Efficient Transduction of Human and Rhesus Macaque Primary T Cells by a Modified Human Immunodeficiency Virus Type 1–Based Lentiviral Vector

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Human immunodeficiency virus type 1 (HIV-1)-based lentiviral vectors efficiently transduce genes to human, but not rhesus, primary T cells and hematopoietic stem cells (HSCs). The poor transduction of HIV-1 vectors to rhesus cells is mainly due to species-specific restriction factors such as rhesus TRIM5 α . Previously, several strategies to modify HIV-1 vectors were developed to overcome rhesus TRIM5 α restriction. While the modified HIV-1 vectors efficiently transduce rhesus HSCs, they remain suboptimal for rhesus primary T cells. Recently, HIV-1 variants that encode combinations of LNEIE mutations in capsid (CA) protein and SIVmac239 Vif were found to replicate efficiently in rhesus primary T cells. Thus, the present study tested whether HIV-1 vectors packaged by a packaging construct containing these CA substitutions could efficiently transduce both human and rhesus primary CD4 T cells. To accomplish this, LNEIE mutations were made in the packaging construct CEM Δ 8.9, and recombinant HIV-1 vectors packaged by Δ 8.9 WT or Δ 8.9 LNEIE were generated. Transduction rates, CA stability, and vector integration in CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /green fluorescent protein cells, as well as transduction rates in human and rhesus primary CD4 T cells by Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vectors, were compared. Finally, the influence of rhesus TRIM5 α variations in transduction rates to primary CD4 T cells from a cohort of 37 Chinese rhesus macaques was studied. While it maintains efficient transduction for human T-cell line and primary CD4 T cells, Δ 8.9 LNEIE-packaged HIV-1 vector overcomes rhesus TRIM5 α -mediated CA degradation, resulting in significantly higher transduction efficiency of rhesus primary CD4 T cells than Δ 8.9 WT-packaged HIV-1 vector. Rhesus TRIM5 α variations strongly influence transduction efficiency of rhesus primary CD4 T cells by both Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vectors. Thus, it is concluded that Δ 8.9 LNEIE-packaged HIV-1 vector overcomes rhesus TRIM5 α restriction and efficiently transduces both human and rhesus primary T cells.

Keywords: rhesus TRIM5 α , lentiviral vector, primary T cells, CA mutation

INTRODUCTION

SINCE THE 1990s, genetic modification of human primary T cells and hematopoietic stem cells (HSCs) has been used in a number of Phase I and II clinical trials for treatment of inherited genetic disorders, acquired immunodeficiency syndrome (AIDS), and cancer.^{1–10} Initially, Moloney murine leukemia virus (Mo-MuLV)-based vectors were used for gene deliv-

ery and appeared to be safe, as long as they were devoid of replication-competent virus. In ensuring years, steady improvements in *ex vivo* gene transfer protocols led to significant advances in the transduction of foreign genes into human primary T cells and HSCs.^{11–21} In a landmark human gene therapy trial that utilized a Mo-MuLV-based vector to correct HSCs derived from patients with X-linked severe

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combined immunodeficiency, proof of principle was established.²² However, subsequent adverse events associated with insertional mutagenesis have led to the reassessment of risks of using Mo-MuLV-based vector.²³ The discovery of a bias of Mo-MuLV-based vectors to integrate near transcription start sites^{24,25} has prompted exploration of alternative vector types.

Human immunodeficiency virus type 1 (HIV-1)-based lentiviral vectors are ideal for delivering potentially therapeutic genes into human primary T cells and HSCs. Unlike Mo-MuLV-based vectors, HIV-1-based vectors can transduce both dividing and non-dividing cells, and display a tendency to integrate into actively transcribed genes.^{26,27} Moreover, the new generation of self-inactivation (SIN) HIV-1-based vectors have been modified in such a way that prevents activation of genes surrounding integration sites.²⁸ Because of these alterations, HIV-1-based vectors are being used in a number of clinical trials for immune deficiencies, hemoglobin disorders, metabolic diseases, and HIV-1/AIDS.²⁹

While HIV-1-based vectors can efficiently transduce genes into human primary T cells and HSCs, they poorly transfer genes into rhesus primary T cells and HSCs.^{30,31} Because rhesus macaques are an extensively utilized preclinical model for evaluating the safety and the efficacy of gene therapy approaches for human diseases, it is useful and important to develop HIV-1-based vectors that can efficiently transduce both human and rhesus macaque primary T cells and HSCs.

