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U937-SCID mouse xenografts: a new model for acute in vivo HIV-1 infection suitable to test antiviral strategies

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Abstract

In this study we attempted to develop a new xenochimeric model for HIV infection in SCID mice, characterized by an easy engraftment of target cells, high levels of viremia and long-lasting HIV-1 infection. SCID mice were injected subcutaneously with uninfected human U937 cells and cell-free HIV-1 (IIIB strain) or HIV-1-infected human peripheral blood lymphocytes (PBL). Mice were evaluated for tumor growth, viral infection at the tumor level (DNA-polymerase chain reaction (PCR), RNA-PCR) and immunostaining for the p55/p18 HIV protein) and p24 antigenemia or serum HIV-1 RNA copies. Pretreatment of mice with antibodies to either mouse-IFN α/β or granulocytes resulted in a tumor take and levels of p24 antigenemia higher than in control mice. In mice treated with these antibody preparations, there was a long-lasting HIV infection with the presence of high levels of circulating infectious virus (serum p24 values up to 4000 pg/ml and serum RNA copies up to 5×10^7/ml over 3 months, with the majority of the cells expressing HIV-antigens at the tumor site). Intraperitoneal treatment of SCID mice with AZT (480 mg/kg per day) resulted in a complete inhibition of both p24 and RNA HIV-1 copies in the serum, together with a marked reduction in the number of infected cells and the levels of virus expression at the tumor site. We conclude that some specific features of this model (i.e. easy establishment, high reproducibility, well defined kinetics of virus infection, massive and long persistent viremia) underline the special advantages of its use for testing new antiviral therapies. © 1997 Elsevier Science B.V.

Keywords: HIV-1; In vivo model; SCID mice; Antiviral therapy

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1. Introduction

Over the past few years, considerable progress has been made in increasing our knowledge of epidemiology of AIDS, its etiologic agent (HIV-1) and the mechanisms of HIV infection. As a result, much research has focused on the development of new molecules and strategies for the therapy of infected patients. However, the definition of the potentially most effective anti-HIV therapies has been hampered by the substantial lack of practical and small animal models, which allow new antiviral treatments to be evaluated on a large scale and for a consistent period of time.

Since the first report of the normal human cell transplantation to severe combined immunodeficiency (SCID) mice (McCune et al., 1988; Mosier et al., 1988) and of the HIV infection of these chimeric animals (Namikawa et al., 1988; Mosier et al., 1991), there was a considerable use of these models in AIDS research. SCID mice transplanted with human peripheral blood lymphocytes (Mosier et al., 1991; Rizza et al., 1996) or hematolymphoid tissues (Namikawa et al., 1988), proved to be valuable models in AIDS research, especially for evaluating some aspects of the pathogenesis of HIV infection, including the virus-induced CD4 T-cell depletion (McCune et al., 1990; Mosier et al., 1991; Rizza et al., 1996). However, in spite of the initial enthusiasm for the possible use of these models for antiviral screening (McCune et al., 1990), their applications for testing antiviral therapies have been limited and somehow controversial. The lack of a widespread use of these animal models in antiviral research for HIV is largely dependent on two major limitations: (i) the restriction of HIV infection to a subset of the transplanted human cells, which does not generally result in high level virus amplification or viremia; (ii) the relatively short persistence of virus infection.

In this study, we describe a new chimeric mouse/human model for HIV infection, where multiple virus infectious cycles occur in human target cells (i.e. human U937 cells) actively replicating in vivo after subcutaneous injection in SCID mice depleted of some residual reactivity by treatment with either anti-mouse interferon (IFN) α/β or anti-mouse granulocyte antibodies. This model exhibits specific features, such as easy establishment, high reproducibility, well defined kinetics of virus infection, massive viremia and long-lasting persistence of the virus infected cells, all of which underline the special advantages of its use for testing the effects of single and combined drug therapies on both the virus and the infected cells.

