Rapid-High, Syncytium-Inducing Isolates of Human Immunodeficiency Virus Type 1 Induce Cytopathicity in the Human Thymus of the SCID-hu Mouse

HIDETO KANESHIMA,^{1*} LISHAN SU,¹ MARK L. BONYHADI,¹ RUTH I. CONNOR,²
DAVID D. HO,² AND JOSEPH M. McCUNE¹

*HIV Group, SyStemix, Inc., Palo Alto, California 94304,¹ and Aaron Diamond AIDS Research Center,
New York University School of Medicine, New York, New York 10016²*

Received 1 July 1994/Accepted 15 September 1994

Clinical deterioration in human immunodeficiency virus type 1 (HIV-1) disease is associated with an increased viral burden in the peripheral blood and a loss of circulating CD4⁺ T cells. HIV-1 isolates obtained prior to this stage of disease often have a “slow-low,” non-syncytium-inducing (NSI) phenotype, whereas those obtained afterwards are often characterized as “rapid-high” and syncytium inducing (SI). Paired NSI and SI isolates from two different patients were inoculated into the human thymus implants of SCID-hu mice. The two slow-low, NSI isolates replicated to minimal levels in the grafts and did not induce thymocyte depletion. In contrast, the two SI isolates from the same patients showed high levels of viral replication and induced a marked degree of thymocyte depletion, accompanied by evidence of programmed cell death. These observations reveal a correlation between the replicative and cytopathic patterns of HIV-1 isolates in vitro and in the SCID-hu mouse in vivo and provide direct evidence that the biological phenotype of HIV-1 switch may be a causal and not a derivative correlate of HIV-1 disease progression.

Clinical and immunological deterioration in human immunodeficiency virus type 1 (HIV-1) disease is associated with an increased viral burden in the peripheral blood and a loss of circulating CD4⁺ T cells (16–18). HIV-1 isolates obtained prior to this stage of disease often have a “slow-low,” non-syncytium-inducing (NSI) phenotype, whereas those obtained afterwards are often found to have a “rapid-high,” syncytium-inducing (SI) phenotype (2, 4, 20). Disease progression may in some cases be caused by a shift in viral phenotype from NSI to SI. Alternatively, the emergence of HIV-1 variants with the SI phenotype may simply be a finding coincident with the enhanced viral replication which occurs as disease progresses.

The heterochimeric SCID-hu mouse was used to discriminate between such alternative possibilities of HIV-1-associated pathogenesis. This animal model is constructed by surgical engraftment of human hematolymphoid organs, which constitute both the hematopoietic system and the primary target organs for HIV-1 infection in humans (10, 14). Coengraftment of human fetal liver and thymus, to produce the conjoint Thy/Liv organ, leads to multilineage human hematopoiesis for periods as long as 6 to 12 months (15). In particular, T-lymphocyte-restricted progenitor cells from the fetal liver differentiate to produce CD4⁺ CD8⁺ cortical thymocytes, mature single positive (CD4⁺ or CD8⁺) medullary thymocytes, and circulating human T cells. The human T-cell subpopulations in SCID-hu mice are phenotypically and functionally similar to those found in humans, with an unperturbed V β repertoire and a CD4/CD8 ratio of 2/1 to 3/1 (9, 22). After infection with primary isolates of HIV-1, T as well as myeloid cells in the Thy/Liv implant are found to be permissive for viral replication and responsive to the antiviral effects of nucleoside analogues such as zidovudine (11). Thereafter, in a time-dependent fashion which varies among different HIV-1 iso-

lates, thymopoiesis is abrogated (1, 1a, 19). As is observed with HIV-1 disease in humans, there is depletion of T cells and an inversion of the CD4/CD8 ratio. These events are associated, at least in part, with HIV-1-facilitated induction of programmed cell death (PCD).

We have used this animal model to test directly the relative in vivo pathogenicities of NSI and SI isolates of HIV-1. Paired samples of NSI and SI isolates were obtained from two patients, either at a time when they were clinically stable or after a time when each experienced a decline in the peripheral CD4 T-cell count and an increase in viral load (3). These isolates were injected into the SCID-hu Thy/Liv organ to evaluate their replicative capabilities and their effects on T lymphopoiesis in vivo.