The poor efficiency of transduction of genes into rhesus primary T cells and HSCs by HIV-1-based vectors is due to species-specific restriction factors in rhesus macaques that inhibit HIV-1 infection.³² Key post-entry restriction factors are the rhesus TRIM5 α and TRIM5-CypA fusion proteins.^{33–35} As a member of a large family of tripartite motif (TRIM) containing proteins, TRIM5 α and TRIM5-CypA contain N-terminal RING, B-box, coiled-coil, and C-terminal SPRY/B30.2 or CypA domain, respectively. Rhesus TRIM5 α and TRIM5-CypA recognize the incoming HIV-1 core by binding its capsid (CA) protein. Subsequently, TRIM5 α or TRIM5-CypA are poly-ubiquitinated, and ubiquitinated TRIM5 α or TRIM5-CypA along with the HIV-1 core complex are degraded.^{36,37} Simian immunodeficiency virus (SIV) CA can escape rhesus TRIM5 α and TRIM5 α -CypA-mediated degradation. Several strategies to modulate these factors have been developed to improve transduction to macaque cells by modified HIV-1 vectors.^{38–40} For example, Kootstra *et al.*³⁸ and Rits *et al.*³⁹ constructed modified HIV-1 vectors with mutations in the cyclophilin A (CypA)-binding domain of the CA sequence in the *gag/pol* packaging

constructs. They demonstrated that the modified HIV-1 vectors could efficiently transduce simian cell lines CV-1 and FrHL2 cells and baboon CD34⁺ HSCs, however, transduction rates in rhesus peripheral blood mononuclear cells (PBMCs) were <10%. Uchida *et al.*⁴⁰ screened a panel of chimeric HIV-1 vectors containing various combinations of HIV-1 and SIV elements in their transduction efficiency in human and rhesus lymphocytic cell lines CEMx174 and LCL8664, and selected a chimeric HIV-1 (χ HIV) vector, in which the vector genome was packaged with SIV CA. They demonstrated that the χ HIV vector efficiently transduces both human and rhesus CD34⁺ HSCs *ex vivo*. *In vivo* competitive repopulation experiments showed that this chimeric vector had better marking levels than either an unmodified HIV-1 vector⁴¹ or SIV-based vector.⁴² However, in the rhesus lymphoblast cell line LCL8664, transduction rates with this chimeric vector were <25%, even though in CEMx174 transduction rates were >90%,⁴⁰ and no transduction efficiency in rhesus primary T cells has been reported with this χ HIV vector. Besides, several groups developed SIV-based vectors and demonstrated that SIV-based vectors can efficiently transduce rhesus CD34⁺ HSCs,^{43–45} and non-myeloablative conditioning regimen increases engraftment of gene-modified HSCs in young rhesus macaques.⁴⁵ However, no transduction to rhesus primary T cells by SIV-based vectors has been reported in these studies. Thus, it appears that although these modified HIV-1 vectors or SIV-based vectors can efficiently transduce rhesus CD34⁺ HSCs, transduction rates of rhesus primary T cells by these vectors remain suboptimal.

Recently, in the development of nonhuman primate models for HIV-1 infection, Soll *et al.*⁴⁶ employed an “assisted evolution” approach in which individual CA mutations that reduced rhesus TRIM5 α resistance without fitness penalties were randomly assorted in a library of viral clones containing synthetic CA sequences. They identified viruses that encoded combinations of five mutations in CA, namely LNEIE, that conferred complete resistance to disruptive effects of rhesus TRIM5 α on incoming HIV-1 core. As a result, HIV-1 variants encoding these CA substitutions and SIVmac239 Vif replicated to high levels in rhesus primary lymphocytes. It was hypothesized that HIV-1 vectors packaged by a HIV-1 *Gag/Pol* expression construct with these CA substitutions could improve transduction of both human and rhesus primary T cells and HSCs.

