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Development and Application of HIV Vectors Pseudotyped with HIV Envelopes

Koichi Miyake and Takashi Shimada
Department of Biochemistry and Molecular Biology, Division of Gene Therapy Research Center for Advanced Medical Technology, Nippon Medical School Japan

1. Introduction

Retroviral vectors derived from the mouse moloney leukemia virus (MLV) are widely used for gene therapy of blood disorders because they are capable of permanently integrating a therapeutic gene into hematopoietic cells and achieving long-term expression. However, a limitation of MLV retroviral vectors is the inability to infect growth-arrested cells. It has been demonstrated that a passage through mitosis of infected cells is required for productive infection of MLV (Lewis & Emerman 1994). The breakdown of the nuclear membrane at mitosis may be necessary for nuclear transport of the oncoretroviral preintegration complex which is too large to enter a nuclear pore in the intact nuclear membrane. Because most of the target cells of gene therapy such as hematopoietic stem cells and lymphocytes are quiescent or rarely dividing, the requirement for cell division limits the use of MLV vectors for therapeutic gene transfer.

For clinical trial of gene therapy, the best-established therapeutic vector is a gamma retroviral vector. This retroviral vector system has successfully been used for gene transfer to hematopoietic cells to treat X-linked severe combined immunodeficiency (X-SCID) and adenosine deaminase deficiency (ADA-SCID). However, in these successful clinical trials, genotoxicity mediated by integrated vector proviruses has led to clonal expansion, and in 5 of 20 of these X-SCID patients have developed T-cell leukemia (Hacein-Bey-Abina et al. 2008; Hacein-Bey-Abina et al. 2003; Howe et al. 2008). Molecular analyses of the leukemia cases showed that these retroviral vectors were integrated into a small number of T-cell proto-oncogenes, especially LMO2, leading to its aberrant and high-level expression caused by the strong enhancer elements located in the U3 region of the MLV long-terminal repeat (LTR). Moreover, the 2 patients with X-linked chronic granulomatous disease (X-CGD) exhibited striking clonal dominance with most of their gene-corrected cells having insertions into or near of the MDS1-EVI1, PRDM16, or SETBP1 loci (Ott et al. 2006). These retroviral vector genotoxicity emphasize that next-generation vectors should further improve the safety and the genotoxicity of retroviral integration is a relevant factor to be considered in designing vectors for future clinical trials.

Human immunodeficiency virus type-1 (HIV-1), an etiological agent of acquired immunodeficiency syndrome (AIDS), is a member of lentivirinae which is a subfamily of retroviruses. We have previously established a packaging system which generates helper

free recombinant HIV vectors (Shimada et al. 1991). Compared with widely used amphotrophic retroviral vectors, HIV vectors have several interesting features. First, since the major receptor for HIV infection is the CD4 molecules of helper T lymphocytes, the HIV vectors are capable of targeted gene transfer into $CD4^+$ cells (Miyake et al. 1996; Shimada et al. 1991). Second, since gene expression from the HIV-LTR is dependent upon the HIV encoded transacting factor TAT (Sodroski et al. 1984), in the presence of TAT, the HIV-LTR functions as a very strong promoter. However, because there is no TAT molecule in target cells, the HIV-LTR of the integrated provirus is inactive. Inactivation of the LTR of the retroviral vector is thought to be important to minimize the chance of insertional activation of cellular proto-oncogenes and promoter interference between the LTR and the internal promoter. Third, unlike oncoretroviruses, HIV and other lentiviruses have the ability to infect and integrate in non-dividing cells. Therefore, HIV based vectors can be used for gene transfer into non-dividing cells (Miyake et al. 1998). This possibility is particularly important, because none of the current vectors can efficiently integrate into chromosome of non-dividing cells.

In this chapter, we describe about HIV vector pseudotyped with HIV envelopes. Replication-incompetent HIV vectors pseudotyped with HIV envelopes (BH10 or SF162) were developed and were shown to be capable of targeted gene transfer into $CD4^+$ T cells and macrophages. This strict T cell tropism is important for the development of gene therapy of AIDS or adult T-cell leukemia (ATL). We also developed an improved strategy for preparation of high titer HIV vectors. Since primary un-stimulated T cells and terminally differentiated macrophages were transduced with high titer HIV vectors efficiently, HIV vectors are useful for the manipulation of gene expression in HIV infectable cells and the development of gene therapy targeting lymphocytes and macrophages. Finally, we present the application of HIV vectors for AIDS and ATL.

2. Production and concentration of HIV vectors

2.1 Plasmid construction

The first generation packaging plasmid (pCGPE) provided all gag and pol sequences, the viral regulatory genes tat and rev and the accessory genes vif, vpr and vpu from T-cell tropic HIV strain (BH10) (Ratner et al. 1987). To improve the safety, the single packaging plasmid (pCGPE) was divided into two plasmids. One is the pCAGgpR, which contains the CAG promoter and HIV gag, pol and RRE; another one is the T-cell tropic HIV env expression vector (penvBH10). We also developed macrophage tropic HIV env expression vector (penvSF162), which contains the macrophage tropic HIV env fragment from SF162 (Collman et al. 1989)(Figure 1A).

The transfer plasmid consists of an expression cassette and the HIV *cis*-acting factors necessary for packaging together with the packaging signal (Ψ). pHXN transfer vector plasmid contains the TK promoter and neomycin resistance gene (neoR). To improve the packaging efficiency and safety, we modified splicing donor (SD) site (TGGT -> TCGC) and initiation codon of gag gene (ATG -> ATC) (pHXNm). Since promoter activity of HIV-LTR is Tat dependent, we also developed Tat independent transfer vector by replacement of 5′ U3 region to CMV promoter (pCHXN). To improve the safety, self-inactivating (SIN) transfer vector was developed by deletion of 3′ U3 region (κB-κB-sp1-sp1-sp1-TATA) (pCHXNΔ) (Figure 1B).

