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Protection of hu-PBL-SCID/beige mice from HIV-1 infection by a 6-mer modified oligonucleotide, R-95288

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Abstract

We analyzed the anti-HIV-1 activity of an oligonucleotide derivative, R-95288, in severe combined immunodeficient (SCID/beige) mice transplanted with normal human peripheral blood leukocytes (PBLs), designated hu-PBL-SCID/beige mice. The human chimeric mice were inoculated with HIV-1_{CC1} 3 weeks after the transplantation and sacrificed 2 weeks later. Virus infection was determined by coculture of splenocytes with fresh human PBLs and also by detection of HIV- specific DNA sequences using the polymerase chain reaction. No evidence of infection was observed in mice treated with R-95288 (100 mg/kg/day) using intraperitoneal delivery by osmotic minipumps starting 1 day before virus challenge. In contrast, virus infection was observed in over 80% of the saline-treated control mice. In addition, partial inhibition of HIV-1 infection was obtained in mice treated subcutaneously with R-95288 (100 mg/kg/day). Toxicity towards the engrafted human cells was not observed by flow cytometric analysis. Moreover, R-95288 failed to inhibit lymphocyte proliferation ($CC_{50} > 400$ μg/ml), while 90% inhibition of HIV-1 replication was achieved at 3.1 μg/ml in vitro. These results suggest the ability of R-95288 to protect the human chimeric mice against HIV-1 infection. © 1997 Elsevier Science B.V.

Keywords: Oligonucleotide; HIV-1; Immunodeficient mouse; SCID

1. Introduction

Previously, we reported that a 15-mer oligonucleotide, which contains a guanine-rich nucleoside sequence, exhibits anti-HIV-1 activity upon cova-

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lent linking at the 5′-end with dimethoxytrityl group (Furukawa et al., 1994). In addition, we found that the essential nucleoside structure of the 5′-end modified oligomer is only a 6-mer nucleotide consisting of TGGGAG nucleosides; the 6-mer oligomer covalently linked to dimethoxytrityl residue at the 5′-end, SA-1080 can inhibit HIV-1 replication at a comparable concentration as the 15-mer oligonucleotide (Furukawa et al. accepted). On the basis of these studies, the oligonucleotide derivative R-95288, which contains the same 6-mer nucleotide coupled with dibenzyloxybenzyl and an hydroxyethyl residues at the 5′- and 3′-ends, respectively, has been established as a potent anti-HIV-1 compound in vitro with a favorable toxicity, stability and pharmacokinetic profile in vivo (Fig. 1) (Koga et al., 1996). Although some oligonucleotide compounds have been reported to inhibit the replication of HIV-1 by an antisense mechanism and/or by inhibition of viral reverse transcriptase activity (Matsukura et al., 1989; Majumdar et al., 1989; Stein et al., 1991; Ojwang et al., 1994), the R-95288 oligonucleotide appears to neutralize HIV-1 infection through interference with the interaction of the virus envelope glycoprotein, gp120, with the virus receptor(s) on the CD4-positive host cells (Agatsuma et al., 1997 submitted). Moreover, it has been recently reported that R-95288 can form a parallel tetrameric structure and the tetramer formation seems to be required for anti-HIV-1 activity (Watanabe et al., 1996). Because of the unique mechanism of action of this oligonucleotide derivative, we felt it was quite meaningful to analyze the anti-HIV-1 activity of R-95288 in vivo.

The use of severe combined immunodeficient (SCID) mice implanted with human peripheral blood leukocytes (PBLs), designated hu-PBL-SCID mice, has been used as an animal model for investigating the pathogenesis of HIV infection (Mosier et al., 1988, 1991; Torbett et al., 1991). The hu-PBL-SCID model has been validated by the observation that implantation of PBL derived from HIV-1 gp120 vaccinated donors (Mosier et al., 1993), and passive transfer of HIV-1 neutralizing antibody (Safrit et al., 1993; Gauduin et al., 1995; Parren et al., 1995) or cytotoxic T cells (Kuyk et al., 1994) can protect hu-PBL-SCID mice against HIV-1 infection. Moreover, inhibitors of HIV reverse transcriptase and protease have been shown to inhibit HIV-1 infection in this model (Ussery et al., 1995, 1996; Mosier and Sieberg, 1995; Bridges et al., 1996).