2. Materials and methods

2.1. Reagents

Polyclonal sheep anti-murine-IFN-α/β (sheep 1) was prepared and purified and had a neutralizing titer of 6.4×10^6 against 4–8 U of murine-IFN-α/β (Gresser et al., 1976). Monoclonal anti-mouse granulocyte antibodies (RB6-8C5 hybridoma) was prepared as previously described (Ferrantini et al., 1994). AZT (Sigma-Aldrich, Milwaukee, WI) was dissolved in prewarmed (40°C) PBS at a concentration of 9 mg/ml and filter-sterilized. Mice were daily injected intraperitoneally (i.p.) with 1 ml of a freshly prepared AZT solution (480 mg/kg per day [maximal tolerated dose]).

2.2. Growth of U937 cell-tumors in scid mice and in vivo HIV-1 infection

CB.17 SCID/SCID female mice (Charles River, Milan, Italy) were used at 4–5 weeks of age and were kept under specific pathogen-free conditions. SCID mice were housed in microisolator cages and all food, water and bedding were autoclaved prior to use. The animal studies were performed in biosafety level 3 facility. Mice were injected subcutaneously (s.c.) in the shoulder with 2×10^6 uninfected U937 cells (Sunstrom and Nilsson, 1976) resuspended in 0.2 ml saline. The two major diameters of each tumor nodule were measured by calliper and the mean tumor diameter of each tumor was calculated.

To deplete animals of some residual reactivity,

SCID mice were injected i.p. with 0.2 ml of a 1:20 dilution of a polyclonal sheep anti-murine IF*N* α/β (320 000 neutralizing units/mouse) or 0.2 ml (100 μg/mouse) of an anti-mouse granulocyte mAb as follows: (i) anti-murine IFN α/β, 3 h before, 6 and 12 days after tumor cell injection; (ii) anti-mouse granulocyte mAb, 1 day before, 3 and 7 days after tumor cell injection.

The in vivo HIV infection of U937 SCID mice was performed using three different approaches: (i) simultaneous s.c. injection of 2×10^6 uninfected U937 cells and 2×10^4 in vitro HIV-1 infected normal human PBMC; (ii) simultaneous s.c. injection of 2×10^6 uninfected U937 cells with 10^6 $TCID_{50}$ of cell-free HIV-1 IIIB; (iii) peritumoral injection of established (20 days post implantation) uninfected. U937 cells tumors with 10^6 TCID50 of cell-free HIV-1 IIIB. In all conditions, the HIV-infected chimeras were sacrificed when the tumors reached 20–25 mm mean diameter and analyzed for the virus replication at the tumor site and p24 antigenemia.

2.3. Detection of viral infection

At sacrifice, the U937-cell tumors were excised, carefully minced and gently stirred for 30 min at 37°C in trypsin-EDTA solution (ICN Pharmaceutical, Amsterdam, Netherlands) to obtain cell suspensions. Cell suspensions underwent: (i) HIV-1-DNA PCR as previously described (Folks et al., 1986; Locardi et al., 1992); (ii) HIV-1-RT-PCR (Santini et al., 1995), using specific primers for HIV RNA, as described elsewhere (Zack et al., 1990); (iii) immunocytochemistry for the expression of HIV-p55/p18 (Cordell et al., 1984; Fais et al., 1984); (iv) cocultivation with C8166 cells for syncytium formation.

Sera of infected animals were tested for HIV p24 antigen by an antigen capture enzyme-linked immunosorbent assay (Dupont, B-1130 Brussels, Belgium), HIV-1 RNA copies by Nucleid Acid Sequence-Based Amplification assay (NASBA HIV-1 RNA QT kit, Organon Teknika, Turnhout, Belgium) and also for syncytium formation in C8166 cell cultures.