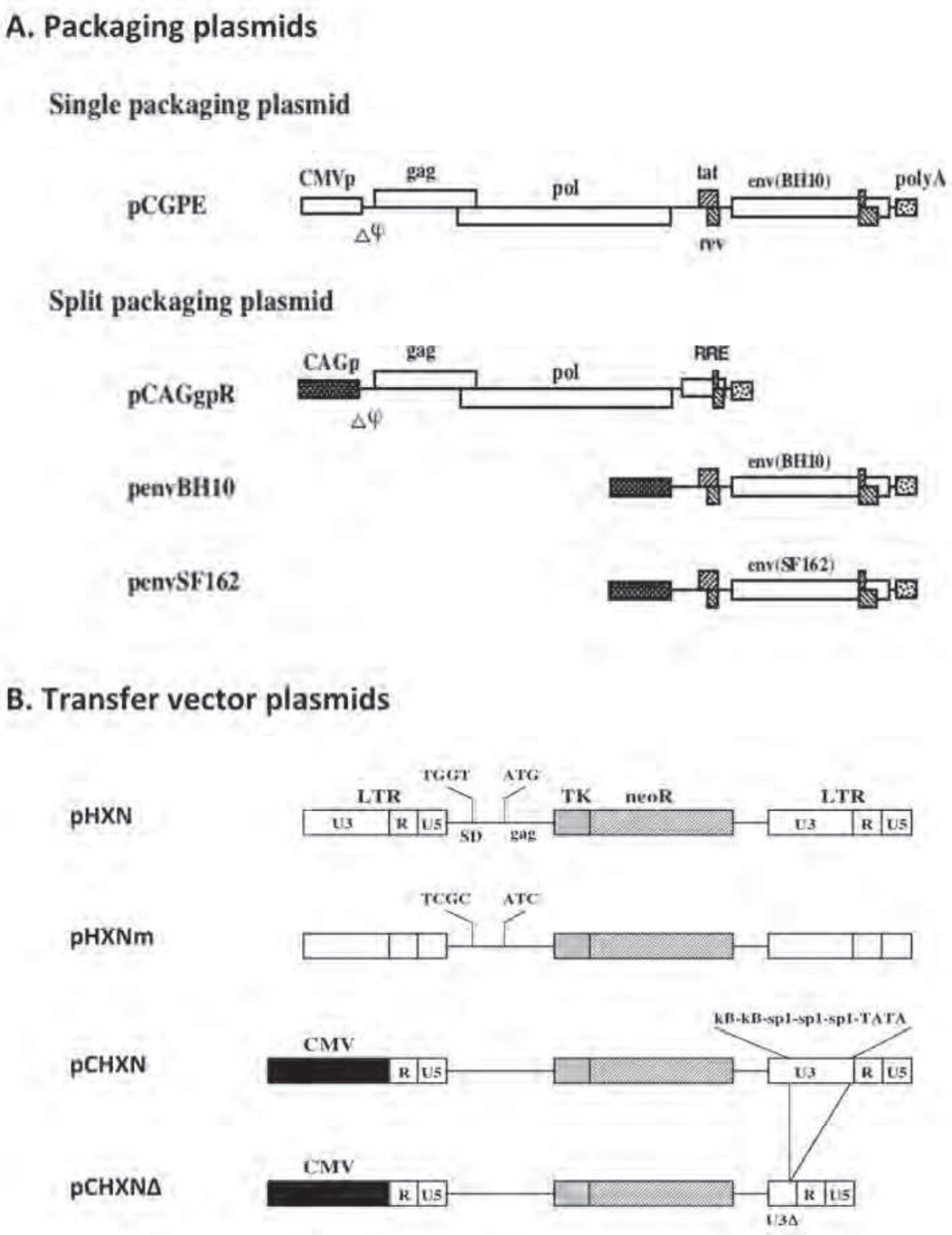

Fig. 1. Construction of recombinant HIV packaging (A) and transfer vector (B) plasmids.

2.2 Production of HIV vectors

Recombinant HIV vectors were prepared by transiently transfecting Cos cells ($2x10^6$ cells/10 cm dish) with 10 μg of packaging plasmid (pCGPE) and 10 μg of transfer vector plasmid (pHXN) using the $CaPO_4$ coprecipitation method described previously (Miyake et al. 1996; Shimada et al. 1991). For a separate packaging system, 7 μg of pCAGgpR, 7 μg of penvBH10 or penvSF162, and 7 μg of vector plasmids were used. After 48 h, the supernatant was collected, passed through a 45-μm filter to remove cell debris. The conditioned medium of transfected Cos cells were used as the HIV vector stock (Figure 2). To assess the transduction efficiency and cell specificity of the vectors, HeLa, CD4H ($CD4^+$ HeLa), and CD4H5 ($CD4^+$ and $CCR5^+$ HeLa) cells were incubated with various dilutions of the viral supernatants and cultured in the presence of 1,000 μg/ml active G418 for two weeks. G418 resistant colonies were calculated and the titer of HIV vector was presented as colony forming unit (cfu)/ml.