The present study focused on testing whether HIV-1 vectors packaged by a LNEIE CA modified

Gag/Pol expression construct would overcome restriction by TRIM5 α and TRIM-CypA and efficiently transduce both human and rhesus primary CD4 T cells. To carry out these studies, LNEIE mutations were introduced in the CA region of the *gag/pol* expression construct pCEM Δ 8.9,²⁶ and vesicular stomatitis virus glycoprotein G (VSV-G) pseudotyped HIV-1 vectors packaged using either Δ 8.9 WT (wild-type pCEM Δ 8.9) or Δ 8.9 LNEIE (pCEM Δ 8.9 containing LNEIE mutations) *gag/pol* expression constructs were generated. The study demonstrates that while the Δ 8.9 LNEIE-packaged HIV-1 vector enables efficient transduction of human T-cell lines and primary CD4 T cells, it overcomes rhesus TRIM5 α -mediated CA degradation, resulting in significantly higher transduction efficiency of rhesus primary CD4 T cells than Δ 8.9 WT-packaged HIV-1 vector. It also demonstrates that variation of rhesus TRIM5 α strongly influences transduction efficiency of rhesus CD4 T cells by both Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vectors.

MATERIALS AND METHODS

Cell lines

The packaging cell line 293FT was purchased from Invitrogen Life Technologies (Carlsbad, CA) and maintained in complete Dulbecco's modified Eagle's medium (DMEM; *i.e.*, high glucose DMEM supplemented with 10% fetal bovine serum [FBS], 2 mM of L-glutamine, 1 mM of sodium pyruvate, 100 IU/mL of penicillin, and 100 μ g/mL of streptomycin). CEMss-CCR5 cells were generated previously,⁴⁷ and CEMss-CCR5-rhTRIM5 α /green fluorescent protein (GFP) cells were generated in this study (see below) and maintained in complete DMEM.

Human and rhesus macaque primary CD4 T cells

Human PBMCs were obtained from healthy anonymous donors through the blood bank of the Changhai Hospital, Shanghai, China. Human primary CD4 T cells were enriched from PBMCs by negative selecting magnetic beads according to the manufacturer's instructions (Cat# 11346D; Thermo Fisher Scientific, Waltham, MA). Rhesus PBMCs were provided by the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences Beijing, China (Animal Experimental Approval# XJ16006). Rhesus primary CD4 T cells were isolated from PBMCs by positive selecting magnetic beads according to the manufacturer's instructions (Cat# 130-091-102, Miltenyi Biotec,

Bergisch Gladbach, Germany). Both human and rhesus primary CD4 T cells were re-suspended in the complete RPMI 1640 medium (*i.e.*, RPMI 1640 medium supplemented with 15% FBS, 2 mM of L-glutamine, 1 mM of sodium pyruvate, 100 IU/mL of penicillin, and 100 μ g/mL of streptomycin) supplemented with human rIL-2 (100 IU/mL; R&D Systems, Minneapolis, MN) before being activated and transduced with Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vectors (see below).

Gene constructs

Δ 8.9 LNEIE *gag/pol* packaging construct was directly synthesized by Genewiz, a service company in Suzhou, China, in which five mutations (M10L/I91N/A92E/M96I/G116E) in the capsid protein were introduced in the pCMV Δ 8.9 *gag/pol* packaging construct.⁴⁸ The resulting packaging constructs were designated as Δ 8.9 WT and Δ 8.9 LNEIE, respectively (Fig. 1A).

The fusion gene encoding the glycosylphosphatidylinositol (GPI)-scFv X5 was generated by polymerase chain reaction (PCR) using pRRL-GPI-scFv X5⁴⁷ as a template and ligated between the Bam HI and Sal I sites of a third-generation lentiviral transfer vector, pRRLsin-18.PPT.EF1 α .2A.GFP.Wpre.⁴⁹ The resulting lentiviral transfer constructs were designated pRRL-GPI-scFv X5/2A/GFP (Fig. 1A).

Fusion gene encoding the GPI-mCherry/his tag was generated by overlapping PCR and ligated between the Bam HI and Sal I sites of a third-generation lentiviral transfer vector, pRRLsin-18.PPT.PGK.Wpre.⁵⁰ The resulting lentiviral transfer constructs were designated pRRL-GPI-mCherry (Fig. 1A).

