bethesda Research Laboratories). For detection of HIV RNA, the priming oligonucleotide 661 was used for first-strand synthesis; the return primer 667 was then used in combination with *Taq* polymerase for PCR.

***In Situ* Hybridization (ISH) and Histochemistry.** The engrafted human LN were harvested and immunostained by the avidin–biotin–peroxidase method as described (7). ISH was performed as described (11). Cells that showed hybridization signals by autoradiography were illuminated with a dark-field condenser (see Fig. 2). The mouse monoclonal antibody Leu-4 (Becton Dickinson) to human CD3 was used for detection of T cells, and the α-naphthyl acetate esterase kit (Sigma) with fast-red-violet LB base was used for detecting nonspecific esterase activity of monocytic lineage cells.

Treatment of SCID-hu Mice with Antiviral Compounds. AZT and ddIno were provided by P. Sager and J. McGowan of the Division of AIDS, National Institute of Allergy and Infectious Diseases. The compounds were administered to SCID-hu mice for 24 hr prior to i.v. infection with JR-CSF. Treatment was continued for 14 days. AZT was dissolved in sterile water and administered in the water bottle. The ddIno was dissolved in sterile water with 0.005 M NaOH and injected i.p.. For the day prior to infection with HIV and for 3 days thereafter, the total daily dose was divided into two equal injections every 12 hr; for days 4–14, the total daily dose was injected once a day. The given dosages (mg/kg of body weight per day) were calculated after determination of the average water consumption per mouse per day (3.3 ml) and the average body weight per mouse (20 g). The dose–response curves were fitted by linear × logistic regression (Glim statistical package).

RESULTS

Injection i.v. of HIV Leads to Selective Infection of Human LN in the SCID-hu Mouse. Two weeks after SCID-hu mice construction, the HIV isolate JR-CSF was injected i.v. Two weeks later, the human and murine lymphoid organs were examined for evidence of infection by using DNA PCR (Fig. 1). HIV-specific PCR products were identified in the human LN (lanes HL) of SCID-hu mice 3, 6, and 7. In contrast, murine spleen (lanes MS) was negative for both human β-globin and HIV. Human thymus (lanes H-T) and human connective tissue (not shown) were also uninfected. Thus, i.v. administration of HIV spares murine lymphoid organs and results in selective infection of human LN.

Human T Cells and Macrophages Are Infected with HIV. To confirm that the cells infected by HIV in the SCID-hu mouse were similar to those infected in man, the human LN were analyzed by immunohistochemistry and ISH. The LN were replete with HLe-1 (CD45)-positive human leucocytes (Fig. 2*A*), many of which were reactive with an ^{35}S-labeled HIV RNA probe against the 3′ end of the viral genome by ISH (Fig. 2*B*). Cells within an adjacent mouse LN (M in Fig. 2*B*) showed no evidence of HIV transcription by this technique. High-power views of the sections analyzed with a combination of immunohistochemistry and ISH identified infected cells with several morphologic and phenotypic characteristics. Within the human LN in the SCID-hu mice, both T cells expressing the marker CD3 (Fig. 2*C*) and tissue macrophages with nonspecific esterase activity (Fig. 2*D*) were found to be infected.

Viremia Develops in SCID-hu Mice after i.v. Injection of HIV. Two weeks after the inoculation of JR-CSF, plasma fractions were obtained by venipuncture, centrifuged to pellet free virus, and assayed for HIV by RNA PCR. In one experiment with nine mice (Fig. 3 *Upper*), HIV-specific PCR products were not detected in the plasma of normal SCID mice 2 weeks after infection with HIV (lanes N). At the same time, six of seven HIV-infected SCID-hu mice were viremic as measured by RNA PCR. The one animal that was not viremic by that technique (SCID-hu mouse 7) was clearly infected as assayed by DNA and RNA PCR of the engrafted LN (see Fig. 1).