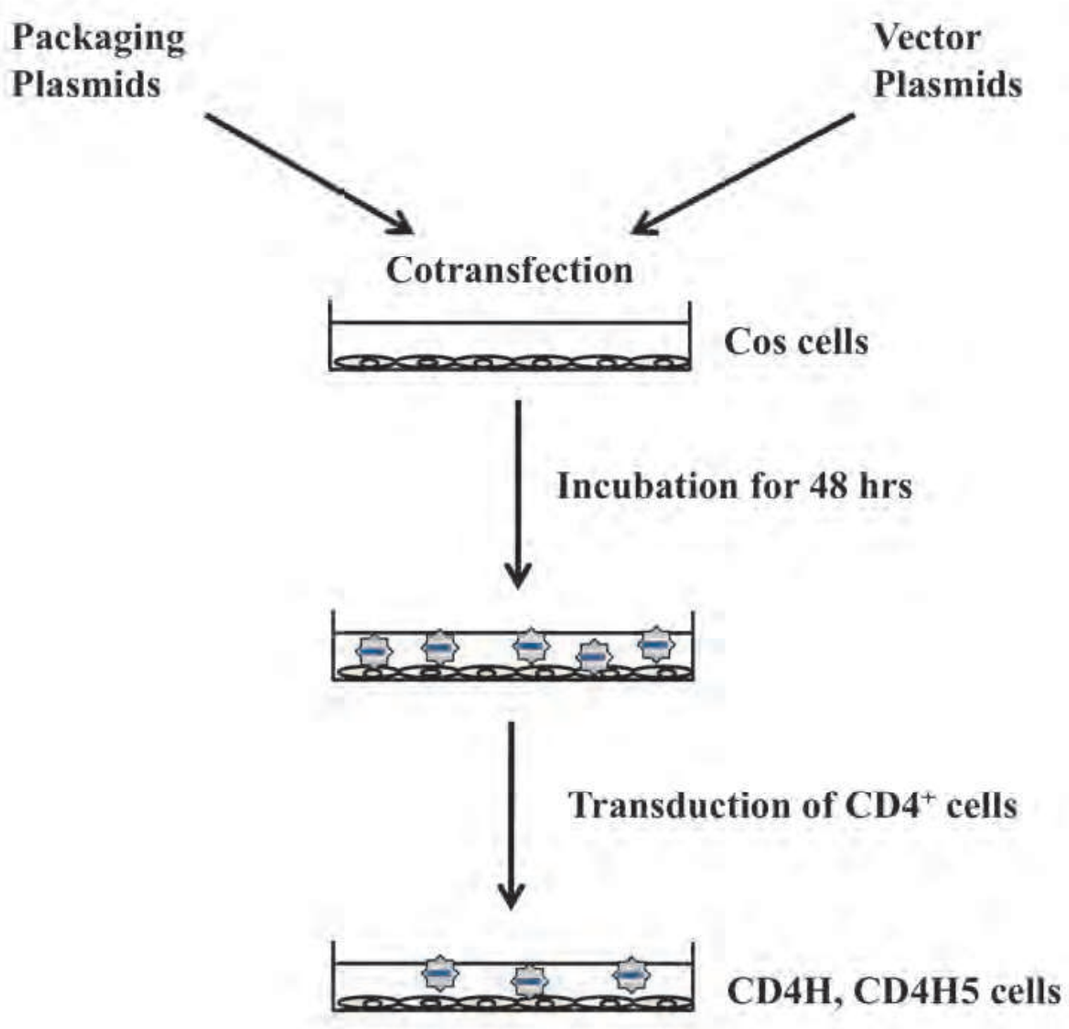

Fig. 2. Production of HIV vector pseudotyped with HIV envelope.

2.3 Concentration of HIV vectors

The titer of the HIV vector in Cos cell supernatants is approximately 10^4 cfu/ml when assayed on CD4H cells. This titer is too low to transduce primary $CD4^+$ cells. To overcome this problem, we concentrated HIV vectors using sulfonated cellulose column chromatography, thereby increasing the titer more than 20-fold (Matsuoka et al. 1998). For further improvement, among several possible procedures, we found that ultrafiltration using CENTRIPREP columns was highly effective to concentrate HIV particles (Miyake et al. 2007b). The titer could be increased four orders of magnitudes (Figure 3). The total recovery was more than 80 %. No replication competent cytopathic HIV was detected in concentrated vector preparation when assayed on MT2 cell-based syncytium formation assay, which is highly sensitive to the cytopathic effect of HIV.

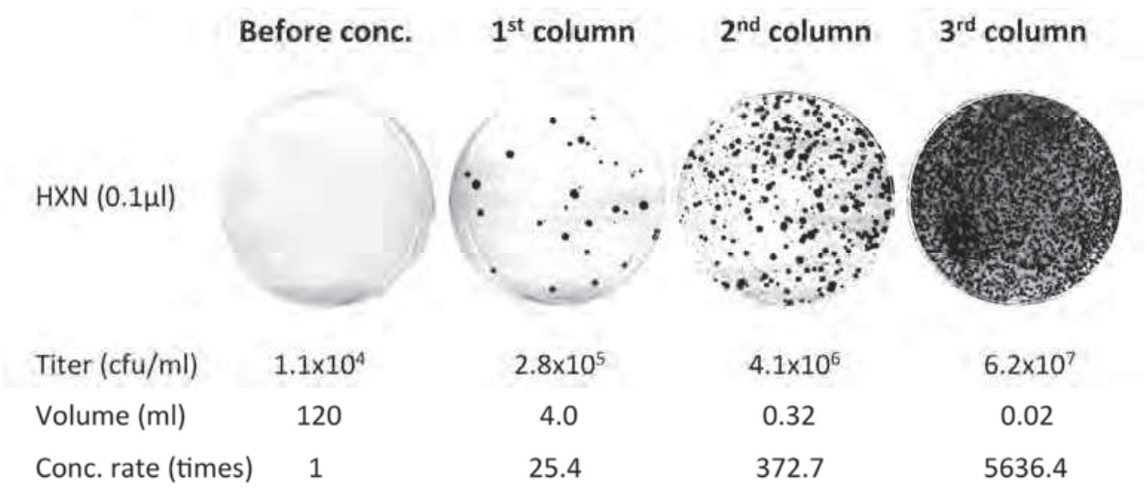

Fig. 3. Concentration of HIV vectors by ultrafiltration. By repeating the concentration step several times, we were able to obtain highly concentrated HIV vectors.

3. Characterization of HIV vectors

3.1 Targeted gene transfer into CD4-positive cells and macrophages

To examine the tissue specificity of the vectors, $CD4^-$ parental HeLa, CD4H, and CD4H5 ($CCR5^+$ CD4H) cells were incubated with the T-cell tropic HIV vector containing the neoR gene (HXN-T) or the macrophage tropic HIV vector (HXN-M). The T-cell tropic HIV vector selectively transduced $CD4^+$ and $CXCR4^+$ HeLa cells, while the macrophage tropic HIV vector transduced $CD4^+$ and $CCR5^+$ HeLa cells. Thus, the HIV vector has the same tissue specificity as wild type virus. The titer of HIV vectors generated from split packaging plasmids were slightly lower than those generated from single packaging plasmids (Table 1). Figure 4 shows the result of transduction of CD4H cells with MLV or HIV vector expressing enhanced green fluorescent protein (EGFP) gene (MLVG or HXG-T). Selective $CD4^+$ cells were transduced by HXG-T compared to MLVG vector.

	HeLa	CD4H	CD4H5
CD4	-	+	+
CXCR4	-	+	+
CCR5	-	-	+
pCGPE	0	$1.2x10^4$	$1.1x10^4$
pCAGgpR+penvBH10	0	$0.8x10^4$	$0.7x10^4$
pCAGgpR+penvSF162	0	0	$0.4x10^4$

Table 1. Selective transduction of CD4-positive HeLa cells by HIV vectors

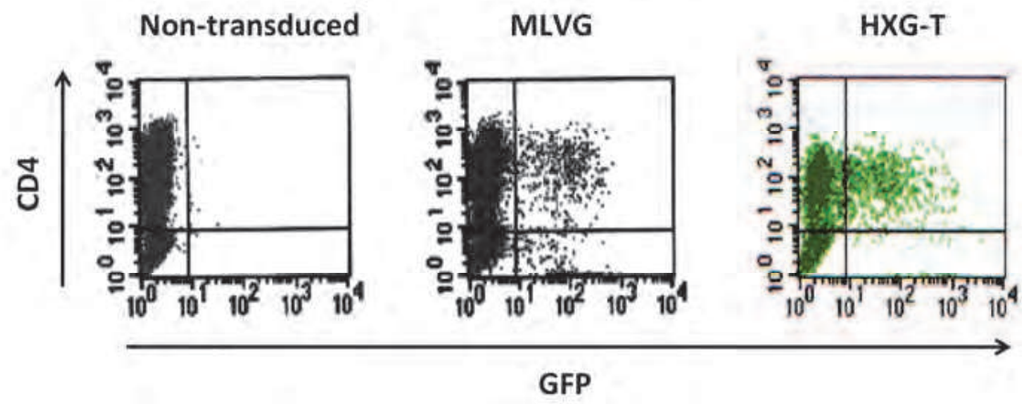

Fig. 4. Transduction of CD4-positive HeLa cells by MLV and HIV vectors expressing GFP.