3. Results

3.1. Definition of the optimal conditions for the growth of 'in vivo' infected U937 cell-tumors producing HIV in SCID mice

In a first set of experiments, we determined the optimal experimental conditions for obtaining a highly reproducible growth of human U937 cells after s.c. transplantation into SCID mice. In particular, in order to decrease the residual mouse resistance to human cell transplantation, we used two approaches: (i) neutralization of endogenous mouse IFN-α/β by the i.p. injection of a potent polyclonal antibody (Gresser et al., 1976; Puddu et al., 1991); (ii) neutralization and depletion of mouse granulocytes (which actively participate in the early phases of the SCID mouse reaction to the engraftment of human cells (Santini et al., 1995) by the i.p. inoculation of a specific mAb. As shown in Fig. 1 (panel A), U937 cell tumors appeared earlier and grew more rapidly in SCID mice injected with either of the two antibody preparations as compared to the control mice. When in vivo HIV-1 infection of U937 cells was performed, the tumors grew somewhat slower than the uninfected controls. Here, a progressive tumor growth was consistently observed only in mice treated with either anti-IFN antibody or anti-granulocyte mAb (Fig. 1B). All the HIV-infected U937-SCID mice exhibited detectable serum levels of p24, though HIV antigenemia was directly related to the tumor growth (Fig. 1, panel C). No major differences were observed in the kinetics of tumor growth and p24 antigenemia between mice treated with antibody to IFN or with anti-granulocyte mAb. Thus, in the subsequent experiments, mice were treated with anti-granulocyte antibodies in order to obtain a highly reproducible tumor growth and p24 antigenemia.

3.2. Different approaches for HIV-1 infection of U937-SCID mice

We used three different approaches of infection: (i) in vitro HIV infection of PHA-stimulated human PBMC and subsequent s.c. co-injection with U937 cells; (ii) simultaneous s.c. injection of cell-

free virus and U937 cells; and (iii) peritumoral HIV-1 injection in mice with established U937 cell tumors (≈ 20 days after the s.c. tumor cell transplantation). The HIV-1 infection of the U937-SCID mice was evaluated by measuring: (i) the number of proviral copies in the infected tumors, by DNA-PCR; (ii) the levels of serum p24 antigenemia; and (iii) the percentage of tumor cells positive for the anti-HIV I p55/p18 staining, by immunocytochemistry. The results showed that all these infection protocols gave a high number of proviral copies in the tumor, as well as very high levels of p24 antigenemia (Table 1). These data were confirmed by immunocytochemical analysis of tumor cells (90–98% of tumor cells were positive for HIV-p55/p18) (Fig. 2). Moreover, the infectivity of circulating virus was shown by both determination of serum HIV-1 RNA copy number (Table 1) and cultivation of either tumor cells and sera with the C8166 cell line (data not shown). Notably, both HIV-1 infection at the tumor level and viremia were detectable up to 3 months post-infection, which corresponds to the mean survival time of U937-SCID mice (data not shown).

DNA-PCR analysis for HLA-DQ and HIV-1 sequences showed that neither human nor HIV-1 DNA were detectable in the blood of the U937-SCID mice, while low but fairly detectable human and viral DNA sequences were present in the lung, brain and lymph nodes of the xenochimeras (data not shown).