In the present study, we analyzed the anti-HIV-1 activity of R-95288 using the immunodeficient mice with the genotype *scid/scid.bg/bg* (Croy and Chapeau, 1990) as an animal model for HIV-1 infection. We then present data that demonstrate the ability of R-95288 to inhibit HIV-1 infection in vivo.

2. Materials and methods

2.1. PBLs and virus

Human PBLs were isolated from buffy coats (North London Blood Transfusion Centre, UK) by centrifugation with lymphoprep (Nycom) and a CD8+ cell pellet was depleted with anti-CD8 antibody-coupled magnetic beads (M450, Dynal) according to manufacturer's instruction. The cells were stimulated with 10 μg/ml of PHA and 10 U/ml of interleukin 2 (IL2) for 3 days in RPMI 1640 medium (Gibco BRL) supplemented with 10% fetal calf serum (Gibco BRL), 1 mM of glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin at 37°C in 5% CO_2. The HIV-1 clinical isolate, HIV-1_{CC1} (syncytium-inducing type) was kindly donated by Dr. D.L. Taylor of the MRC Collaborative Centre, London, UK. Cell-free virus was harvested on day 3 or 4 from the supernatant of HIV-1-infected PBL cultures supplemented with 10 U/ml of IL2. Virus stocks were titrated on uninfected PBLs for 7 days and the infectious dose of each virus stock was expressed as the 50% tissue culture infectious dose ($TCID_{50}$/ml).

2.2. Anti-HIV-1 activity in vitro

The antiviral activity of R-95288 against HIV-1_{CC1} was assessed either against acute infection of uninfected PBLs with the cell-free virus, or against virus spread from infected cells with virus-

infected PBLs. PHA (10 μg/ml)- and IL2 (10 U/ml)-stimulated PBLs (2×10^5/well) were either infected with 400 $TCID_{50}$/ml of the virus stock or mixed with virus-infected PBLs at 10:1 ratio and distributed into duplicate well of 96-well plates (25 860, Corning) containing serial 2-fold dilutions of R-95288 in RPMI1640 with 10 U/ml of IL2. After being cultured for 7 days, cell-free supernatants were collected and the anti-HIV-1 activity was determined by measuring levels of HIV-1 p24 core antigen using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Coulter).

2.3. Lymphocyte proliferation assay

Freshly isolated PBLs were stimulated with 2.5 μg/ml of Concanavalin A (ConA) (Sigma, UK) in the presence of different concentrations of R-95288. Lymphocyte proliferation was determined by counting the viable cell number under a microscope after staining with trypan blue (Sigma, UK) to distinguish dead cells.

2.4. Pharmacokinetic studies

DDY mice (male, 8-week old) were implanted intraperitoneally with osmotic minipumps, model 2002 (ALZA), containing 200 mg/ml of R-95288 in saline in order to deliver R-95288 into the recipient mice at the rate of 100 mg/kg/day. The mice were sacrificed and blood was obtained from the transverse cervical artery of the mice on either days 1 ($n = 2$), 2 ($n = 2$), 7 ($n = 2$) or 14 ($n = 3$) after implantation and the plasma concentration was determined by HPLC using an internal control method.