MATERIALS AND METHODS

HIV-1 stocks. As reported previously (3), isolates A6/87 and B5/85 were obtained from patients A and B, respectively, prior to a decline in the CD4 T-cell count (Table 1). These isolates replicate slowly in peripheral blood mononuclear cells (PBMC), do not replicate in leukemic cell lines, do not form syncytia in vitro, and, hence, are of a slow-low, NSI phenotype. In contrast, isolates A7/88 and B11/88, obtained after clinical deterioration in patients A and B, respectively, were observed to be syncytium-inducing viruses with high replicative potential in both PBMC and MT-2 cells in vitro. All viral isolates were propagated by short-term (<2-week) cocultivation of patient PBMC with phytohemagglutinin-activated PBMC from healthy seronegative donors. Titers of each stock were then determined on phytohemagglutinin-activated PBMC in vitro to obtain relative 50% tissue culture infective doses (TCID₅₀s) as described previously (8).

Infection of SCID-hu Thy/Liv mice. SCID-hu mice carrying human Thy/Liv implants were prepared as described previously (15). Between 3 and 6 months posttransplantation, animals were inspected visually for robust Thy/Liv organ

* Corresponding author. Mailing address: HIV Group, SyStemix, Inc., 3155 Porter Dr., Palo Alto, CA 94304. Phone: (415) 813-5010. Fax: (415) 856-1486.

TABLE 1. Characteristics of HIV-1 clinical isolates used in this study

Patient	Isolate (mo/yr)	CD4 ⁺ T-cell count/mm ³	TCID ₅₀ /10 ⁶ CD4 cells	HIV DNA copies/10 ⁶ CD4 cells	Phenotype in vitro ^a	Replication in:		Syncytium induction in MT-2 cells ^b
						PBMC	MT-2 cells ^b	
A	6/87	926	19	314	NSI	Slow	—	—
	7/88	660 ^c	1,022	1,210	SI	Rapid	+	+
B	5/85	528	29	228	NSI	Slow	—	—
	11/88	117	2,285	11,557	SI	Rapid	+	+

^a Summarized from primary data published elsewhere (3).^b +, present; —, absent.^c Levels fell rapidly.

growth and HIV-1 isolates (20 to 2,000 TCID₅₀s in 25 μ l, as indicated in the tables) were injected directly into the organ. All animals used for a given experiment (e.g., those shown in Table 2 or Table 3) carried human fetal tissues derived from a single donor. At various times after inoculation, the animals were sacrificed and samples from the implants were taken for histological analysis, p24 enzyme-linked immunosorbent assay (ELISA), and flow cytometry.

Cellular p24 analysis. Suspended thymocytes (4×10^6 in 200 μ l) were solubilized in phosphate-buffered saline (PBS) containing 1% Triton X-100, and p24 levels in the supernatant were measured by using a p24 ELISA kit (Dupont NEN, Boston, Mass.), as described elsewhere (1a). Data were obtained from the linear region of the standard calibration curve and standardized as picograms of p24 per 10⁶ input thymocytes.

Flow-cytometric analysis. Single cell suspensions were prepared from the Thy/Liv grafts and stained with phycoerythrin-conjugated monoclonal antibodies against CD4 (Becton Dickinson, Mountain View, Calif.) and TRI-COLOR-conjugated monoclonal antibodies against CD8 (Caltag Laboratories, South San Francisco, Calif.) in PBS with 2% fetal calf serum. Cells were washed, resuspended in PBS containing 1% paraformaldehyde, and analyzed by single-laser flow cytometry (FACScan; Becton Dickinson Immunocytometry Systems, Mountain View, Calif.). Dead cells were gated out by forward scatter and side scatter, and the remaining cells were analyzed for CD4 and CD8 expression as described in detail previously (1a). The ratio of CD4/CD8 was obtained by dividing percent CD4⁺ CD8[−] by percent CD4[−] CD8⁺.

Detection of DNA strand breaks (PCD) by TdT reaction. Single cell suspensions from uninfected and infected Thy/Liv grafts were analyzed for DNA strand breaks consistent with

PCD. Terminal deoxynucleotidyl transferase (TdT) was used to transfer deoxynucleotides to the 3' OH ends of fragmented DNA in cells undergoing PCD; necrotic cells are not labelled under these conditions (5). Briefly, paraformaldehyde-fixed thymocytes were permeabilized by 70% ethanol and 3' OH DNA ends were labelled with biotinylated dUTP (Boehringer Mannheim, Indianapolis, Ind.) by using TdT (Boehringer Mannheim). The incorporation of dUTP was then detected by FACScan after incubation with fluorescein-streptavidin (Vector Laboratories, Burlingame, Calif.).