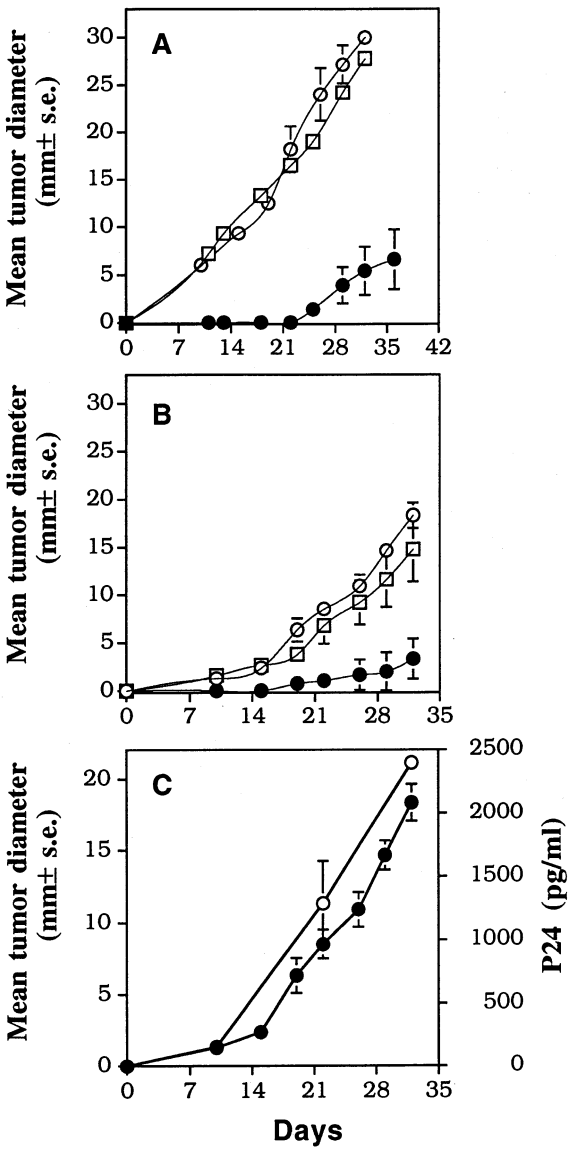

Fig. 1.

Fig. 1. Growth of uninfected and in vivo HIV-infected U937-cell tumors, and analysis of the relationship between tumor growth and HIV p24 antigenemia in SCID mice. (A) Effect of antibodies to murine IFN α/β (purified polyclonal sheep anti-murine-lFN-α/β with a neutralizing titer of 6.4×10^6 against 4–8 U of murine-IFN-α/β (Gresser et al., 1976) and to murine granulocytes (monoclonal anti-mouse granulocyte antibodies, clone RB6-8C5 (Ferrantini et al., 1994) on tumor growth in SCID mice transplanted s.c. with uninfected U937 cells. (●) Untreated U937 tumors; (○) U937 tumors treated with antibodies to murine IFN-α/β (□) U937 tumors treated with antibodies to murine granulocytes. (B) Effect of antibodies to murine IFN-α/β (○) and to murine granulocytes (□), as compared to untreated controls (●), on tumor growth in SCID mice transplanted s.c. with uninfected U937 cells and infected in vivo with HIV-1. (C) Relationship between tumor growth (●) and p24 antigenemia (○) in SCID mice treated with antibodies to mouse granulocytes, transplanted s.c. with uninfected U937 cells and infected s.c. with HIV. Data are mean of six mice per each group $\pm$ S.E.M. (where not shown, error bars fall within the symbol height).

Table 1
Effectiveness of different modalities of HIV-1 infection of U937 cell-tumors implanted s.c. in SCID mice

Days after U937 cell injection:	Day 0		Day 20
Type of HIV infection:	HIV infected PBMC	Cell-free virus	Cell-free virus
Proviral copy number/2×10^5 tumor cells	$>10^4$	$>10^4$	$>10^4$
P24 serum levels (pg/ml)	1290 ± 332	843 ± 187	1040 ± 236
Serum HIV-1 RNA (copies/ml)	N.D.	$35.8\times10^6\pm9.8\times10^6$	N.D.

The in vivo HIV-1 infection of U937 SCID mice was performed using three different approaches: (i) simultaneous s.c. injection of 2×10^6 uninfected U937 cells and 2×10^4 in vitro HIV-1 infected normal human PBMC, prestimulated with phytohemoagglutinin (PHA) 2.5 mg/ml and 5 U/ml of recombinant human interleukin-2 (IL-2); (ii) simultaneous s.c. injection of 2×10^6 uninfected U937 cells with 10^6 $TCID_{50}$ of cell-free HIV-1(IIIB) strain; (iii) injection of established (20 days post implantation) uninfected U937 cells tumors with 10^6 $TCID_{50}$ of cell-free HIV-1(IIIB) strain. In all conditions the UC937-HIV-SCID mouse chimeras were sacrificed when the tumors reached 20–25 mm mean diameter (2–3 months post-infection) and analyzed for the in vivo HIV-1 infection of U937 tumors, for p24 antigenemia end serum HIV-1 RNA.
Results derived from one representative experiment (mean $\pm$ S.E.M. are calculated on five mice/group). In all conditions, cultivation of both tumor cells and sera with C8166 cells resulted in syncytium formation. N.D., not done.