2.5. SCID/beige mice reconstitution with PBLs

SCID/beige (*scid/scid.bg/bg*) mice used in this study were purchased from the Centre for Applied Microbiological Research (CAMR), UK and maintained under specific pathogen-free conditions (in a containment 2 laboratory) at the National Institute for Medical Research (NIMR), UK. Non-leaky phenotype mice (8–20 weeks old) were implanted by intraperitoneal injection with 2×10^7 viable PBLs in 0.5 ml of phosphate-buffered saline (PBS). The PBLs had been stimulated with PHA (10 μg/ml) and IL2 (10 U/ml) in RPMI 1640 medium for 18–24 h and then washed three times with PBS. At 2 weeks after the PBL injection, reconstitution was confirmed by analysis of the mouse sera for the presence of human immunoglobulin G (IgG) using a sandwich ELISA. The capture antibody was goat anti-human polyvalent Ig (Sigma), and the detection antibody was goat anti-human IgG peroxidase conjugate (Dako). Only human IgG positive mice which expressed over 800 μg/ml of human IgG 2 weeks after reconstitution were used for HIV-1 infection. About 70% mice in total were human IgG positive in this system.

2.6. R-95288 delivery and virus challenge of hu-PBL-SCID/beige

All procedures for infection and maintenance of the infected hu-PBL-SCID/beige mice were performed in a containment 3 facility in NIMR under supervision of the Virology Group of the MRC Collaborative Centre, UK. The hu-PBL-SCID/beige mice were inoculated intraperitoneally with 2 000 $TCID_{50}$ of HIV-1_{CC1} 3 weeks after PBL reconstitution. For the protection experiment, osmotic minipumps filled with R-95288 (200 mg/ml) or saline control were implanted subcutaneously into anaesthetized mice, 24 h prior to virus inoculation, and arranged to deliver R-95288 into the peritoneal cavity or tela subcutanea.

2.7. Detection of HIV-1 by coculture

At 2 weeks after virus challenge, the mice were sacrificed and cells were recovered from the spleens. Serial 10-fold dilutions of the cells were

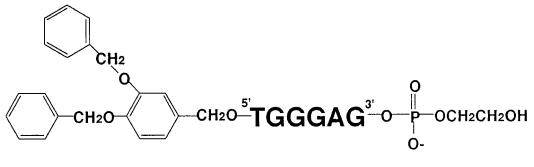

Fig. 1. Structure of the anti-HIV-1 oligonucleotide R-95288.

made in 96 well round-bottomed plates (3077, Falcon) and 5×10^5/well of PHA (10 μg/ml) and IL2 (10 U/ml)-stimulated PBLs were added. Cell free supernatant was collected after 7 days incubation and analyzed for the presence of HIV-1 p24 antigen using an HIV p24 ELISA kit (Coulter, UK).

2.8. Detection of human cells by flow cytometry after HIV-1 infection

Splenocytes (2×10^6) from hu-PBL-SCID/beige mice were stained with a FITC-conjugated anti-HLe-1(CD45) monoclonal antibody (mAb) (Becton–Dickinson, UK) or an isotype control for 1 h at 4°C and then red blood cells were eliminated by FACS lysing solution (Becton–Dickinson, UK). After washing with PBS containing 0.1% bovine serum albumin, the stained cells were fixed with 4% formalin-PBS overnight at 4°C and analyzed by FACScan (Becton–Dickinson, UK). Results were expressed as percentage of CD45+ cells.