When JR-CSF was used, p24 antigen was not detectable in the plasma even when the same specimens were positive for HIV by RNA PCR. Therefore, circulating virus was at a concentration < 30 pg of p24 per ml (the lower limits of sensitivity of ELISA). To show that HIV could be rescued from HIV-infected SCID-hu mice, plasma fractions were cultivated *in vitro* with PHA-activated PBMC for 14–28 days; in 6 of 60 cases, culture supernatants turned positive for HIV p24 at that time (not shown).

Viremia Is Dependent upon Time, Dose, and Viral Isolate. To further define the parameters of this model for acute infection, SCID-hu mice were prepared with human LN alone. Different doses of different isolates of HIV were

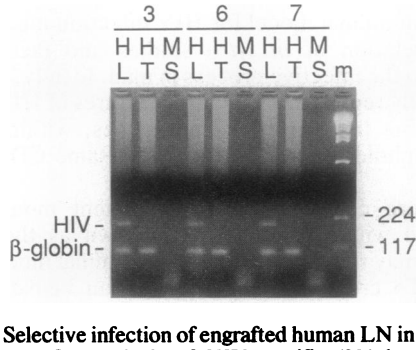

FIG. 1. Selective infection of engrafted human LN in the SCID-hu mouse. DNA PCR analysis of HIV-specific (201 base pairs) and human β-globin-specific (110 base pairs) products in the engrafted human LN (lanes HL) and thymus (lanes HT) of SCID-hu mice 3, 6, and 7, 2 weeks after i.v. inoculation of JR-CSF. As a negative control, murine spleen (lanes MS) fragments from the same animals were analyzed by DNA PCR. Lane m, molecular weight standards.