The gene encoding rhesus TRIM5 α was amplified by PCR using pLPCX-rhTRIM5 α vector⁵¹ as a template and a pair of primers (5'-GGACTAGTTCC ACCATGGCTTCTGGAATCCTGCTTAATGT-3' and 5'-CCGCTCGAGTCAAGCGTAGTCTGGGACGTCG TATGGGTA-3') and ligated into the Spe I and Xho I sites of the lentiviral transfer vector, pTRIP-MND-NeffinB6-IRES-GFP,⁵² and sequenced. The resulting lentiviral transfer constructs were designated pTRIP-rhTRIM5 α /IRES/GFP (Fig. 1C). The lentiviral transfer vector containing the correct rhesus TRIM5 α sequence was used to generate recombinant lentiviruses.

Generation of recombinant lentiviruses

Recombinant lentiviruses were generated as described previously.⁵³ Briefly, 4×10^6 293FT cells were seeded onto a P-100 dish in 10 mL of complete DMEM. After culturing overnight, cells were

co-transfected with transfer construct (20 μ g) (one of the pTRIP-rhTRIM5 α /IRES/GFP, pRRL-GPI-mCherry, or pRRL-GPI-scFv X5/2A/GFP constructs), *gag/pol* packaging construct Δ 8.9 WT (7 μ g), or Δ 8.9 LNEIE (20 μ g; the different amounts of Δ 8.9 WT or Δ 8.9 LNEIE used here were based on the results of our initial assay opti-

mization, in which various amounts of Δ 8.9 WT or Δ 8.9 LNEIE had been tested) and plasmids encoding the VSV-G protein envelope (pLP/VSV-G; 3 μ g) using a calcium phosphate precipitation method. Sixteen hours later, culture supernatants were removed and replaced with fresh complete DMEM plus 1 mM of sodium butyrate (Cat#

Figure 1. Lentiviral transfer and packaging constructs and transduction of CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /green fluorescent protein (GFP) cells with Δ 8.9 wild type (WT) or Δ 8.9 LNEIE-packaged human immunodeficiency virus type 1 (HIV-1) vectors. **(A)** Schematic diagram of the lentiviral transfer vectors pRRL-GPI-mCherry and pRRL-GPI-scFv X5/2A/GFP, as well as packaging constructs Δ 8.9 WT or Δ 8.9 LNEIE and vesicular stomatitis virus glycoprotein G (VSV-G). The numbers are the amino-acid residue substitutions in capsid protein of *gag/pol* packaging construct pCMV Δ 8.9. **(B)** The list of three batches of Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vectors. **(C)** Schematic diagram of pTRIP-rhTRIM5 α /IRES/GFP vector. **(D)** Western blot analysis of TRIM5 α /HA fusion gene and GFP expression in CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /GFP cells. Anti-HA, anti-HA-tag antibody. **(E)** Fluorescence-activated cell sorting analysis of GFP expression in CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /GFP cells. **(F)** The gating strategy of GPI-mCherry-positive cells in CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /GFP cells transduced with Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vectors. **(G)** The transduction rates of CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /GFP cells with Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vectors in various indicated multiplicities of infection (MOI). Color images available online at www.liebertpub.com/hum

Figure 1. (Continued)

B58875G; Sigma–Aldrich, St. Louis, MO). Eight hours later, supernatants were again removed and replaced with fresh DMEM plus 4% FBS. After another 20 h, the culture supernatants were harvested and concentrated by ultracentrifugation as described previously.⁴⁷ The vector pellets were resuspended in a small volume of DMEM and stored in aliquots in a -80°C freezer. Vector titers before and after the concentration were determined as previously described.⁴⁷

Generation of CEMss-CCR5-rhTRIM5 α /GFP cell line

To generate a stably transduced CEMss-CCR5-rhTRIM5 α /GFP cell line, 1×10^5 CEMss-CCR5 cells and 2×10^6 transducing units (TU) of the recombinant lentiviruses containing pTRIP-rhTRIM5 α /IRES/GFP were added to a 24-well tissue culture plate in the presence of $8 \mu\text{g/mL}$ of polybrene (Cat# H9628; Sigma–Aldrich). Twenty-four hours later, cells were extensively washed and cultured in complete DMEM. The expression of fusion gene rhTRIM5 α /IRES/GFP was determined by Western blot and by fluorescence-activated cell sorting (FACS) analysis (see below).