3.3. Effect of AZT treatment on in vivo HIV infection of U937-SCID mice

To assess the effectiveness of typical antiviral treatment on HIV-1 infection in this model, SCID mice were treated i.p. with AZT for 2 days before HIV injection and daily thereafter for 2 weeks. This set of experiments was performed by co-injecting s.c. uninfected U937 cells and HIV-1. AZT treatment dramatically reduced the p24 antigenemia and the serum levels of HIV-1 RNA copies, while, in some animals, few HIV-1 infected cells persisted at the tumor level (Table 2). However, in the majority of the U937-SCID mice, AZT entirely suppressed HIV infection at the tumor level, as shown by both HIV-DNA (Fig. 3A) and HIV-RNA analysis (Fig. 3B). The AZT antiviral effect was not due to toxicity to the implanted target cells, since the tumor size in the AZT treated mice was always greater than that of the untreated animals (data not shown). Furthermore, AZT treatment, extended over 2 weeks, did not show any toxic effect on both SCID mice and U937 tumors (data not shown).

4. Discussion

It is generally accepted that any substance shown to exhibit anti-HIV activity using in vitro cell systems, before its possible clinical use, has to be tested in an animal model, in which drug bioavailability, metabolism, clearance, toxicity and antiviral effectiveness can be taken into consideration.

In this study, we describe a new xenochimeric model in SCID mice, in which multiple cycles of HIV-1 infection can occur in vivo and high virus levels are continuously produced over a prolonged period of time. This model exhibits some properties (low costs, reproducibility, high levels of viremia) which render it particularly suitable for testing new anti-HIV therapies (Namikawa et al., 1988; Mosier et al., 1993; Alder et al., 1995; Hesselton et al., 1995; Sato et al., 1995).

It is well known that the reproducibility of the human cell engraftment might be impaired by the natural residual reactivity of SCID mice against xenotransplanted cells (Murphy et al., 1987), but treatment with anti-mouse asialo GM-1 antibodies greatly improves the engraftment of both normal and tumor human cells (Shpitz et al., 1994; Lacerda et al., 1996). Little information is available, however, on the role of other host reactive components which may significantly affect the engraftment of human cells in SCID mice. We previously described a model in which nude mice were only successfully transplanted with chronically HIV-1-infected U937 cells by repeated injections of antibodies to mouse IFN α/β (Puddu et

al., 1991). Moreover, we have recently shown that SCID mice react to human cell engraftment with an acute inflammatory response characterized by an impressive neutrophil recruitment and mouse cytokine production (Santini et al., 1995). Thus, the first step of our study was to define the optimal conditions for obtaining a continuous growth and infection of human target cells in the SCID mouse host by neutralizing endogenous type I IFN and mouse granulocyte functions.

We found that injections of either antibodies to mouse IFN α/β or an anti-mouse-granulocyte mAb were essential to obtain high levels of p24 antigenemia, HIV production and the persistence/growth of virus-infected cells. These results indicate that a strong natural reactivity, partially mediated by endogenous type I IFN and host granulocytes, can strongly affect U937 cell tumor growth, HIV-1 production and persistence of virus-infected cells in SCID mice. The effects of the anti-granulocyte and the anti-type I IFN antibodies may be related to either a direct inhibition of SCID mouse granulocyte recruitment (Santini et al., 1995) or to the inhibition of type I IFN endogenous production (conferring to mouse NK cells or macrophages some antitumor activity), as previously discussed elsewhere (Puddu et al., 1991). Moreover, our results suggest that antibodies to type I IFN or to mouse granulocytes can be useful for increasing human cell engraftment in the existing SCID mouse models.