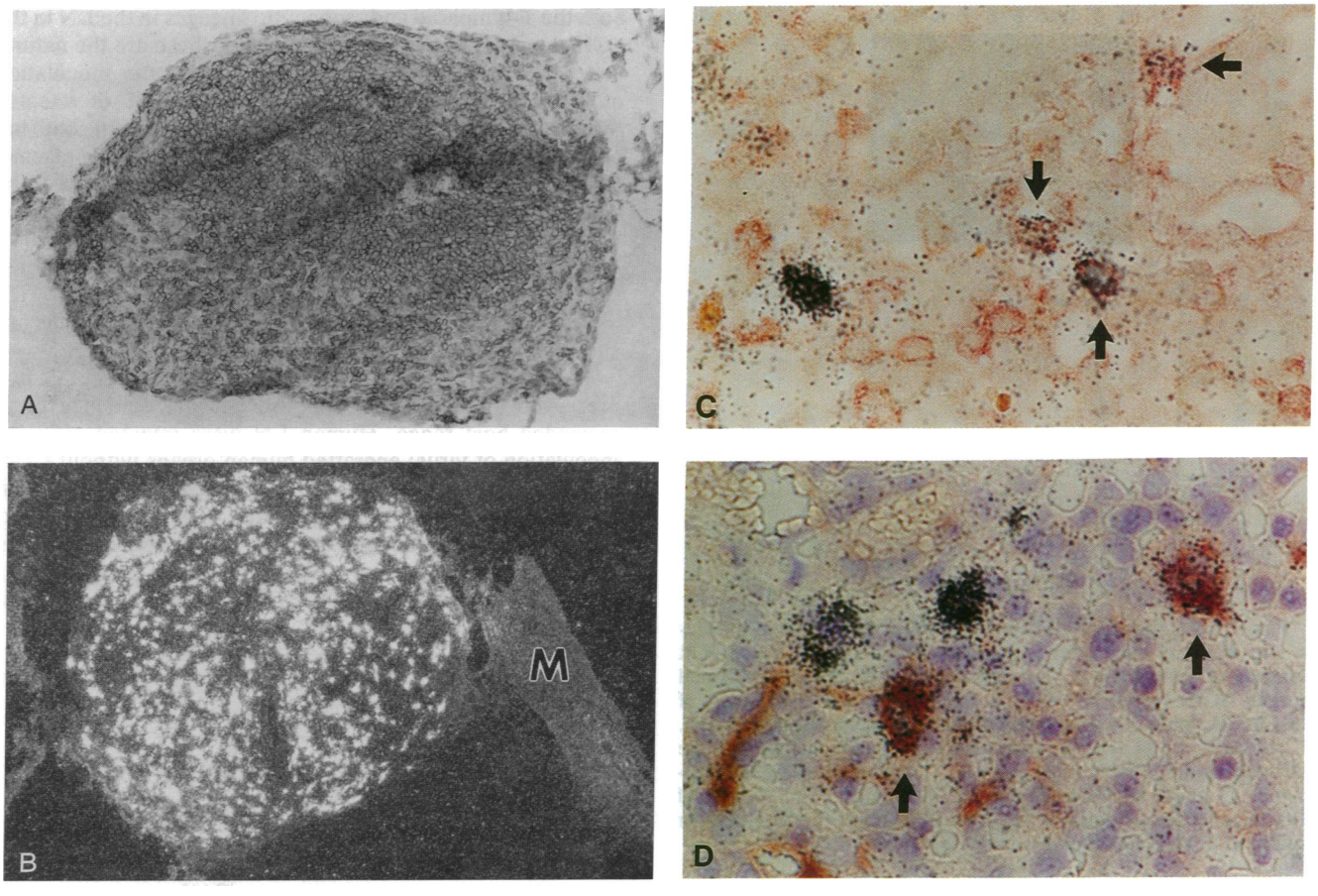

FIG. 2. Histological examination of HIV-infected human LN in the SCID-hu mouse. (*A*) The distribution of HLe-1(CD45)-positive human leukocytes within the transplanted human LN as detected by immunohistochemistry. Sections processed without the HLe-1 antibody showed no cells with dark reaction signals. (×75.) (*B*) ISH with an ^{35}S-labeled HIV RNA probe against the 3′ end of the viral genome. The picture was taken under the dark-field condenser. Cells with HIV transcripts are marked by white grains. No positive cells are found in an adjacent mouse inguinal LN (M) or when a negative control (sense) RNA probe was used. (×40.) (*C* and *D*) Histochemistry with antibodies against human CD3 (*C*) or with stains for nonspecific esterase activity (*D*), followed by ISH for cells with HIV transcripts. Colocalization of both signals can be seen (arrows) in some of the human CD3-positive T cells (red-brown cell-surface staining and grains) and in some of the human myelomonocytic cells (red cytoplasmic staining and grains). (×390.)

injected i.v., and mice were followed for signs of viremia later. When normal SCID mice were injected with 120,000 $TCID_{50}$ of JR-CSF, circulating virus was cleared within 12 hr (as detected by RNA PCR) and never observed later. When an equivalent inoculum was injected into SCID-hu mice with human LN implants, viremia later developed (Fig. 3 *Lower*). By day 7 after infection, 30% of infected SCID-hu mice were viremic as measured by RNA PCR; by days 10–14, >95% (61 of 63 mice) were viremic. Free HIV has been detectable in the peripheral circulation of infected SCID-hu mice for periods of time as long as 16 weeks.

Infection itself was dependent upon the input dose of virus. Inoculation i.v. of 120,000 $TCID_{50}$ of JR-CSF resulted in the infection of 100% of SCID-hu mice, and doses as low as 120 $TCID_{50}$ infected at least some animals (Fig. 4); ≈12,000 $TCID_{50}$ of JR-CSF were required to produce viremia in 50% of SCID-hu mice after 2 weeks. Infection was also dependent upon the source of the virus inoculum.[§] In addition to JR-CSF, 10 other primary HIV isolates derived from patients resulted in infection of 100% of SCID-hu mice 2 weeks after i.v. inoculation. In contrast, a prototype strain of HIV which has been propagated in human T-cell leukemic lines for several years (HTLV-IIIb) did not replicate in the engrafted human lymph nodes of the SCID-hu mouse, even when doses as high as 1,200,000 $TCID_{50}$ were inoculated (data not shown).