Comparison of transduction efficiency between $\Delta 8.9$ WT- or $\Delta 8.9$ LNEIE-packaged HIV-1 vector in CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /GFP cell lines

To compare transduction efficiency between $\Delta 8.9$ WT- or $\Delta 8.9$ LNEIE-packaged HIV-1 vector, CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /GFP cells (10^5 cells per well) were transduced with $\Delta 8.9$ WT- or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors expressing GPI-mCherry at a multiple of infection (MOI) of 0.625, 1.25, 2.5, 5, and 10 in $500 \mu\text{L}$ of complete DMEM with $8 \mu\text{g/mL}$ of polybrene. The cells were centrifuged at $1,500 g$ for 3 h at 37°C to permit virus attachment. After overnight culture, cells were washed twice and re-suspended in fresh complete DMEM. Seven days later, the GPI-mCherry and/or GFP expression was evaluated by flow cytometry.

Western blot

To measure stability of CA proteins, 10^6 CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /GFP cells were transduced with $\Delta 8.9$ WT- or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors expressing GPI-mCherry at a MOI of 10 in $500 \mu\text{L}$ of complete DMEM with $8 \mu\text{g/mL}$ of polybrene and centrifuged at $1,500 g$ for 1 h at 4°C to permit virus attachment and to synchronize the transduction. After washing cells with DMEM twice to remove unattached virus, cells were incubated at 37°C . At 0, 1, 2, and 4 h post

transduction, cells were harvested and lysed using the lysis buffer (100 mM Tris-HCl, pH 8.0, and 1% NP40) containing protease inhibitor cocktail (Cat# 539134; Calbiochem, Billerica, MA). The lysis samples were incubated for 15 min at 90°C in loading buffer containing SDS and 0.6 M of dithiothreitol and loaded onto 12% SDS-PAGE. Gels were transferred onto polyvinylidene difluoride membranes, and the membranes were blocked with Tris-buffered saline containing 0.1% Tween-20 (TBST) and 5% nonfat dry milk for 1 h at room temperature. The membranes were subsequently incubated with 3 mL ($0.5 \mu\text{g/mL}$) of mouse anti-Gag p24 antibody (1:1,000) and rabbit anti-hemagglutinin (HA) antibody (1:1,000) overnight at 4°C . After being washed twice with TBST, the membranes were probed with HRP-conjugated goat anti-mouse antibody (1:5,000) and HRP-conjugated goat anti-rabbit antibody (1:4,000), respectively. Mouse anti-Gag p24 antibody (183-H12-5C) was obtained from the NIH ARRRP. Goat anti-HA antibody was purchased from Sigma–Aldrich (Cat# H6980). HRP-conjugated goat anti-mouse antibody and HRP-conjugated goat anti-rabbit antibody were purchased from ICL.

Quantitative PCR

Before the transduction, the recombinant lentiviruses were treated by DNase I (Cat# D7073; Beyotime, Haimen, China) according to the manufacturer's instructions. To synchronize the infection, CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /GFP cells were exposed to $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors at a MOI of 5, centrifuged at $1,500 g$ for 1 h at 4°C to permit virus attachment, and washed twice with DMEM to remove unattached virus. Cells were then incubated at 37°C . At 0, 4, 8, and 16 h post transduction, 1×10^5 cells per sample were harvested and frozen in -20°C . Genomic DNA was extracted by the MiniBEST Universal Genomic DNA Extraction Kit (Cat# 9765; TaKaRa, Otsu, Japan). Equal amounts of genomic DNA were used in triplicate and amplified with the THUNDERBIRDTM SYBR qPCR Mix provided by TOYOBO (Osaka, Japan). The level of expression of integration ratio was determined using the ABI 7900HT. Amplification efficiencies were validated and normalized against host-keeping gene *GAPDH*, and fold increases were calculated using the comparative CT method for quantification. The primers for transfer constructs were: WPRE F (5'-GGCACTGACAATTCGGTGGT-3'), WPRE R (5'-AGGGACGTAGCAGAAGGACG-3'); *GAPDH*: *GAPDH*-gF (5'-CTAGGGCTGCTCACATATTCTGG-3'), *GAPDH*-gR (5'-TAGTTGAGGTCAATGAAGGGGTC-3').