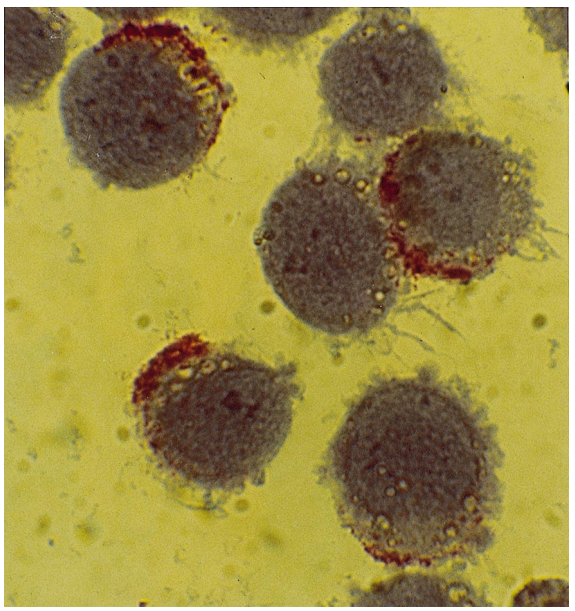

Fig. 2. Staining for HIV-1 p55/p18 matrix protein in U937 cells obtained from tumors grown and infected with HIV in SCID mice. Poly-L-lysine coated chamber-slide preparation of U937 cells obtained by disaggregation of tumors grown and infected with HIV-1 in SCID mice. (Immunocytochemistry, anti-p55/p18-alkaline phosphatase anti-alkaline phosphatase (APAAP) complex; final magnification, $\times 1000$). Cell suspensions from the tumors were spun onto glass slides (Shandon, Cheshire, UK) or attached to L-poly-lysine-covered glass chamber slides (Labtek Naperville, IL) (Fais et al., 1984) and stained by immunocytochemistry for the expression of HIV-p55/p18 (anti HIV-p55/p18 IgG1, clone 11H9, from Medical Research Council AIDS Reagent Project, London, UK), using the APAAP method (Cordell et al., 1984), as described (Fais et al., 1984).

The human SCID mouse xenochimeric model described in our study may represent an improved approach for the preclinical testing of some anti-HIV treatments. In fact, in the current xenochimeric models (Namikawa et al., 1988; Mosier et al., 1991; Alder et al., 1995; Hesselton et al., 1995; Sato et al., 1995) the extent and duration of virus infection and production is limited and HIV-1 viremia has only occasionally been described. In the present model, substantial levels of p24 antigenemia (up to 4000 pg/ml) and HIV-1 RNA copies (up to 50×10^6 ml), as well as a consistent number of HIV-1-infected cells at the tumor level (90–98%), can be detected in the HIV-1-infected xenochimeras and both HIV-1 infection and viremia can persist up to 3 months post infection. In our previously described xenochimeric model of nude mice transplanted with chronically HIV-1-infected U937 cells (Puddu et al., 1991), treatment with AZT did not affect the levels of p24 antigenemia, probably reflecting the inability of AZT to affect HIV production in chronically infected cell systems (Poli et al., 1989). Conversely, in the present model of acute in vivo HIV-1 infection, i.p. injection of SCID mice with AZT resulted in a potent systemic antiviral effect, virtually abolishing p24 antigenemia and greatly reducing the number of HIV-1-infected cells at the tumor level (about 70% reduction). This impressive antiviral effect was not the result of toxicity towards the implanted