We next attempted to transduce human primary cells using the high titer HIV vector carrying the EGFP gene. After enrichment of $CD4^+$ cells, the cells were stimulated with IL2 and PHA. Two days after stimulation, transduction was performed using the concentrated T-cell tropic HIV vector (HXG-T), and up to 84% of the stimulated T cells were found to be transduced. Samples of these transduced lymphocytes were analyzed to determine the duration of GFP expression, which was detected for more than one month with no decline in the percentage of GFP^+ cells (Miyake et al. 2007b). It thus appears that HIV vectors were integrated into the chromosomes, and the growth rate of vector-transduced cells was the same as that of non-transduced cells, making this highly concentrated HIV vector potentially useful for gene transfer into human primary lymphocytes.

We have reported that HIV vectors are capable of mediating gene transfer into non-dividing cells (Miyake et al. 1998). Therefore, we next attempted to transduce freshly isolated, un-stimulated $CD4^+$ T cells and terminally differentiated macrophages. The un-stimulated T cells were also efficiently transduced by HXG-T, with from 20 % to 54 % of the un-stimulated T cells being GFP positive, while these un-stimulated T cells could not transduced by MLV based retroviral vector. In addition, approximately 20 % of terminally differentiated, monocyte-derived macrophages were transduced using concentrated the macrophage tropic HIV vector expressing EGFP (HXG-M), indicating that macrophage trophic HIV vector should also be useful for targeted gene transfer into macrophages (Miyake et al. 2007b). We also construct HIV vectors containing LacZ gene and transduced with primary $CD4^+$ T cells and macrophages. Figure 5 shows the result of transduction of $CD4^+$ T cells and macrophages with HIV vector expressing LacZ gene. X-gal staining showed that both primary T cells and macrophages are efficiently transduced by HIV vectors.

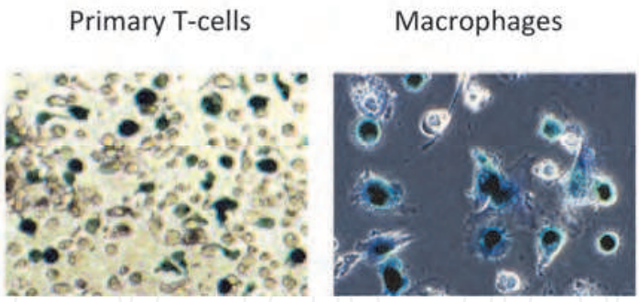

Fig. 5. Transduction of CD4+ T cells and macrophages with HIV vector expressing LacZ gene

3.2 *In vivo* targeted gene transfer

Targeted gene transfer is essential for safe and efficient gene therapy. Systemic injection of targeting vector is considered to be an ideal method of gene transfer. Since HIV vector was capable of targeted and efficient gene transfer into CD4+ human cells including non-stimulated primary CD4+ T-cells, it should be potentially useful for *in vivo* gene transfer into T lymphocytes. To analyze whether the HIV vector could be used for *in vivo* targeting gene transfer, Hu-PBL-NOD-scid mice were prepared by intraperitoneal injection of 1 x 10^7 human PBL into NK depleted NOD-scid mice. One ml of the HIV vector stock (HXG-T: 1 x 10^8 transduction unit (TU)/ml) was inoculated into the intraperitoneal cavity, and the mouse was sacrifices after 10 days. FACS analysis showed that 2-4 % of CD4+ T-cells recovered from the peritoneal cavity, peripheral blood, and spleen were transduced with the HXG-T vector (Figure 6).

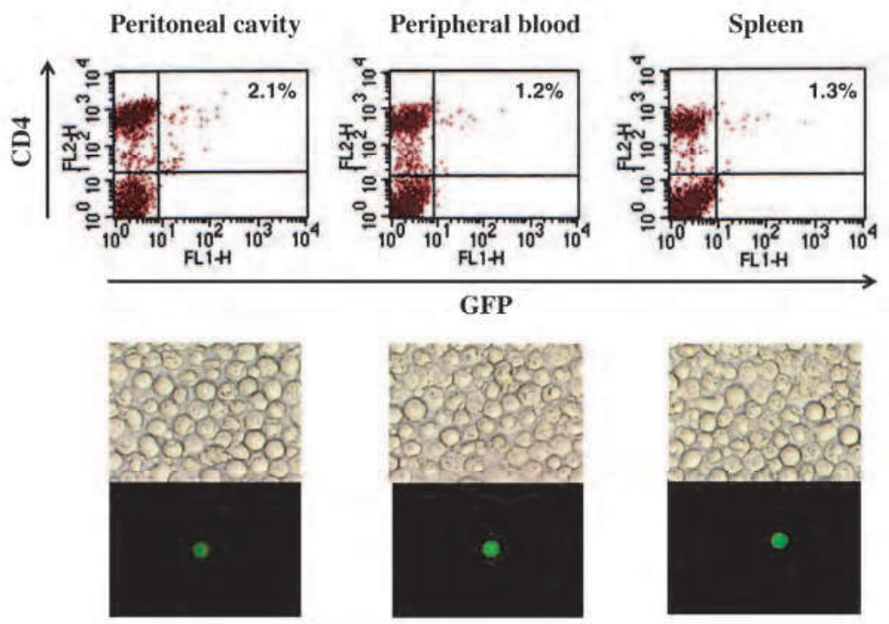

Fig. 6. *In vivo* targeted gene transfer by HIV vector. One ml of the HIV vector containing GFP stock (1 x 10^8 TU/ml) was inoculated into the intraperitoneal cavity of Hu-PBL-NOD-scid mice, and the mouse was sacrifices after 10 days. Expression of CD4 and GFP were analyzed by FACS.

These results indicate that HIV vector could be used for *in vivo* targeted gene transfer into $CD4^+$ T-cells. Lymphocytes are important target cells for gene therapy of various disorders. Targetable and injectable HIV vectors would greatly facilitate the development of such gene therapy strategies.