2.9. Analysis of HIV-1 and human tissue specific sequence by polymerase chain reaction

DNA was extracted from 2×10^7 splenocytes of hu-PBL-SCID/beige mice using QIAmp Blood Kit (QIAGEN, UK) and amplified using the polymerase chain reaction (PCR) in an automated thermal cycler (LEP scientific) using two primer pairs for detection of HIV-1 *gag* (SK38 and SK39) and HLA-DQ-α (GH26 and GH27) (Lee et al., 1991), under the following reaction conditions: 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 3 min. The amplified product (10 μl) was then electrophoresed through a 2% agarose gel and transferred onto nylon membrane Hybond-N+ (Amersham). The blot was hybridized with the biotinylated oligonucleotides SK19 and GH38 for detection of both the HIV-1 *gag* and HLA-DQ-α gene (Lee et al., 1991) at 65°C. Hybridized probes were detected by treatment with streptavidin–biotinylated horseradish peroxidase complex (Amersham) and ECL detection reagents, and then autoradiographed with Hyperfilm-ECL (Amersham, UK). DNA from the HIV-1 infected cell line 8E5/LAV (MRC AIDS Reagent Project, UK) was used as a standard after amplification under the same conditions since this cell line contains one copy of proviral HIV-1 DNA per cell. Signals were quantified with a densitometer (ULTROSCAN XL Enhanced Laser Densitometer; LKB-Produkter AB, Sweden) and HIV copy No./human cell was calculated using the following formula: HIV copy No./human cell = (HIV signal of a sample/HLA signal of the same sample)/(HIV signal of 8E5/LAV cells/HLA signal of 8E5/LAV cells.).

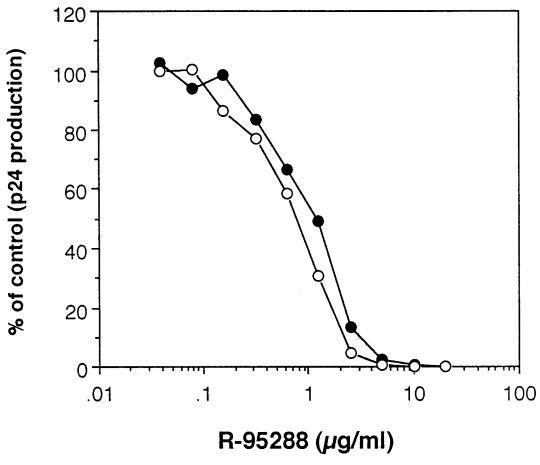

Fig. 2. Antiviral activity of R-95288 against HIV-1_{CC1} in vitro. PHA- and IL2-stimulated PBLs were infected with cell-free virus (●) or virus-infected PBLs (○) and then incubated in the presence of different concentrations of R-95288. p24 Virus antigen in the cell-free supernatant after 7 days was monitored by ELISA.

3. Results

3.1. In vitro anti-HIV-1 activity and toxicity of R-95288

To determine the optimal concentration of R-95288 for subsequent animal experiments, we first measured the activity of R-95288 against the replication of HIV-1_{CC1} and its toxicity against lymphocyte proliferation in vitro. R-95288 inhibited infection of PBLs with cell-free virus stock of HIV-1_{CC1} (virus-to-cell infection) at an IC_{50} (50%

inhibitory concentration) of 1.2 μg/ml and an IC_{90} (90% inhibitory concentration) of 3.1 μg/ml (Fig. 2). Similar endpoints were obtained in coculture experiments with HIV-1_{CC1}-infected PBLs and fresh PBLs (cell-to-cell infection), with IC_{50} and IC_{90} values of 0.78 and 2.1 μg/ml, respectively (Fig. 2). In the lymphocyte proliferation assay, R-95288 did not affect the cell number of Con A-stimulated PBLs from two different donors at concentrations up to 200 μg/ml. The CC_{50} (50% cytotoxic concentration) values for the two donors were 500 and 800 μg/ml (Fig. 3). Therefore, R-95288 should be effective at concentrations of 3–200 μg/ml against HIV-1_{CC1} with no effect on lymphocyte proliferation.

3.2. Pharmacokinetics of R-95288 delivered by an osmotic minipump

In order to deliver R-95288 efficiently and also to eliminate the risk of infection through an accident, we used osmotic minipumps for the delivery of R-95288 to the mice. The plasma concentration of R-95288, after implantation of a minipump, was measured prior to the HIV-1 challenge experiments. Minipumps filled with 220–250 μl of R-95288 (200 mg/ml) in saline were implanted intraperitoneally into nine male DDY mice and the plasma concentration was monitored on day 1 ($n = 2$), day 2 ($n = 2$), day 7 ($n = 2$) or day 14 ($n = 3$). The plasma concentration plateaued by day 1 and was maintained at a concentration greater than 35 μg/ml until at least day 14 (Fig. 4). The average plasma concentration was 73.1 ± 23.4 μg/ml which produced anti-HIV-1 activity without inhibition of lymphocyte proliferation in vitro.