Transduction of human and rhesus primary CD4 T cells as well as rhesus PBMCs

Human CD4 T cells (2.5×10^5 per well) were activated with anti-CD3/CD28 antibody-coated microbeads (Cat# 11131D; Thermo Fisher Scientific) at a 1:1 ratio in 500 μ L of complete RPMI 1640 medium supplemented with human rIL-2 (100 IU/mL; Cat# 202-IL; R&D Systems) in 48-well plates. After 24 h, 5×10^6 TU Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vectors expressing GPI-scFv x5/2A/GFP in complete RPMI 1640 supplemented with human rIL-2 (100 IU/mL) and 8 μ g/mL polybrene were added into the cell suspension at a final volume of 750 μ L and various indicated MOIs. The plates were centrifuged at 1,500 *g* for 3 h at 37°C to facilitate transduction. The anti-CD3/CD28 antibody-coated microbeads in human CD4 T cells were removed by DynaMag magnet after 4 days of activation. The transduced human primary CD4 T cells were then cultured in complete RPMI 6140 supplemented with human rIL-2. The transduction efficiency was measured by FACS analysis.

To transduce rhesus primary CD4 T cells or PBMCs, anti-CD3 antibody (clone# FN-18; Thermo Fisher Scientific) and anti-CD28 antibody (clone# CD28.2; eBioscience, San Diego, CA) were pre-coated at a 1:1 ratio in 500 μ L of phosphate-buffered saline (PBS) in 24-well non-tissue culture plates (Cat# 3738; Corning, Corning, NY) at 4°C overnight. The coated plates were then blocked with 1% bovine serum albumin (BSA) in PBS for 30 min at 37°C, washed twice with PBS, and used for stimulation. Rhesus CD4 T cells (5×10^5) or PBMCs were added onto the anti-CD3/CD28-coated plate in 1 mL of complete RPMI 1640 medium supplemented with human rIL-2 (100 IU/mL). After 24 h, 10×10^6 TU Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vectors expressing GPI-scFv x5/2A/GFP in complete RPMI 1640 supplemented with human rIL-2 (100 IU/mL) and 8 μ g/mL of polybrene were added into the cell suspension at a final volume of 2 mL and various indicated MOIs. The plates were centrifuged at 1,500 *g* for 3 h at 37°C to facilitate transduction, and were cultured for an additional 3 days. The transduced rhesus primary CD4 T cells or PBMCs were then cultured in complete RPMI 6140 supplemented with human rIL-2. The transduction efficiency was measured by FACS analysis.

FACS analysis

To study the transgene expression, CEMss-CCR5 or CEMss-CCR5-rhTRIM5 α /GFP cells transduced with Δ 8.9 WT- or Δ 8.9 LNEIE-packaged HIV-1 vectors expressing GPI-mCherry or human and rhesus primary CD4 T cells transduced with Δ 8.9 WT- or

Δ 8.9 LNEIE-packaged HIV-1 vectors expressing GPI-scFv X5/2A/GFP (2×10^5 per sample) were harvested, washed twice with FACS buffer (PBS containing 1% BSA), and fixed with 1% formaldehyde in 0.5 mL of FACS buffer. FACS analysis was performed on Fortessa (Becton Dickinson, Mountain View, CA).

To study the transgene expression in transduced rhesus PBMCs, rhesus PBMCs transduced with Δ 8.9 WT- or Δ 8.9 LNEIE-packaged HIV-1 vectors expressing GPI-scFv X5/2A/GFP (2×10^5 per sample) were harvested, washed twice with FACS buffer, and stained with APC-conjugated anti-CD4 and PE-conjugated anti-CD8 antibodies (BD Biosciences, San Jose, CA). After the staining, cells were washed twice with FACS buffer and fixed with 1% formaldehyde in 0.5 mL of FACS buffer. FACS analysis was performed on Fortessa.