4. Applications of HIV vectors

4.1 Gene therapy for AIDS

A number of gene therapeutic strategies have been proposed for the treatment of AIDS. One approach, intracellular immunization (Baltimore 1988), entails rendering otherwise HIV-1-permissive cells resistant to HIV-1 infection by introducing such anti-HIV molecules as siRNA, antisense RNAs, ribozymes, RNA decoys, and trans-dominant negative mutants. A second approach entails vaccination using expression vectors for viral proteins, and a large scale clinical trial based on MLV vector-mediated transfer of the HIV-1 env and rev genes was examined. Finally, a third approach entails the use of a suicide gene to induce the death of HIV-1 infected cells, thereby preventing virus spread. The herpes simplex virus thymidine kinase (HSV-TK) gene is a most used typical suicide gene. HSV-TK acting in concert with cellular kinase specifically converts the prodrug gancyclovir (GCV) into highly toxic GCV-triphosphate, which causes DNA polymerase chain termination and eventually cell death. To minimize unwanted side effects, it is essential to develop a technique that enables tissue-specific gene transfer and expression only in targeted cells.

HIV vectors transduce only human $CD4^+$ lymphocytes and macrophages. Further, when used in conjunction with the HIV-LTR, an inducible promoter dependent on the virus encoded *trans*-activator Tat, HIV-based vectors pseudotyped with HIV envelope have the potential to mediate targeted gene transfer into HIV-1-permissive cells and for specific expression in HIV-1 infected cells. This would appear to make such vectors ideal for use in gene therapies against AIDS. Advantages of HIV vector for gene therapy of AIDS are summarized in Table 2.

1. Specific gene transfer into HIV infectable cells
2. Inducible expression on HIV infected cells
3. HIV vector sequences were packaged into newly synthesized virions
4. Useful for *in vivo* targeting gene transfer

Table 2. Advantages of HIV vector for gene therapy of AIDS

We constructed an HIV-based vector pseudotyped with HIV envelope containing the HSV-TK suicide gene under the control of the inducible HIV-LTR promoter (Figure 7A). This vector was selectively transferred into $CD4^+$ cells, and the HSVTK gene was subsequently expressed only in HIV-1-infected cells. Consequently, subsequent exposure to GCV selectively killed HIV-1 infected cells (Figure 7B). After transduction of H9 cells with HXTKN (HXTKN/H9), GCV-sensitivity was analyzed by MTT assay. Prior to HIV-1 infection, the GCV IC_{50} in HXTKN/H9 cells was approximately 100 μg/ml. On the other hand, HXTKN/H9 cells became highly sensitive to GCV after infection with HIV-1. The GCV IC_{50} of HIV-1-infected HXTKN/H9 cells were 0.1 μg/ml or about 1,000 times greater than in uninfected cells (Figure 7C). We next tested the extent to which HIV-1 replication was inhibited by the selective suicide of HIV-1-infected cells. In a model experiment,

HIV-1 infected HXTKN/H9 cells were mixed with uninfected HXTKN/H9 cells to a ratio of 1:100, and virus replication was studied by measuring p24 (Figure 7D). High levels of p24 were detected when the mixed cells were grown in the absence of GCV, but the amount of detected p24 declined dramatically following addition of 10 μg/ml GCV, indicating that selective suicide of HIV-infected cells effectively inhibited spread of the virus. In addition, on HIV-1 infection, HIV vector sequences were packaged into newly synthesized virions that were transferred into surrounding untransduced cells. These results support the rationale for treatment of HIV infection with HIV-based vectors (Miyake et al. 2001).

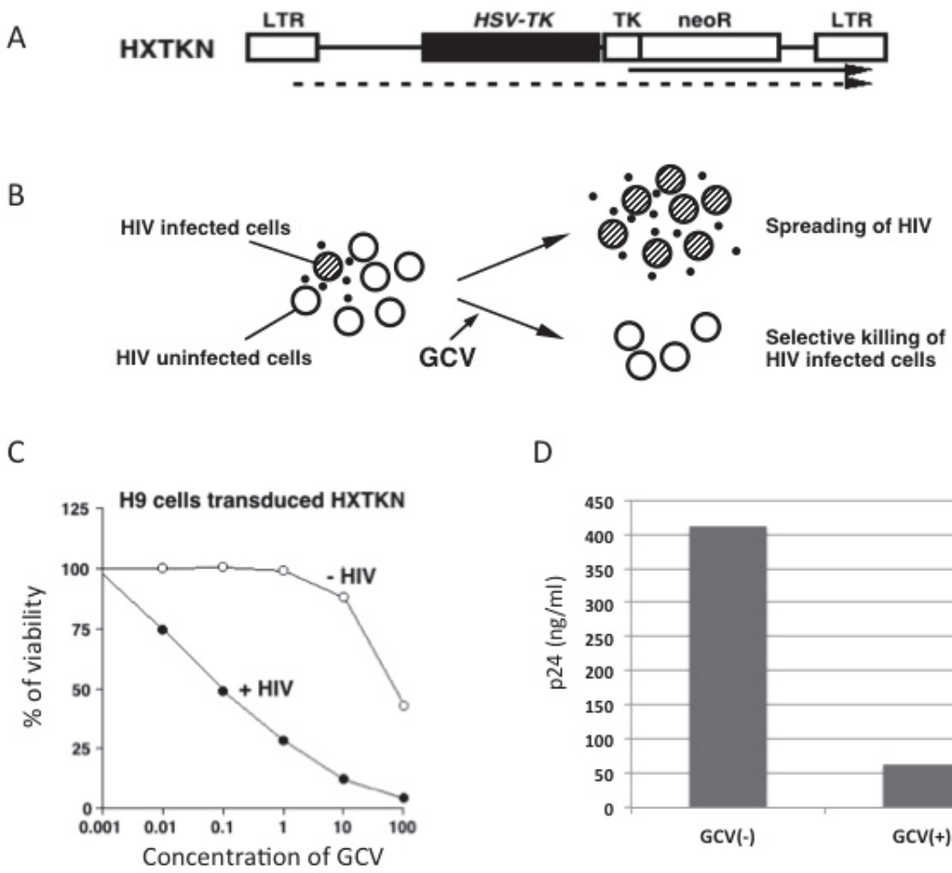

Fig. 7. Selective killing of HIV infected cells. (A) Inducible HIV vectors containing the HSV-TK suicide gene. (B) Rationale for treatment of HIV infection with HIV-based vectors. (C) HXTKN transduced H9 cells were incubated for 5 days with various concentrations of GCV (0.001-100 μg/mL), after which cell growth was estimated by MTT assay. (D) Effect of GCV on HIV-1 replication. A mixture of HIV-1-infected and uninfected HXTKN/H9 cells (1:100) were cultured with or without 10 μg/ml GCV. After 7 days, p24 levels in the culture supernatant were measured by ELISA.