HIV-Infected SCID-hu Mice May Be Used to Test the Efficacy of Antiviral Compounds. The experiments above demonstrate that SCID-hu mice engrafted with human LN can be reproducibly infected by i.v. injection of JR-CSF. To test the ability of this system to measure antiviral efficacy, SCID-hu mice were infected with HIV in the presence of the reference compound, AZT. The drug was added to the drinking water at varying concentrations, starting 1 day prior to infection with HIV and continuing for 2 weeks thereafter. As a first test for efficacy, peripheral blood was obtained and assayed for signs of viral RNA. Viremia was completely suppressed at a concentration of 1000 μg/ml (not shown). To confirm that cells within the LN were also protected, tissue samples from the same animals were tested by DNA PCR. At concentrations of AZT ≥ 500 μg/ml, none of the lymph nodes (0 of 14) were positive by this assay. As the daily dose of AZT decreased, the percentage of infected animals increased; 50% of animals were protected by concentrations of 245 μg/ml (Fig. 5 *Upper*), corresponding to an average dose of 40.4 mg/kg per day for a mouse weighing 20 g drinking 3.3 ml of water per day (95% confidence interval: 199–302 μg/ml or 32.8–49.8 mg/kg per day).

The efficacy of ddIno was also tested. When administered to the SCID-hu mouse 1 day before and 14 days after i.v. infection with HIV, ddIno protected animals in a dose-dependent manner (Fig. 5 *Lower*). At doses ≥ 40 mg/kg per day, no animals (0 of 39) were infected. Between 40 and 0

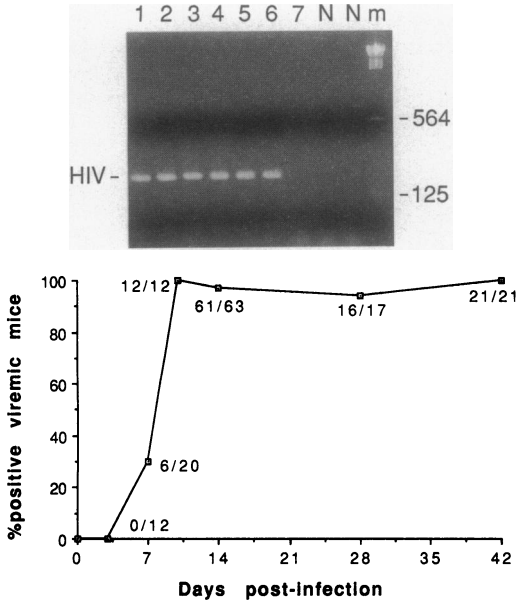

FIG. 3. Reproducible and time-dependent development of viremia in SCID-hu mice after i.v. infection with HIV. (*Upper*) PCR analysis of HIV RNA in the plasma of SCID-hu (lanes 1–7) and normal SCID (lanes N) mice, 2 weeks after i.v. inoculation of JR-CSF. Animals 3, 6, and 7 were the same as those described in Fig. 1. HIV-infected SCID mice (lanes N) were caged together with the HIV-infected SCID-hu mice. (*Lower*) Time course of the development of viremia in HIV-infected SCID-hu mice. The percent of viremic mice (as detected by RNA PCR) amongst the total number analyzed at each time point is indicated.

mg/kg per day, HIV infection was apparent in progressively more animals. The dose of ddIno that protected 50% of animals in three separate experiments was 13.7 mg/kg per day (95% confidence interval:11.2–16.8 mg/kg per day).