RESULTS

Distinct patterns of replicative kinetics and of cytopathicity were observed after infection of SCID-hu Thy/Liv grafts with NSI or SI isolates of HIV-1 (Tables 2 and 3). At 4 and 8 weeks postinoculation, NSI isolates obtained from patients A and B (A6/87 and B5/85, respectively) produced low levels of p24 and no change in the percentage of CD4⁺ CD8⁺, CD4⁺, or CD8⁺ thymocyte subpopulations relative to uninfected controls. In contrast, after inoculation of the SI isolates from patients A and B (A7/88 and B11/88, respectively), high levels of p24 production and marked cytopathicity to thymocyte subpopulations were observed. At 4 weeks, the mean p24 levels were 5-fold (patient A) or 17-fold (patient B) higher than those obtained after injection of the corresponding NSI isolates. At 8 weeks, the mean p24 level in the Thy/Liv grafts infected with the patient A SI isolate (A7/88) was lower. This decline in viral production corresponded to the pattern of thymocyte depletion shown in Fig. 1 and summarized in Table 2. Even by 4 weeks, the subpopulation of CD4⁺ CD8⁺ cells was significantly reduced and the CD4/CD8 ratio was inverted. By 8 weeks, CD4⁺ cell depletion and inversion of the CD4/CD8

TABLE 2. Results for SCID-hu mice infected with HIV-1 isolates from patient A

Incubation time (wks)	Isolate	Inoculum TCID ₅₀ /graft	Amt (pg) of p24/10 ⁶ cells ^a (mean $\pm$ SE) as determined by ELISA	% Cells			CD4/CD8 ratio
				CD4 ⁺ CD8 ⁺ ^b	CD4 ⁺	CD8 ⁺	
NA ^c	None (uninfected)	None	0, 0, 0 (0 $\pm$ 0)	84, 86, 75	9, 10, 18	7, 4, 6	1.3, 2.5, 3.0
4	A6/87	2,000	32, 46, 64 (47 $\pm$ 9.3)	82, 81, 73	9, 10, 19	6, 8, 7	1.7, 1.2, 2.7
	A7/88	2,000	157, 276, 334 (256 $\pm$ 52)	74, 7, 15	10, 34, 28	13, 52, 42	0.8, 0.7, 0.7
8	A6/87	2,000	38, 25, 35, 46 (36 $\pm$ 4.3)	86, 82, 84, 83	8, 11, 9, 10	6, 7, 7, 6	1.3, 1.5, 1.3, 1.4
	A7/88	2,000	96, 53 (75 $\pm$ 22)	3, 4	9, 15	75, 70	0.1, 0.2

^a Each number represents data obtained from a single SCID-hu Thy/Liv mouse.^b Comparing the mean percentage of CD4⁺ CD8⁺ cells remaining at 4 and 8 weeks after infection with the NSI isolate A6/87 ($n = 7$, 82% $\pm$ 2%) with the mean percentage remaining after infection with the SI counterpart A7/88 ($n = 5$, 21% $\pm$ 14%), the difference is significant at the level of $P = 0.0003$ by the unpaired t test.^c NA, not applicable.

Incubation time (wks)	Isolate	Inoculum TCID ₅₀ /graft	Amt (pg) of p24/10 ⁶ cells ^a (mean ± SE) as deter- mined by ELISA	% Cells			CD4/CD8 ratio
				CD4 ⁺	CD8 ⁺	CD4 ⁺ CD8 ⁺	
NA ^c	None (uninfected)	None	0, 0, 0 (0 ± 0)	84, 84, 79	10, 9, 14	4, 7, 6	2.5, 1.3, 2.3
4	B5/85	200	34, 8.5, 1.2 (14 ± 9.8)	74, 81, 85	11, 12, 10	10, 6, 5	1.1, 2.0, 2.0
	B11/88	20	387, 115, 209 (235 ± 78)	64, 74, 20	20, 15, 49	13, 8, 25	1.5, 1.9, 2.0
8	B5/85	200	8.0, 4.7 (6.4 ± 1.7)	85, 88	9, 7	5, 5	1.8, 1.4
	B11/88	20	362, 620, 224 (402 ± 116)	41, 40, 2	30, 25, 27	23, 29, 51	1.3, 0.9, 0.5

^c NA, not applicable.

HIV-1-induced T-cell destruction has been associated with PCD, both in the context of mature circulating T cells from

FIG. 2. Histogram for fluorescein isothiocyanate (FITC) channel of thymocytes after incubation with TdT followed by streptavidin-FITC. The small debris and completely dead cells were gated out by forward scatter and side scatter. The data shown were obtained from one uninfected animal and from two animals 6 weeks postinfection with either A6/87 or A7/88. The percentage of cells whose TdT positivity falls above background level is indicated to the right of each plot. Variation in the mean fluorescence intensity of the apoptotic peaks (e.g., for the A7/88-infected samples) is occasionally observed.