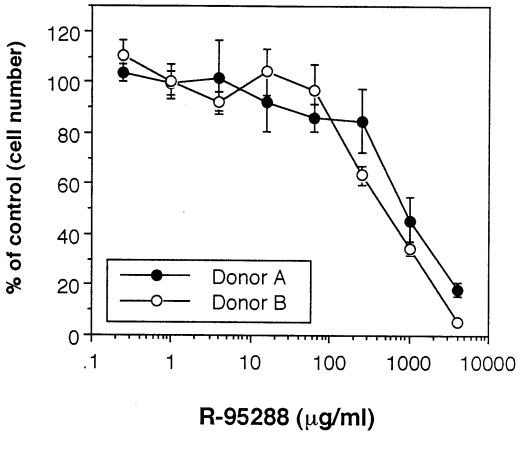

Fig. 3. Effect of R-95288 on human lymphocyte proliferation induced by ConA. Freshly isolated PBLs from two different donors (●, ○) were stimulated with 2.5 μg/ml of ConA and then incubated in the presence of different concentrations of R-95288 for 3 days. Lymphocyte proliferation was determined by counting the viable cell number in each cell culture.

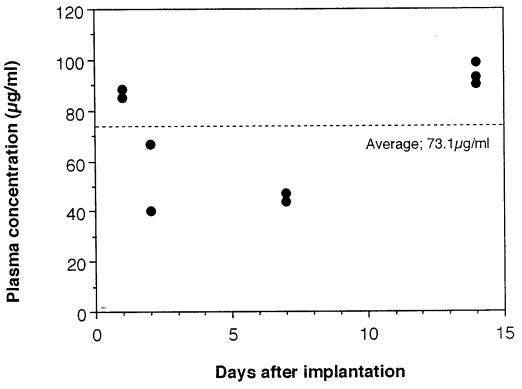

Fig. 4. Mouse plasma concentration of R-95288 delivered intraperitonealy from an osmotic minipump. DDY mice were implanted intraperitoneally with osmotic minipumps containing 200 mg/ml of R-95288 in saline. The blood was obtained from the mice on either days 1, 2, 7 or 14 after implantation and the plasma concentration was determined by HPLC.

3.3. Protection against HIV-1 infection of hu-PBL-SCID/beige mice by R-95288

At 2 weeks after virus challenge, spleen cells were isolated from the mice and analyzed for the presence of infectious virus by coculture with fresh PBLs. HIV-1 was recovered from control groups: 6 out of 7 mice (86%) treated intraperitoneally with saline or 4 out of 5 mice (80%) dosed subcutaneously with saline in control groups (Table 1, Fig. 5). The titers of the isolated virus in these two control groups were not significantly different (Mann–Whitney's U-test). In contrast, no evidence of HIV-1 infection was observed in mice treated intraperitoneally with R-95288 as indicated by the failure to recover HIV-1 from the spleen after coculture (Table 1). The

Table 1
Protection of hu-PBL-SCID/beige mice against HIV-1 infection by R-95288 delivered from osmotic minipumps as determined by virus recovery from spleen cells

Treatment (route[a])	HIV-1 inoculation	Virus recovery	Human cell recovery[b]
Saline (i/p or s/c)	−	0/5[c] (0/4)[d]	4/5
Saline (i/p)	+	6/7 (5/5)	5/5
Saline (s/c)	+	4/5 (4/5)	5/5
R-95288 (i/p)	+	0/6 (0/4)	4/5
R-95288 (s/c)	+	6/10 (6/10)	10/10