DISCUSSION

After i.v. inoculation of HIV into SCID-hu mice, 100% of engrafted human LN showed signs of infection within 10–14 days. Infection was dependent upon the origin of the viral isolate used for infection, the virus dose, the target organ, and time. Thus, doses of 120,000 $TCID_{50}$ of the clinical isolate JR-CSF were required to produce detectable infection in all animals by day 14. Infection of cells within the human LN was demonstrated by DNA PCR for integrated proviral genome and ISH for viral transcripts. The entry of HIV into

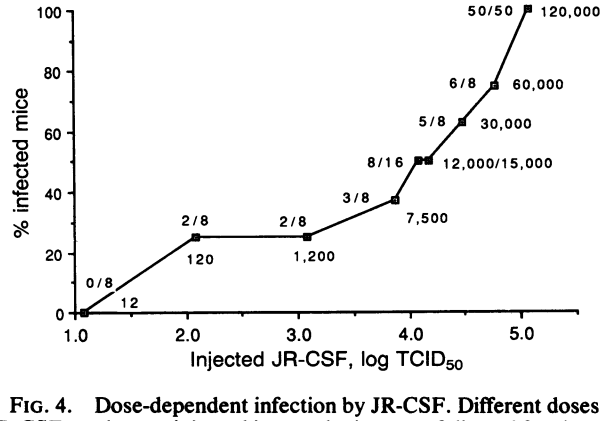

FIG. 4. Dose-dependent infection by JR-CSF. Different doses of JR-CSF stock were injected i.v., and mice were followed for signs of infection by DNA PCR in the human LN 2 weeks later. The percentage of infected mice amongst the total number analyzed at each dose is indicated.

both the T-lymphoid and monocytic lineages in the LN in the SCID-hu mouse has been documented; these are the natural target cells in man (16, 18). By 10–14 days after inoculation of virus, viremia could be detected in >95% of infected SCID-hu mice by RNA PCR analysis of plasma. In some but not all cases, infectious virus could be isolated from plasma. Finally, when antiviral compounds such as AZT and ddIno were administered to SCID-hu mice prior to inoculation of virus, infection and viremia were later suppressed in a dose-dependent manner. The HIV-infected SCID-hu mouse thus encompasses some of the salient features of acute HIV infection in man (19, 20), including tropism for LN cells and subsequent viremia (21).

The tropism of HIV for human organs with $CD4^+$ cells is preserved in the SCID-hu mouse without evidence of an expanded host range. Human LN were infected after i.v. inoculation of virus; engrafted human organs without $CD4^+$ cells (e.g., connective tissue) and mouse hematolymphoid organs were spared. Thus, although phenotypic mixing or pseudotype formation between HIV and endogenous murine viruses may occur *in vivo* (22), it does not happen in the SCID-hu mouse with a frequency that is high enough to result