We also investigated the utility of the HIV vector by intracellular immunization strategy. The chemokine receptors, CXCR4 and CCR5 (Alkhatib et al. 1996; Deng et al. 1996; Feng et al. 1996), have been identified as co-receptors for HIV-1 infection (Figure 8A). These cellular genes are attractive targets for intracellular immunization, because the mutation rate of the cellular gene is much less than that of the viral genome. We examined the feasibility of antisense inhibition of CXCR4 expression using HIV vector. We constructed the HIV vector containing the antisense sequence (738-429 nt) of CXCR4 driven by the CAG promoter and transduced CEM cells (Figure 8B). The level of CXCR4 in antisense expressing (HXCXAN-transduced) CEM cells was reduced up to 20 % of that in control (HXN-transduced) cells (Figure 8C). Inhibition of T-cell trophic HIV replication also observed in transduced CEM cells (Figure 8D). These results suggest that antisense inhibition of CXCR4 expression is effective to block HIV-1 infection at the early step and may be useful for gene therapy of HIV-1 infection.

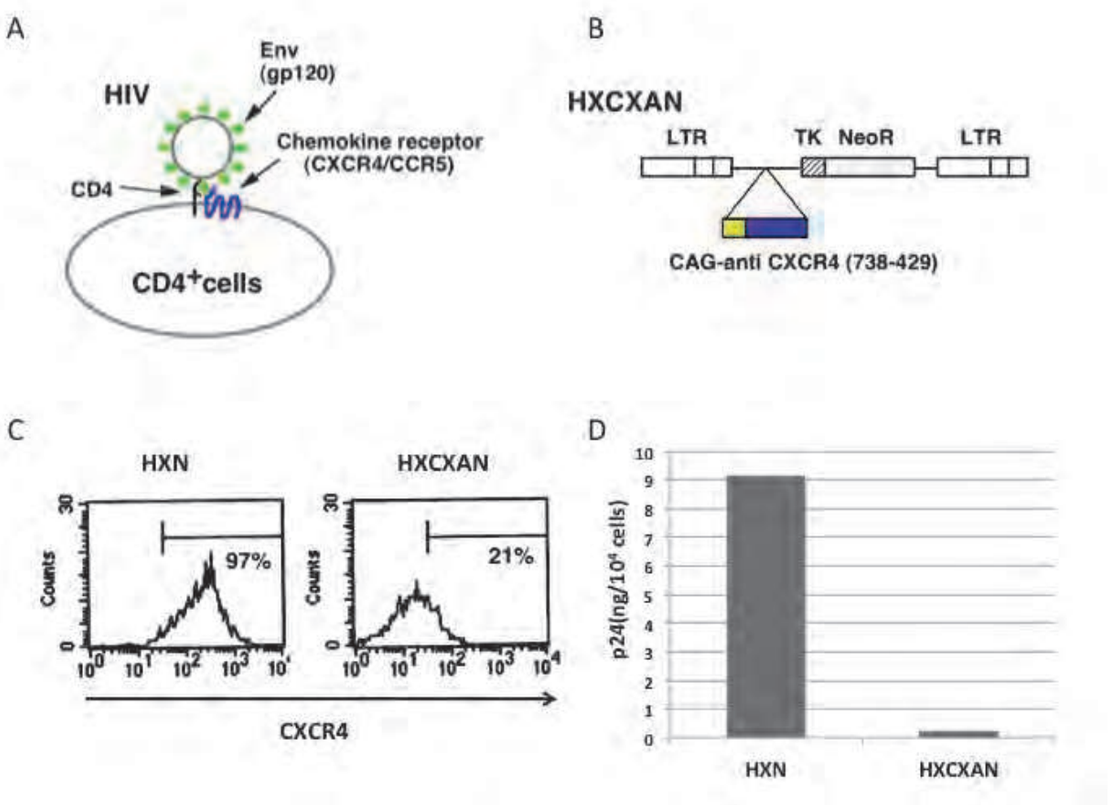

Fig. 8. Suppression of HIV-1 replication by HIV vector containing antisense CXCR4. (A) CXCR4 or CCR5 is a co-receptor of HIV-1 infection. (B) Construction of HIV vector expressing antisense CXCR4. (C) Expression of CXCR4 on HXN (control) or HXCXAN-transduced CEM cells. Expression of CXCR4 was analyzed by FACS. (D) Effect of antisense CXCR4 on HIV-1 replication. After HIV-1-infected HXN- or HXCXAN-transduced CEM cells were cultured for 7 days, p24 levels in the culture supernatant were measured by ELISA.

4.2 Development of selective transduction of HIV-1-infected cells

HIV based vectors bind specifically to the CD4 antigen and are capable of targeted gene transfer into CD4+ cells. The strict cell specificity of HIV vectors should be important for development of gene therapy of AIDS. However, HIV vectors may not be useful for gene transfer into cells already infected with HIV-1, because CD4 expression is usually down-regulated and in contrast, the HIV envelope glycoprotein (HIV-Env) is overexpressed in such cells (Hoxie et al. 1986; Maddon et al. 1986). Therefore, using a special feature of the HIV vectors, we have developed a novel strategy for targeted gene transfer into HIV-1 infected cells based on two-step gene transfer. Figure 9A shows the concept of two-step gene transfer. The first step involves the stable introduction of the HIV vector containing the ecotrophic MLV receptor (EcoRec) gene into human CD4+ T cells as a molecular switch.