[a]i/p, intraperitoneal route; s/c, subcutaneous route.
[b]No. of mice containing over 1% of CD45+ cell in spleen (CD45+ mice)/No. of mice analyzed for CD45 antigen in the spleen. Some samples were not analyzed because of insufficient cell number.
[c]No. of HIV-1 infected mice (infected mice = HIV recovered from spleen by coculture)/No. of tested.
[d]No. of HIV-1 infected mice/No. of CD45+ mice.

virus was only recovered from 6 out of 10 mice when R-95288 was delivered subcutaneously. In addition, the viral titers after coculture of spleen cells in this group were significantly lower than those from the control group (Fig. 5) ($P < 0.05$ for both comparisons; Mann–Whitney's U-test).

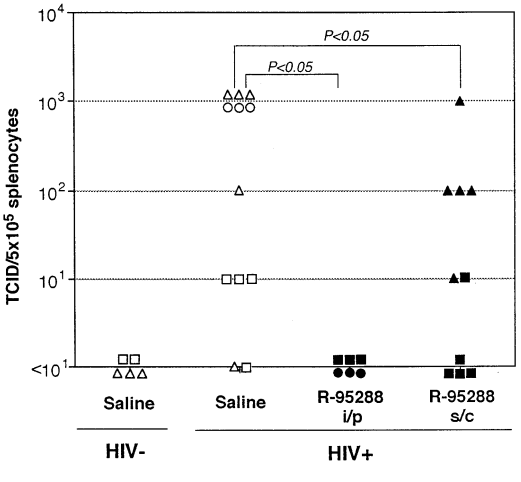

Fig. 5. Titer of infectious HIV-1 recovered from spleen cells of hu-PBL-SCID/beige mice. R-95288 (100 mg/kg/day) (closed) or only solvent (open) was delivered intraperitoneally or subcutaneously into hu-PBL-SCID/beige mice by an osmotic minipump which was implanted 1 day before virus challenge. Titers of infectious HIV-1 recovered from spleen cells at sacrifice 2 weeks later from three different experiments (circle, triangle or square) are indicated on the vertical axis. At titer of less than 10 $TCID_{50}/5 \times 10^5$ splenocytes was considered negative. Statistical comparison was done by the Mann–Whitney's U-test.

We also investigated human CD45 antigen expression on the spleen cells from the mice at the end of the experiments, in order to assess whether R-95288 might affect the engrafted human PBLs. Although the percentage of human CD45+ cells in each sample varied, HIV-1 was consistently recovered from mice with greater than 1% human CD45+ cells in the spleen (data not shown). Therefore, mice with more than 1% human CD45+ cells present by flow cytometry were considered capable of supporting HIV-1 infection. On this basis, more than 80% of mice from each group were assumed to contain enough CD45+ cells to become infected in this experiment (Table 1 and Table 2). Moreover, treatment with R-95288, given intraperitoneally or subcutaneously, did not markedly decrease the percentage of CD45+ cells (Table 2). These results suggest that R-95288 did not affect the reconstitution of mice with PBLs but did inhibit HIV-1 infection.

PCR amplification of 0.1 μg of DNA from the spleen cells of hu-PBL-SCID/beige mice was performed to detect HIV-1 proviral DNA as well as the human leukocyte marker HLA-DQ-α. This was done to calculate the HIV copy number per cell. Spleens from the mice in the control groups had 0.64 ± 0.24 copies of HIV/human cell, while a background level of HIV (< 0.28 HIV copy/human cell) was obtained for the group intraperitoneally treated with R-95288 (Fig. 6). Although a high level of HIV DNA, comparable to that in the control groups, was obtained for three out of ten

Table 2
Recovery of reconstituted human leukocytes from spleens of hu-PBL-SCID/beige mice at the end of experiment

Treatment	HIV-1 inoculation	No. of mice (% in a group)		
		CD45+cell (%)[a]		
		0–1	*1–20*	*20–100*
Saline	−	1 (20)[b]	2 (40)	2 (40)
Saline	+	0 (0)	7 (78)	2 (22)
R-95288 (i/p)	+	1 (20)	3 (60)	1 (20)
R-95288 (s/c)	+	1 (11)	5 (56)	3 (33)

