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Suppression of HIV Infection in AZT-Treated SCID-hu Mice

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The SCID-hu mouse, engrafted with human hematolymphoid organs, is permissive for infection with the human immunodeficiency virus (HIV). This mouse model was used to test compounds for antiviral efficacy. Two weeks after infection with HIV, 100 percent (40/40) of SCID-hu mice were positive for HIV by the polymerase chain reaction. When first treated with 3′-azido-3′-deoxythymidine (AZT), none (0/17) were HIV-positive by this assay. However, AZT-treated SCID-hu mice did have a few infected cells; after AZT treatment was stopped, viral spread was detected by polymerase chain reaction in such mice. Thus, the SCID-hu mouse provides a means to directly compare new antiviral compounds with AZT and to further improve antiviral efficacy.

MUCH HAS BEEN LEARNED ABOUT the epidemiology of the acquired immunodeficiency syndrome (AIDS) and its etiologic agent, HIV. Little is known, however, about the pathophysiology of infection in man. It has thus been difficult to evaluate prophylactic measures, such as vaccines, that might prevent infection or to establish therapies that might alter the course of established disease.

A relevant animal model for HIV infection would provide a system to evaluate the pathogenesis of AIDS and to analyze potential antiviral agents or vaccine preparations before the initiation of clinical trials. The SCID-hu mouse (*1*) was constructed precisely for this purpose. Immunodeficient C.B-17 *scid/scid* mice (*2*) are engrafted with those organs of the human hematolymphoid system that are requisite for both hematopoietic stem cell differentiation and immune function. Human hematopoietic precursor cells in the fetal liver differentiate to mature human T and myelomonocytic cells in SCID-hu mice engrafted with human fetal thymus. Implants of human fetal lymph node provide human antibody responses. Direct intrathymic or intranodal injection of HIV resulted in signs of viral replication in the SCID-hu mouse (*3*). Our data indicate that HIV infection is suppressed in SCID-hu mice that are treated with the antiviral compound, 3′-azido-3′-deoxythymidine (AZT).

Four to eight weeks after engraftment, SCID-hu mice were infected with 400 to 4000 infectious units (IU) of $HIV_{JR\text{-}CSF}$ or $HIV_{JR\text{-}FL}$ by direct intrathymic injection. This dose of virus had been shown to result in infection of 100% of SCID-hu mice by 2 weeks, as assayed by in situ hybridization (*3*). Two weeks after infection, the human thymus implants were biopsied and analyzed by the polymerase chain reaction (PCR) for the presence of HIV (Fig. 1A). PCR products for both human β-globin and for HIV were present. Cells from these biopsy specimens were also positive for immunohistochemical stains for envelope and gag epitopes (*3*). The HIV and β-globin PCR products were not detectable in biopsy specimens derived from the murine spleen (M) (Fig. 1A) or the murine lymph nodes or thymus of the same animals; nor was the HIV PCR product found in uninfected littermates kept within the same cage (Fig. 1A). Thus, direct intrathymic injection of HIV results in detectable and reproducible levels of HIV infection in the human, but not the murine, lymphoid organs of the SCID-hu mouse.

Table 1. Cumulative results of HIV infection of SCID-hu mice by $HIV_{JR\text{-}CSF}$ or $HIV_{JR\text{-}FL}$ in the presence (+) or absence (−) of AZT. At 2 weeks after infection, thymic sections were analyzed by DNA PCR for the presence of human β-globin and HIV, as described in the legend to Fig. 1.

Virus strain	AZT treatment	HIV	β-globin
JR-CSF	−	34/34	34/34
JR-FL	−	6/6	6/6
JR-CSF	+	0/12	12/12
JR-FL	+	0/5	5/5

To test the possibility that such evidence of HIV infection could form the basis of an in vivo assay of antiviral efficacy, AZT was administered to SCID-hu mice with human thymic implants for 24 hours before HIV infection by intrathymic injection. These mice then received continued AZT treatment for 2 weeks. At that time, the thymus implants were biopsied for PCR analysis (Fig. 1B). After 2 weeks of AZT treatment, HIV-infected SCID-hu mice (number 5, 6, 7, and 8) had no detectable HIV-specific PCR products. This antiviral effect was reproducible with larger numbers of animals (Table 1). All (40/40) of the SCID-hu mice infected with HIV in the absence of AZT were positive by PCR after 2 weeks. None (0/17) of the SCID-hu mice treated with AZT had HIV-specific PCR products after 2 weeks. Infection was suppressed when either a T-lymphotropic isolate of HIV (JR-CSF) or a monocytotropic isolate (JR-FL) was used.