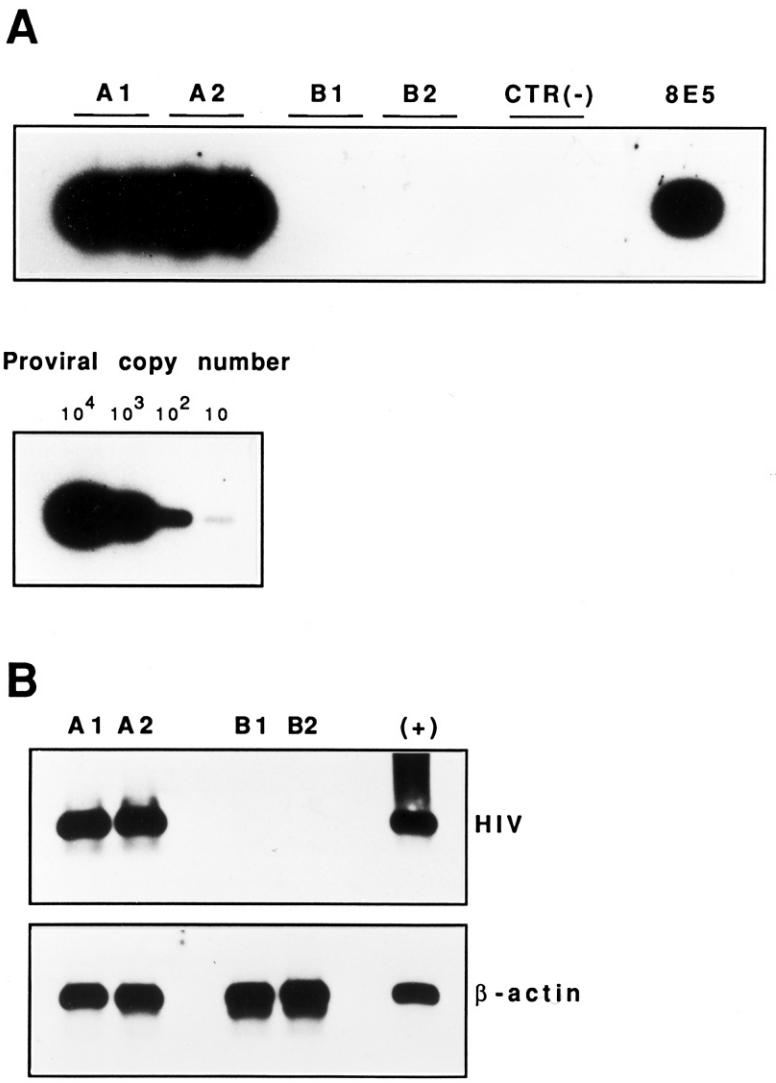

Fig. 3. Proviral copies and HIV-RNA at the tumor level in SCID mice transplanted s.c. with U937 cells, infected in vivo with HIV and treated or not with AZT. Treated and control-untreated HIV-1-infected animals were sacrificed at 2 weeks and tumors analyzed for in vivo HIV-1 infection. At sacrifice, the U937-cell tumors were excised, carefully minced and gently stirred for 30 min at 37°C in trypsin-EDTA solution to obtain cell suspensions. Cells were counted and 2×10^5 and 10^6 cells underwent both DNA and RNA extraction, respectively. (A) DNA extracted by standard procedures and amplified for gag specific sequences (Locardi et al., 1992), from U937-cell tumors transplanted s.c. in SCID mice and infected with HIV-1 in vivo, untreated (A1, A2) and treated i.p. with AZT (B1, B2). AZT (Sigma-Aldrich, Milwaukee, WI) was dissolved in pre-warmed (40°C) PBS at a concentration of 9 mg/ml and filter sterilized. Mice were injected daily i.p. with 1 ml of a freshly prepared AZT solution (480 mg/kg per day [maximal tolerated dose]). Data represent duplicate experiments in four different animals. Sensitivity of the assay was tested by amplifying DNA, prepared from 8E5 T-cells (Folks et al., 1986), which was serially diluted into SCID mouse cell DNA. The proviral copy number after amplification is indicated; (B) RT-PCR analysis of HIV-1 and human β-actin mRNAs in U937 cells obtained from the same animals analyzed for proviral copies in (A). Total RNA was isolated by RNAzol B (Biotech, Houston, TX) and then subjected to phenol/chloroform extraction and isopropanol precipitation. RNA was purified and quantified and 1 μg of total RNA underwent RT-PCR as described (Santini et al., 1995). Specific primers for HIV RNA has been previously described (Zack et al., 1990). A1 and A2 are SCID mice transplanted s.c. with U937 cells infected with HIV-1 and untreated with AZT, B1 and B2 are treated i.p. with AZT.