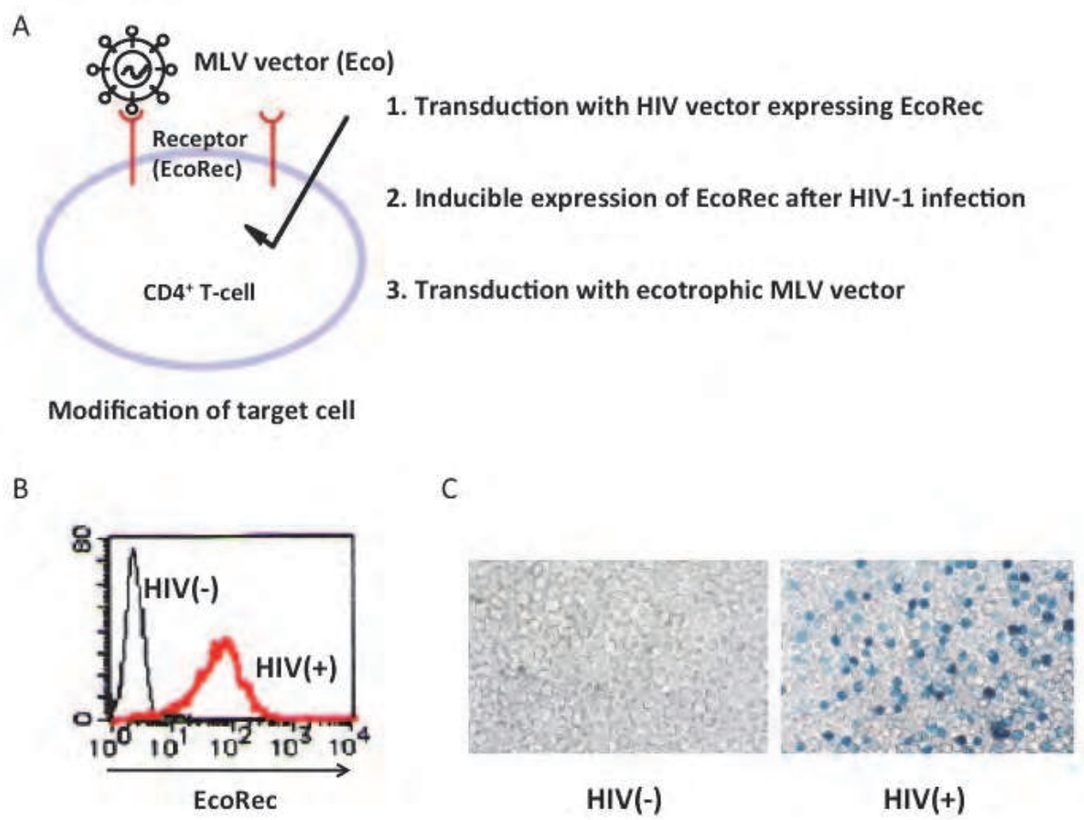

Fig. 9. Targeted gene transfer into HIV-1 infected cells. (A) Concept of two-step gene transfer for selective transduction of HIV-1 infected cells. (B) Inducible expression of EcoRec on HXERN transduced CEM cells. The cells were infected (HIV(+)) or uninfected (HIV(-)) with HIV-1. (C) HXERN transduced CEM cells were infected (HIV(+)) or uninfected (HIV(-)) with HIV-1 and then, transduced with the ecotrophic retroviral vectors containing LacZ gene. Expressions of β-galactosidase were assessed by X-gal staining.

Since the HIV-LTR is Tat-inducible, it is expected that EcoRec is expressed only after HIV-1 infection. High levels of EcoRec expression were observed only in HIV-1 infected cells.

These cells became highly susceptible to ecotrophic MLV infection and therefore, in the second step, HIV-1 infected cells were selectively transduced with ecotrophic MLV vectors.
CEM cells were transduced with the HIV vector containing the EcoRec gene under the control of the HIV-LTR (HXERN). Inducible expression of EcoRec in transduced cells was studied by the virus binding assay. Prior to HIV-1 infection, no detectable EcoRec was observed on the cell surface. However, after HIV-1 infection, high levels of induced EcoRec expression were detected by FACS analysis (Figure 9B). These findings indicated that HXERN transduced cells were ecotrophic MLV non-permissive, but became highly sensitive to ecotrophic MLV infection, once infected with HIV-1. In the next step, HXERN transduced cells were incubated with the ecotrophic MLV vector containing the beta-galactosidase gene (MLVLacZ). X-gal staining showed that HXERN transduced cells were totally resistant to retroviral infection. However, HIV-1 infected HXERN transduced cells were selectively transduced with MLVLacZ (Figure 9C). These findings indicate that this two-step method can be used for efficient, selective and stable gene transfer into HIV-1 infected cells (Sakai et al. 2001).
A possible clinical application of this strategy is as follows. Targets should be HIV seropositive but disease free patients. Most $CD4^+$ T cells in these patients are HIV negative. HXERN is stably inserted into these T cells (1st step). After a variable asymptomatic period, the virus apparently starts replication and spreads within the whole body. During this stage, EcoRec is expressed on the surface of only HIV-1 infected cells. Therefore, HIV-1 infected cells can be selectively attacked with the ecotrophic MLV vector carrying the therapeutic gene.

4.3 Gene therapy for ATL

Adult T cell leukemia (ATL) is a malignant disease etiologically associated with human T cell leukemia virus type I (HTLV-I). The prognosis of acute- or lymphoma-type ATL is far worse than that of other lymphoid malignancies, and effective therapy has not been established. HIV based vectors are capable of targeted and highly efficient gene transfer into $CD4^+$ T cells regulated by the HIV envelope glycoprotein gp120 and CD4 interaction. Because almost all ATL cells express CD4, HIV based vectors are useful for targeting therapeutic genes to ATL cells. We investigated the potential efficacy of treating ATL cells using a gene therapeutic approach involving the use of an HSV-TK-mediated suicide system. HIV vectors containing the HSV-TK gene under the control of CMV promoter (Figure 10A) were constructed to achieve targeted gene transfer into $CD4^+$ ATL cells, after which the transduced cells were selectively killed by treatment with GCV. To examine the utility of HIV vectors *in vivo*, ATL-NOD-SCID mice were prepared by intraperitoneal injection of 1×10^7 MT2 cells into NK-depleted NOD-SCID mice. Thereafter, 1 ml of concentrated HIV vector expressing HSV-TK (HXCTKN) or GFP (HXG) stock was injected into the intraperitoneal cavity, and GCV was administered twice a day for 5 days (Figure 10B). After three weeks, plasma sIL2-Rα levels, which are indicator of growth of MT2 cells, were significantly lower in mice administered HXCTKN than in those administered control HXG. Moreover, HXCTKN-injected mice survived significantly longer than HXG-injected mice (Miyake et al. 2007a) (Figure 10C). Taken together, these findings suggest that HIV vectors could be used for *in vivo* targeted gene transfer into ATL cells and thus could serve as the basis for the development of effective new therapies for the treatment ATL.