More sensitive assays were used to evaluate the qualitative nature of this antiviral effect. Thymic biopsy specimens from HIV-infected SCID-hu mice treated with AZT for 2 weeks were subjected to in situ hybridization with a ^{35}S-labeled RNA probe specific for the 3′ end of the HIV genomic transcript (Fig. 2). Fewer cells were infected in the presence of AZT and each infected cell had fewer HIV transcripts. Nonetheless, infection by HIV had occurred. Because continued rounds of viral infection might spread from such cells, SCID-hu mice that had been treated with AZT for 2 weeks and were negative for HIV (by PCR) were then switched to a regimen without AZT for an additional 4 weeks. At that time, thymic biopsy specimens were found to be positive

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for HIV by the PCR technique (Fig. 1C). Thus, under these conditions, AZT treatment suppressed viral infection of the SCID-hu mouse, but its effect was not absolute.

Antiviral agents against HIV are now first tested in vitro, often in the context of $CD4^+$ human T or myelomonocytic cell lines or human peripheral blood cells. The SCID-hu mouse represents a biological system of greater relevance to in vivo infection; it may thus offer experimental opportunities unique among other animal models for HIV infection. Acute infection, latency, and the interaction of HIV with multiple human cell types and in diverse human tissue microenvironments can be observed and followed in many animals as a function of time or HIV isolate. These parameters may be more difficult to study in models that use lentiviruses other than HIV (*4*), larger animals (*5*), or SCID mice reconstituted with discrete subsets of mature human peripheral blood cells (*6*).

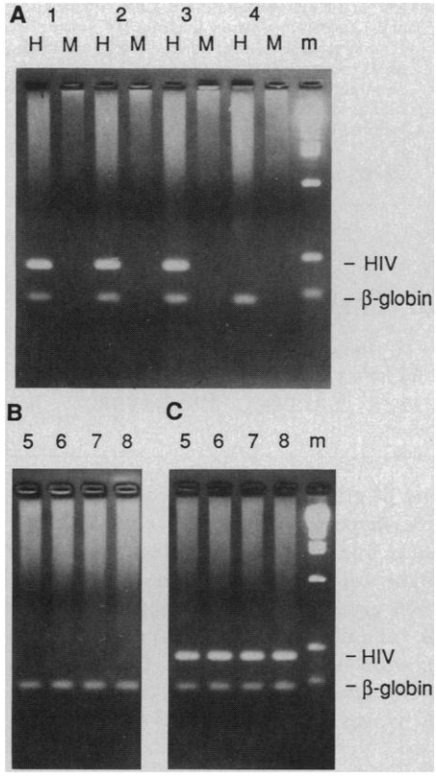

Fig. 1. Effect of AZT on HIV infection of the SCID-hu mouse. (**A**) Two weeks after infection with $HIV_{JR\text{-}CSF}$, sections from the human thymus (H) and murine spleen (M) of SCID-hu mice number 1, 2, and 3 were analyzed by DNA PCR, by using primer pairs specific for HIV and human β-globin. Similar analyses were conducted on a control, uninfected littermate (SCID-hu mouse number 4). (**B**) SCID-hu mice number 5, 6, 7, and 8 were treated for 24 hours with AZT, infected with $HIV_{JR\text{-}CSF}$, and maintained for 2 weeks on AZT; DNA PCR for HIV and human β-globin was then conducted on sections from the human thymus and the mouse spleen. Thereafter, these mice were taken off AZT and analyzed by DNA PCR 4 weeks later (**C**). SCID-hu mice were prepared as described (*1*). The HIV isolates CSF and FL grow in phytohemagglutinin (PHA)-activated human T cell blasts; JR-FL is also monocytotropic, and neither JR-CSF nor JR-FL can be grown in long-term human T cell lines (*12*). Infection of SCID-hu mice with these isolates was conducted as described (*3*). Twenty-four hours before infection with 400 to 4000 IU of $HIV_{JR\text{-}CSF}$ or $HIV_{JR\text{-}FL}$ (1 to 10 IU corresponds to 1 pg of p24, measured by enzyme-linked immunosorbent assay of culture supernatants 14 days after infection of PHA blasts), SCID-hu mice (number 5, 6, 7, and 8) were treated with water containing AZT (1 mg/ml). On average, the dose of AZT that each mouse consumed was 160 to 200 mg kg^{-1} day^{-1}). At this dose and duration of drug, no overt toxicity to SCID-hu mice was noticed. Sections from the human thymus or murine spleen and lymph nodes of SCID-hu mice were analyzed for the presence of human cells by using PCR and the following primers specific for human β-globin: LA1, 5′-ACACAACTGTGTTCACTAGC-3′; and LA2, 5′-CAACTTCATCCACGTTCACC-3′. Infection of the tissues with HIV was assessed by PCR with the use of primers specific for the conserved U5-*gag* region of HIV: 661 5′-CCTGCGTCGAGAGAGCTCCTCTGG-3′; and 667 5′-GGCTAACTAGGGAACCCACTG-3′. The protocol for tissue preparation and PCR was essentially as described (*13*). After 60 cycles of amplification (95°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min), PCR products were resolved on 3% agarose gels and detected by ethidium bromide staining. We estimate that the lower limit of sensitivity for the PCR assay was ten infected cells per 10^6 uninfected cells. m, size markers from Bst EII–digested phage λ DNA.