Table 2
Effectiveness of AZT treatment on HIV-1 infection of U937 cell-tumors implanted s.c. in SCID mice

	Treatments	
	HIV/PBS	HIV/AZT
Proviral copy number/2×10^5 tumor cells[a]	$>10^4$	$3.5 \times 10^3 \pm 1 \times 10^3$
HIV/p55/p18 +ve cells[b] (% and range)	95 (98–92)	10 (7–13)
P24 serum levels (pg/ml)	913 ± 162	Under detection limits
Serum HIV-1 RNA (copies/ml)	$43.2 \times 10^6 \pm 9.3 \times 10^6$	$<4 \times 10^2$

AZT (Sigma-Aldrich) was dissolved in prewarmed (40°C) PBS at a concentration of 9 mg/ml and filter-sterilized. Mice were injected daily i.p. with 1 ml of a freshly prepared AZT solution (480 mg/kg per day [maximal tolerated dose]) or with PBS for 2 weeks. Treated and control-untreated HIV-1-infected animals were sacrificed at 2 weeks and analyzed for the in vivo HIV-1 infection of U937 tumors, p24 antigenemia and serum HIV-1 RNA. Results are derived from two separate experiments.
Mean $\pm$ S.E.M. are calculated on four mice/group.
[a]DNA was extracted by standard procedures and amplified for gag specific sequences (Locardi et al., 1992). Sensitivity of the assay was tested by amplifying DNA, prepared from 8E5 T-cells (Folks et al., 1986), which was serially diluted into SCID mouse cell DNA. The proviral copy number before amplification is indicated.
[b]Cell suspensions from the tumors were spun onto glass slides (Shandon, Cheshire, UK) or attached to L-poly-lysine-covered glass chamber slides (Labtek Naperville) (Fais et al., 1984) and stained by immunocytochemistry for the expression of HIV-p55/p18 using the APAAP method (Cordell et al., 1984) as previously described (Fais et al., 1984).

target cells since the AZT-treated mice always developed greater tumors than untreated mice. In this model, correlation between the levels of viremia and the number of HIV-1-infected cells can be easily performed. For example, it is of interest to underline that the AZT treatment did not completely eliminate HIV-1-infected cells at the tumor level, even though it totally abolished HIV-1 viremia. Thus, this model may offer a useful tool for shedding some light on issues concerning virus load viremia and blood HIV-1 clearance in treated patients, in view of the fact that a major target in therapy should be the source of the virus (i.e. the virus infected cells) and not only its product (Levy et al., 1996). Furthermore, in this model, tumor cells from HIV-infected and antiviral drug-treated xenochimeric mice can be serially re-implanted into SCID mice, thus allowing investigation of the effects of a long term (single or combined) antiviral treatment on the selection of HIV-1 drug-resistant mutants. Experiments are now in progress to evaluate the possibility of infecting tumor xenografts of U937 cells and other human cells with different strains of HIV and eventually, clinical isolates of PBMC from AIDS patients. Lastly, in addition to its practical use in the testing of antiviral substances, the present xenochimeric model (or its implemented versions) can be successfully utilized in preclinical studies for immunotherapy approaches of HIV-1 infection, such as passive (Parren et al., 1995) (or active) immunization or adoptive transfer of virus-specific CTL (Van Kuyk et al., 1994).

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