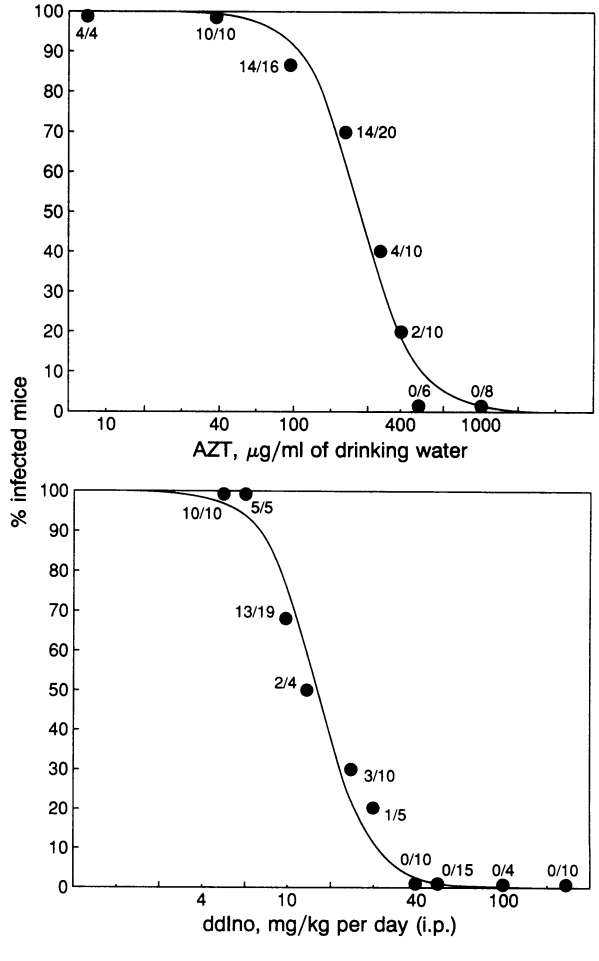

FIG. 5. Dose-dependent inhibition of HIV infection of SCID-hu mice by AZT and ddIno. Groups of SCID-hu mice were treated with various doses of AZT (orally) or ddIno (i.p.) for 1 day prior to infection with HIV and for 14 days thereafter. Human LN specimens were then assayed for HIV by DNA PCR. The percentage of infected animals is plotted as a logarithmic function of the concentration (μg/ml) of AZT in drinking water (*A*) or the actual dose (mg/kg per day) of ddIno (*B*). The dose–response curves were derived from data pooled from three experiments, after examination of the results revealed no significant departure from homogeneity between these experiments.

in detectable infection of organs without $CD4^+$ cells. In fact, the host range of HIV in the SCID-hu mouse is even more restricted than might have been expected. For instance, $CD4^+$ cells in the thymus were not infected after i.v. inoculation, even though they were permissive for viral replication after direct intrathymic injection. This suggests the presence of a "blood–thymus barrier" which may have a physiologically important role in defining the spread of infection in the SCID-hu mouse and, possibly, man.

HIV infection of the SCID-hu mouse was found to be dependent upon the use of primary isolates (such as JR-CSF). A tissue culture-adapted isolate (HTLV-IIIb) was not infectious, even when used at doses as high as 1,200,000 $TCID_{50}$. The primary isolates have been propagated only on PHA-activated human PBMC; most do not grow on human T-cell lines. In contrast, members of the HTLV-IIIb family have been adapted over time to tissue culture growth. It is possible that selective pressures *in vitro* have favored the outgrowth of a cohort of viruses that cannot replicate with efficiency *in vivo*. Such selection has previously been documented for SIV (23) as well as for HIV (3).

The response of the HIV-infected SCID-hu mouse to antiviral therapy followed a pattern similar to that seen in man. When infected with HIV in the presence of a concentration of 245 μg of AZT per ml of drinking water (40.4 mg/kg per day for an average mouse weighing 20 g and drinking 3.3 ml of water per day), 50% of SCID-hu mice were protected (95% confidence interval for a dose protecting 50% of the mice: 32.8–49.8 mg/kg per day). Given the faster clearance of drugs in smaller animals, equivalent daily doses in mice are ≈12 times higher, on a mg/kg basis, than those used in man (6). Accordingly, the minimal efficacious dose for AZT in the SCID-hu mouse is equivalent to a dose of 2.7–4.1 mg/kg per day in man. This dose compares favorably to the dose of AZT currently being used in the clinic (17). It is also notable that AZT was initially dosed in man at much higher levels (30–40 mg/kg per day) and gradually lowered over 3–4 years to minimize toxicity. The AZT dose–response studies in the SCID-hu mouse, on the other hand, took 3–4 weeks to complete.

The efficacy of ddIno against HIV infection in the SCID-hu mouse was tested in a second experiment. Unlike AZT, it has only recently been used in clinical trials, and it might be difficult to test in animal models other than man; different species apparently metabolize the drug differently (24). The dose of ddIno that protected 50% of SCID-hu mice was 13.7 mg/kg per day (95% confidence interval:11.2–16.8 mg/kg per day). The conversion factor of 12 equates this dose to a dose of 0.9–1.4 mg/kg per day in man. In contrast, human trials are now starting at doses as high as 10 mg/kg per day (14); these doses are associated with pancreatitis and peripheral neuropathy. Further experience with ddIno in clinical trials may eventually determine an efficacious, less toxic, and lower dose.

Continued experience with other antiviral compounds in the SCID-hu mouse will establish its general utility in predicting dose requirements in man. In such fashion, the SCID-hu mouse might serve as a relevant preclinical surrogate in which to rationally develop effective strategies for the treatment of HIV infection in man. This process may be carried out with minimal amounts of compound and in a time-efficient manner.

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