[a]The percentage of human leukocytes in the spleen was determined by FITC-conjugated anti-CD45 mAb using flowcytometry and over 1% of CD45+ human leukocytes was considered to indicate the potential to become infected with HIV-1 in this study. Some samples were not analyzed because of insufficient cell number.
[b]The percentage of the applicable mice in the same group is indicated in parentheses.

spleen samples from the mice treated subcutaneously with R-95288, the copy number of HIV DNA/human cell in the remainder was only at the background level or significantly less than in the control groups. The difference in the copy number between the control groups and the groups treated with R-95288 intraperitoneally or subcutaneously was also assessed statistically ($P < 0.05$; student t-test) (Fig. 6).

4. Discussion

A number of anti-HIV agents (e.g. AZT) have been reported to be effective against viruses (other than HIV) that can infect different hosts (Tsai et al., 1993). In these cases, the information on the efficacy of the anti-HIV agents in vivo could be obtained using a different virus/host model system. These model systems, however, may provide misunderstandings of the antiviral activity against HIV because of the different specificities of the test compounds for each virus. This concept might not apply to other potent antiviral agents specific for HIV: for example, antisense oligonucleotides, HIV-1 protease inhibitors, virus-cell entry inhibitors, etc. Therefore, an animal model where HIV itself can replicate is quite important and desired.

In this study, we constructed the immunodeficient mouse model for HIV infection using SCID mice with scid/beige phenotype engrafted with human PBL in order to demonstrate that R-95288 can inhibit HIV-1 infection in this model. In this case, inoculation of HIV-1 led to a detectable level of replicative virus and HIV DNA loads in the spleen. Virus replication, however, was completely suppressed in mice treated intraperitoneally with R-95288 (100 mg/kg/day) since no virus could be recovered. This data is similar to the previous observation that hu-PBL-SCID mice can be completely protected through the passive transfer of an anti-HIV-1 mAb into the peritoneal cavity of the mice (Safrit et al., 1993; Parren et al., 1995). We also found that complete protection against HIV-1 infection was apparent in some mice treated subcutaneously with R-95288 at the same dose, but HIV-1 infection was evident in others and, as such, antiviral activity was only partial with this route of delivery. Inhibitors of HIV reverse transcriptase and protease, clinically used in AIDS patients, do not appear to produce complete inhibition of HIV infection either, but only produce a reduction in the frequency of virus recovery and a decrease in titers of replicative virus in the hu-PBL-SCID or hu-PBL-SCID/beige mice when given orally or subcutaneously (Ussery et al., 1995; Bridges et al., 1996). While the reason for the lack of complete inhibition of HIV-1 infection via a non-intraperitoneal route has not been elucidated, insufficient blood concentration of each compound is a likely explanation. Alternatively, there may be an unusual tissue distribution of human lymphocytes and HIV in the mice.