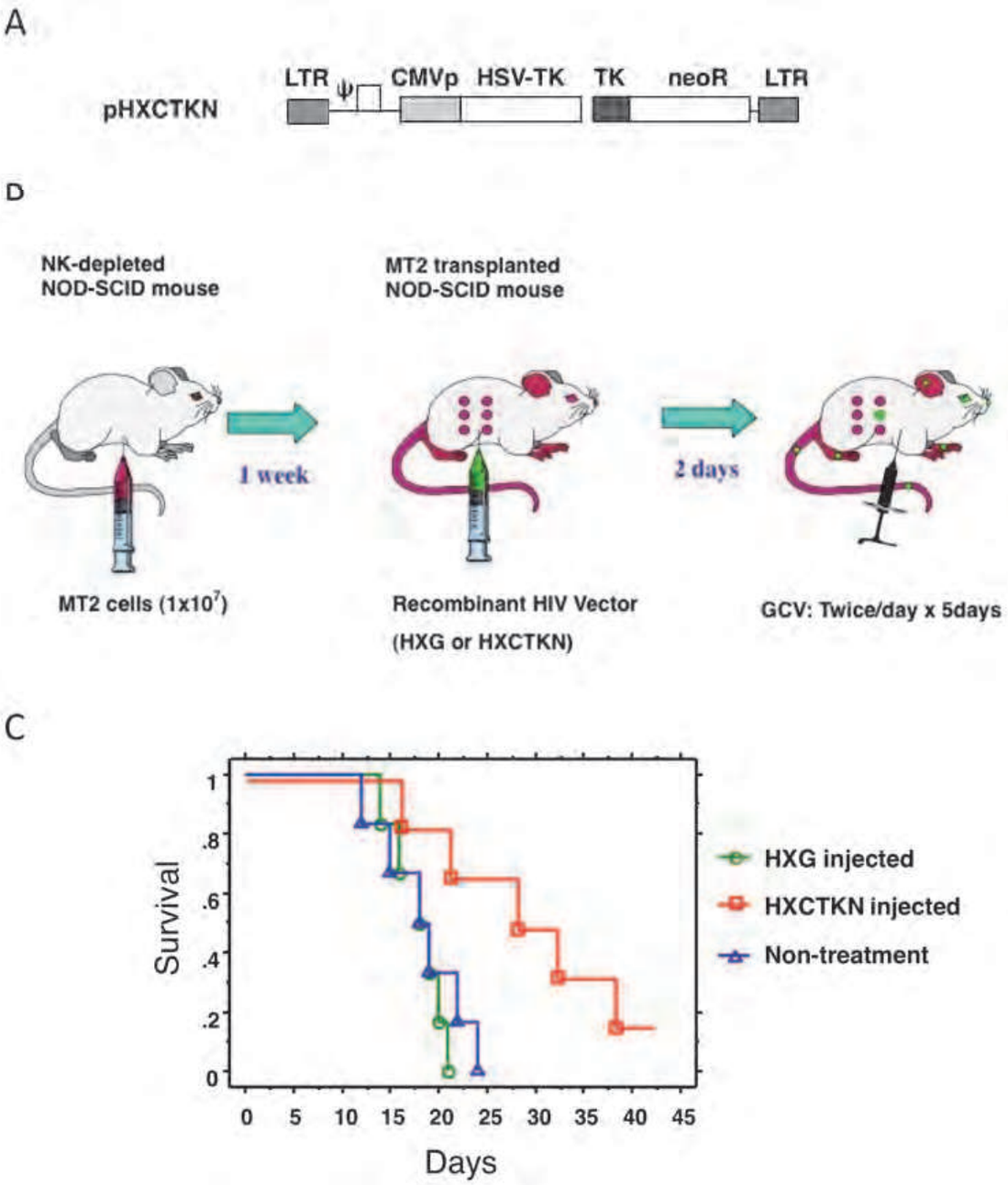

Fig. 10. Selective killing of ATL cells by HIV vector expressing HSV-TK gene. (A) Construction of HIV vector containing the HSV-TK suicide gene. (B) Experimental design of *in vivo* model for ATL. ATL model mice were prepared by intraperitoneal injection of 1×10^7 MT2 cells into NK-depleted NOD-SCID mice. Two days after treatment by HIV vectors (HXG or HXCTKN), GCV was administered twice a day for 5 days. (C) Survival curve of HIV vector treated ATL model mice. Mice administered HXCTKN-injected survived significantly longer than those injected with HXG ($p=0.002$).

5. Summary and future developments

Tissue-specific gene transfer and expression are crucial for the development of safe and effective gene therapy protocols. In that context, HIV-based retroviral vectors pseudotyped with HIV envelope have several advantages compared to MLV or VSV pseudotyped HIV vector. We succeeded in concentration of HIV vectors and primary $CD4^+$ cells were efficiently transduced with high titer HIV vectors. Moreover, HIV vector is potentially useful for *in vivo* gene transfer into T lymphocytes. These findings strongly suggest that concentrated HIV vectors should be useful in gene therapies targeting lymphocytes and macrophages.

HIV based retroviral vectors were originally designed for gene therapy in the treatment of AIDS. And HIV vector have a lot of advantages of for gene therapy of AIDS (Table. 2). Therefore, we developed novel gene therapy strategy for AIDS using our original HIV vectors. Tissue specific transduction into HIV-infectable cells and inducible expression of HIV-infected cells were achieved by using HIV vector. We used an HIV vector containing the HSV-TK gene to selectively kill HIV-1-infected cells and to inhibit HIV-1 replication. Although this system may not represent a complete cure for AIDS, combining gene therapy with other anti HIV-1 therapies may be useful for the long-term control of the disease.

Currently, recombinant HIV vectors were prepared by transiently transfecting Cos cells with packaging plasmid and transfer vector plasmid using the $CaPO_4$ coprecipitation method. Therefore, to make a high titer HIV vector, a large number of cell plate and a lot of plasmids are need. A stable packaging system for producing HIV vectors may be useful for future clinical applications using HIV vectors.

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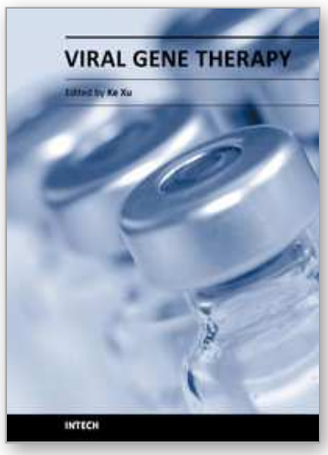

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The development of technologies that allow targeting of specific cells has progressed substantially in recent years for several types of vectors, particularly viral vectors, which have been used in 70% of gene therapy clinical trials. Particular viruses have been selected as gene delivery vehicles because of their capacities to carry foreign genes and their ability to efficiently deliver these genes associated with efficient gene expression. This book is designed to present the most recent advances in viral gene therapy

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