HIV-1-infected patients (6, 12) and after infection of SCID-hu Thy/Liv (1). Differential degrees of PCD were observed after inoculation of the paired NSI and SI isolates from patient A (Fig. 2). In this flow-cytometric analysis, dead cells and debris were excluded by forward- versus side-scatter characteristics and cells falling in the viable gate were analyzed for the TdT-mediated incorporation of biotinylated dUTP into DNA strand breaks. Previous studies have shown that this assay provides reproducible and reliable data about the extent of CD4⁺ CD8⁺ depletion in HIV-infected SCID-hu Thy/Liv animals (19a). At 6 weeks after infection, little if any evidence of PCD was observed in two grafts infected with the slow-low, NSI isolate (A6/87). In contrast, two grafts infected with the rapid-high, SI isolate (A7/88) showed incorporation of dUTP into 72 and 61% of thymocytes, respectively.

DISCUSSION

This study demonstrates a direct relationship between the replicative and cytopathic characteristics of slow-low, NSI and rapid-high, SI isolates in vitro and in the SCID-hu Thy/Liv model in vivo. NSI isolates with slow replicative properties in vitro showed low levels of replication in the Thy/Liv organ and did not induce thymocyte depletion. In contrast, infection with SI isolates from the same patients resulted in high levels of

viral replication and marked degrees of thymocyte depletion in the Thy/Liv organ. As has been observed in other instances of HIV-1-mediated cell death, SI-induced thymocyte depletion was accompanied by evidence of PCD.

These observations are consistent with the possibility that at least some SI virus isolates can accelerate pathogenesis in vivo and are not simply a coincidental by-product of disease progression (3, 21). It is also notable that these isolates are cytopathic in the SCID-hu mouse without the presence of appreciable B- or T-cell responses to viral components and, hence, in the absence of autoimmune reactions (23). It follows that such isolates may have structural or functional attributes that are both different from those of their NSI counterparts and directly responsible for pathogenicity. Detailed molecular mapping of such attributes is now feasible by using the paired NSI and SI variants reported here in the context of thymocyte depletion in the SCID-hu mouse.

Previous studies have shown that the structure and function of the SCID-hu Thy/Liv organ are similar to those of the normal human fetal thymus, with thymopoiesis and T and myeloid function proceeding as would be expected. HIV-1 infection of the organ system also appears to follow a predictable course, with permissivity to primary as opposed to laboratory-adapted isolates of HIV-1 (8), dual tropic infection of T and myeloid cells, thymocyte depletion, and responsiveness to

antiviral compounds such as zidovudine and dideoxyinosine (8). Yet, since the experimental conditions for infection are not identical to any likely to occur in humans (with respect to route, dose, and time course), conclusions reached for the SCID-hu model require ultimate validation in humans before acceptance.

This point is underscored by studies performed with another SCID model, posing the same question and reaching a different conclusion. Thus, Mosier and colleagues (13) have reported that when various HIV-1 isolates are inoculated into hu-PBL-SCID mice (reconstituted with human PBMC intraperitoneally), T-cell depletion appears to occur more readily with NSI than with SI isolates. These data diverge both from those of the current study and from those obtained in multiple studies with HIV-infected patients (2–4, 20, 21). This could be related to differences in a variety of experimental conditions, including the exact virus isolates used as well as the distinct microenvironments provided for human cells in the SCID-hu and the hu-PBL-SCID mouse models (7).

At best, preclinical models of human pathogenesis guide the formulation of hypothesis that can be tested directly with humans. The hypothesis raised most directly by the present study is that the subgenomic region(s) of at least some SI isolates may directly cause accelerated disease progression in humans (3, 21). Further analysis of this hypothesis, by using the SCID-hu mouse and then HIV-1-infected patients, should clarify the relative predictive powers of the two preclinical SCID models and, more importantly, the precise contribution of NSI-SI variation in HIV-1 disease.

ACKNOWLEDGMENTS

We thank the Comparative Medicine Group, SyStemix, Inc., for SCID-hu mouse production and maintenance.

This work was supported partly by grants from the NIH (AI 24030, AI 25541, AI 27742, AI 30358, AI 32427, and AI 27665 to D.D.H. and AI 29323 to J.M.M.), the Ernst Jung Foundation, and the Aaron Diamond Foundation.

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