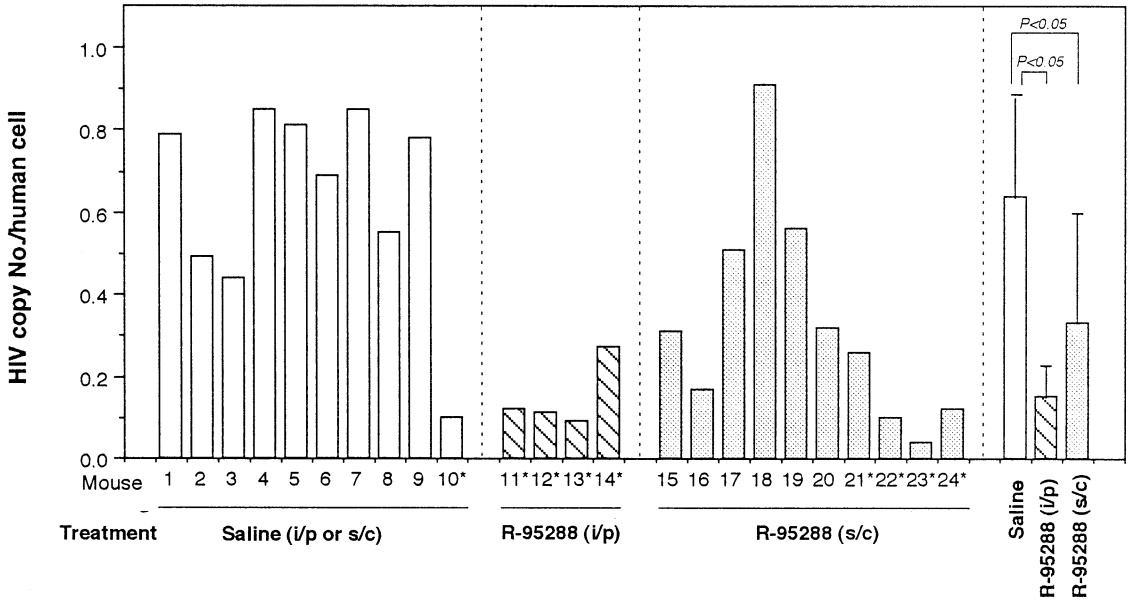

Fig. 6. Reduction of HIV-1 DNA in spleen cells of hu-PBL-SCID/beige mice by R-95288 as determined by PCR. PCR-amplified products of HIV-1 *gag* and HLA-DQ-α from 0.1 μg of spleen DNA of hu-PBL-SCID/beige mice were detected by hybridization with labeled oligonucleotide probes after agarose gel electrophoresis and transfer to a nylon membrane. The intensity of the signal was quantified by densitometry and the copy number of HIV DNA per human cell was calculated as described in Materials and methods. The mice from which HIV-1 was not recovered by coculture are indicated by an asterisk. open bar, saline treated (control) mice; hatched bar, mice treated intraperitoneally with R-95288; gray bar, mice treated subcutaneously with R-95288. Mean value of each group is shown in the right column and statistical comparison was done by the Student *t*-test.

A test compound delivered by the non-intraperitoneal route might not achieve its optimal concentration in the major tissues where human PBL engrafts and HIV replicates. In our case, preliminary data showed that the peak blood concentration of R-95288 after one shot injection through subcutaneus route is about half of that after intraperitoneal injection. On the basis of this observation, it is likely that the delivery from subcutaneously implanted minipumps could provide a sufficient blood concentration of R-95288 to exhibit anti-HIV-1 activity in mice. Precise pharmacokinetic investigations are required to answer this question and these experiments are now underway.

The effect of a test compound on the human leukocytes engrafted in the immunodeficient mice is considered quite important in the analysis of its efficacy because virus recovery might be adversely affected by the toxic elimination of human leukocytes leading to a misinterpretation of data. In this study, we have demonstrated that the reconstitution with human leukocytes in hu-PBL-SCID/beige mice was not altered by R-95288 treatment at the conclusion of the experiments. This result is consistent with the observation that R-95288 did not inhibit human lymphocyte proliferation at concentrations which produced anti-HIV-1 activity in vitro. Although it has been recently suggested that some oligonucleotide compounds containing G residues often modulate lymphocyte functions (Stein, 1995), it is unlikely that the beneficial effect of R-95288 in the hu-PBL-SCID/beige mouse model was due to a toxic effect on the human graft.

Taken together, the results obtained in this study demonstrate that the short oligonucleotide containing only phosphodiester bonds, which exhibits anti-HIV-1 activity in vitro, can achieve optimal blood concentrations in mice. This also provides the first evidence indicating that a short oligonucleotide can produce antiviral effects against HIV-1 infection in hu-PBL-SCID/beige mice without toxicity to human lymphocytes.

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