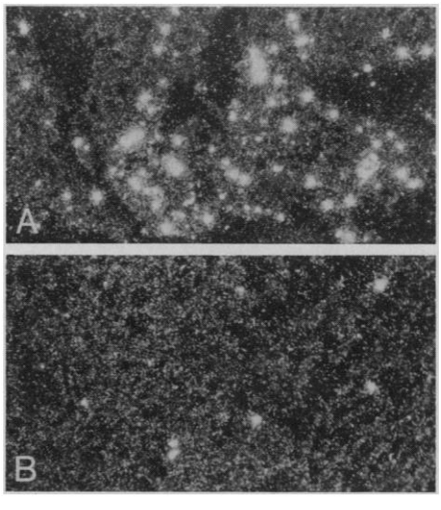

Fig. 2. In situ hybridization of thymus sections from SCID-hu mice infected with HIV in the absence (**A**) or presence (**B**) of AZT. Sections derived from the human thymus of SCID-hu mice were obtained 2 weeks after infection with HIV, fixed with 4% paraformaldehyde in phosphate-buffered saline at 4°C for 4 to 8 hours, and prepared for in situ hybridization by using ^{35}S-labeled probes specific for the 3′ end of the genomic HIV transcript, as described (*3, 14*). All of the viral RNA transcripts (genomic and subgenomic) were detected by this assay without discrimination; under the conditions used, the probes (*3*) did not hybridize to uninfected mouse or human cells, or to HIV DNA. Therefore, cells with grains in the autoradiograph are presumed to be infected with transcriptionally active HIV. In (B), the HIV-infected SCID-hu mouse had been treated with AZT starting 24 hours before infection and for 2 weeks thereafter. Photographs were taken under dark field at ×25.

In studies of prophylactic antiviral efficacy in vivo, AZT has been shown to inhibit Rauscher leukemia virus infection of mice (*7*) and feline leukemia virus (FeLV) infection of cats (*8*). Another nucleoside analog, 2′,3′-dideoxycytidine, failed to inhibit replication of FeLV in feline AIDS (*9*). Our data showed that even when AZT was administered before and after infection of SCID-hu mice with HIV, protection is not complete. A small number of HIV-infected cells can be detected by in situ hybridization. When AZT coverage was discontinued, viral spread from such cells to uninfected cells was observed 4 weeks later by PCR. This result could be related to a quantitative phenomenon: even in the presence of AZT, a few full-length viral genomes might be produced (*10*). It might, however, be due to a qualitative difference in the interaction of AZT with different subpopulations of human cells. Some infected cells, for instance, might phosphorylate the drugs less readily and thereby be less susceptible to protection (*11*).

Because of the relative ease with which HIV-infected SCID-hu mice are made and analyzed, it is possible to construct protocols of drug administration that compare different congeners, different doses, varying routes, and the effect of compounds given in combination on viral infection and spread. The SCID-hu mouse will be useful for measuring the in vivo efficacy of antiviral compounds and may provide a test system in which their administration could be experimentally altered to achieve optimal efficacy. The same techniques that are used to analyze antiviral compounds against HIV should be directly transferable to the analysis of interventions against other human pathogens. The human hematolymphoid organs of the SCID-hu mouse are likely to be permissive for infection with human T cell leukemia virus type 1, cytomegalovirus, and Epstein-Barr virus. As such, this animal model might aid the development of therapeutics for disease states in man that currently do not have treatment options.

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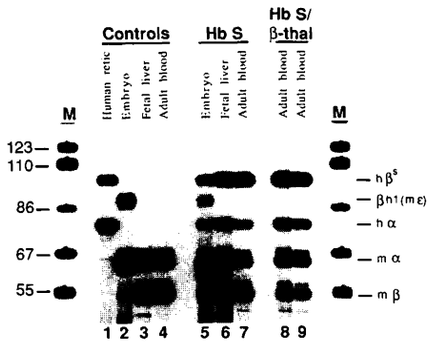

Fig. 2. Primer extension analysis of total RNA from Hb S and Hb S/β-thal mice. Authentic human β^s- and human α-globin primer extension products are 98 and 76 bp, respectively; correct mouse βh1- (ε), α-, and β-globin products are 87, 65, and 53 bp, respectively (*15*). Lanes 1 to 4 are total RNA samples from normal controls, lanes 5 to 7 are from Hb S mice, and lanes 8 to 9 are from Hb S/β-thal mice. Lane 1, human reticulocytes; lanes 2 and 5, 11-day mouse embryo (10 μg); lanes 3 and 6, 16-day mouse fetal liver (1.0 μg); and lanes 4, 7, 8, and 9, adult mouse reticulocytes (0.25 μg). Hb S mice contain five copies of HS I–V α and seven copies of HS I–V β^s. Hb S/β-thal animals were derived by mating Hb S mice with the β-thalassemic mouse line C57BL/6.*Hbb*$^{d\text{-}3thJ}$ (*7*).

Human Sickle Hemoglobin in Transgenic Mice

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DNA molecules that contain the human α- and β^s-globin genes inserted downstream of erythroid-specific, deoxyribonuclease I super-hypersensitive sites were coinjected into fertilized mouse eggs and a transgenic mouse line was established that synthesizes human sickle hemoglobin (Hb S). These animals were bred to β-thalassemic mice to reduce endogenous mouse globin levels. When erythrocytes from these mice were deoxygenated, greater than 90 percent of the cells displayed the same characteristic sickled shapes as erythrocytes from humans with sickle cell disease. Compared to controls the mice have decreased hematocrits, elevated reticulocyte counts, lower hemoglobin concentrations, and splenomegaly, which are all indications of the anemia associated with human sickle cell disease.

SICKLE CELL ANEMIA WAS THE FIRST genetic disease to be understood at the molecular level (*1*). An A to T transversion in the sixth codon of the human β-globin gene causes an amino acid change from a polar glutamic acid residue to a nonpolar valine in the β-globin polypeptide. This change decreases the solubility of deoxygenated Hb S. At low oxygen tensions, Hb S molecules polymerize to form extensive networks of intracellular fibers (*2*). These fibers distort the erythrocytes into a variety of sickled shapes. The sickled cells are rigid and nondeformable and can occlude the microvasculature, which causes local hypoxia and tissue damage (*3*).

High concentrations of functional human hemoglobin can be synthesized in transgenic mice when human α- and β-globin genes are inserted immediately downstream of the erythroid-specific, deoxyribonuclease (DNase) I super-hypersensitive sites normally located 50-kb upstream of the human β-globin gene (*4*). These results suggested that high concentrations of sickle hemoglobin could be produced in transgenic mice if human α- and β^s-globin genes were inserted downstream of the super-hypersensitive (HS) sites and injected into fertilized eggs. Therefore, cosmid clones that contained all five upstream sites (HS I–V) and either the human α- or β^s-globin gene were constructed (Fig. 1) (*5*). The 26-kb fragments containing HS I–V α and HS I–V β^s were purified from vector sequences and coinjected into fertilized eggs (*6*). Injected eggs were implanted into the uteri of foster mothers; tail DNA of the progeny was analyzed for intact copies of the transgenes. Three transgenic animals had head-to-tail tandem arrays of both transgenes. Hemolysates from these animals were analyzed on isoelectric focusing (IEF) gels (*4*). All three mice synthesized Hb S; the highest expressor was mated to control animals to establish a line. These transgenic mice were then bred with a strain of β-thalassemic (β-thal) mice to reduce endogenous mouse β-globin levels (*7*). Hb S/β-thal animals derived from this mating were het-

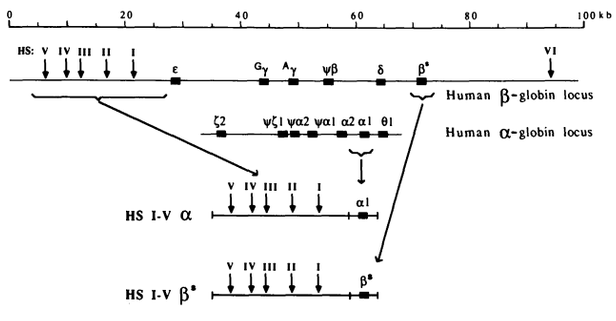

Fig. 1. HS I–V α and HS I–V β^s constructs. One hundred kilobases of the human β-globin locus and 35 kilobases of the human α-globin locus are illustrated. Cosmids containing HS I–V α1 and HS I–V β^s were constructed as described (*5*). The 26-kb inserts were purified from vector sequences, mixed at a 1:1 molar ratio (final DNA concentration was 2 ng/μl) and coinjected into fertilized mouse eggs (*6*).

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