Rhesus TRIM5 α genotyping

Rhesus TRIM5a genotyping was performed using genomic DNA. Genomic DNAs from 37 rhesus macaques were extracted from PBMCs using the MiniBEST Universal Genomic DNA Extraction Kit (Cat# 9765; TaKaRa). A 525 bp fragment was generated by PCR using a pair of primers SPRY-006 (5'-CAGTGCTGACTCCTTTGCTTG-3') and T5 α 1087R (5'-GCTTCCCTGATGTGATAC-3'), as previously reported by de Groot *et al.*⁵⁴ The PCR products were then purified and sequenced directly.

Statistics

Statistical analyses were performed using the PRISM v6 software program. Transduction rates and integration ratio were compared using Student's *t*-test. Correlations were evaluated by R^2 in linear regression and *p*-values in correlation coefficient. A *p*-value of <0.05 was deemed significant. Standard errors of the mean are shown as error bars in all figures.

RESULTS

Δ 8.9 LNEIE-packaged HIV-1 vector efficiently transduces human T-cell lines and overcomes rhesus TRIM5 α restriction

To evaluate whether Δ 8.9 LNEIE-packaged HIV-1 vector could overcome restriction by rhesus TRIM5 α , first Δ 8.9 LNEIE packaging construct was constructed, in which M10L/I91N/A92E/M96I/G116E mutations were introduced into the CA sequence. The Δ 8.9 WT or Δ 8.9 LNEIE and VSV-G packaging constructs along with one of two SIN transfer vectors pRRL-GPI-mCherry or pRRL-GPI-scFv X5/2A/GFP (Fig. 1A) were co-transfected into the packaging cell line 293FT. Several batches of recombinant HIV-1 vectors were generated, and

Figure 2. Resistance of $\Delta 8.9$ LNEIE-packaged HIV-1 vector to TRIM5 α -mediated capsid degradation. **(A)** The Western blot analysis of capsid (CA), actin, and TRIM5 α /HA fusion protein in CEMss-CCR5-rhTRIM5 α /GFP cells transduced with $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vector at 0, 1, 2, and 4 h post transduction. **(B)** Graph presentation of quantitative analysis of Western blot data. Columns represent densitometric quantification of optical density (OD) of CA signal normalized with the OD values of actin, which served as a loading control. The intensities of the bands in **(A)** were quantified using ImageJ software. **(C)** The amounts of proviral DNA isolated at 0, 4, 8, and 16 h post transduction by real-time polymerase chain reaction analysis. Amplification efficiencies were validated and normalized against glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and fold increases were calculated using the comparative CT method for quantification. Results are typical of those obtained in three independent experiments.

they were concentrated and titrated using 293FT cells as targets. Figure 1B shows that the titers of concentrated $\Delta 8.9$ WT-packaged HIV-1 vector stocks ranged from 9.08×10^7 to 1.62×10^8 TU/mL, whereas $\Delta 8.9$ LNEIE-packaged HIV-1 vector stocks ranged from 5.66×10^7 to 8.88×10^7 TU/mL. Thus, while high titers of recombinant virus were obtained with $\Delta 8.9$ LNEIE-packaged HIV-1 vectors, the titers of $\Delta 8.9$ LNEIE-packaged HIV-1 vectors were only half of those of $\Delta 8.9$ WT-packaged HIV-1 vectors.

Meanwhile, a bicistronic expression vector was constructed containing both HA-tagged rhesus TRIM5 α and GFP (Fig. 1C), and the recombinant viruses were produced and used to transduce CEMss-CCR5 cells. The Western blot analysis shows that in transduced CEMss-CCR5 cells, both GFP and HA-tagged rhesus TRIM5 α proteins expressed at detectable levels compared to parental CEMss-CCR5 cells (Fig. 1D). FACS analysis shows that 99.3% of transduced CEMss-CCR5 cells were GFP positive (Fig. 1E).

To compare transduction efficiency of $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors, CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /GFP cells were transduced with various doses of $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors expressing GPI-mCherry (MOI from 0.625 to 10). Figure 1F shows the gating strategy of representative CEMss-CCR5 and CEMss-CCR5-rhTRIM5 α /GFP cells transduced with $\Delta 8.9$ LNEIE-packaged HIV-1 vector expressing GPI-mCherry at a MOI of 10. Clearly, both $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors efficiently and comparably transduced CEMss-CCR5 cells. At a MOI of 0.625, 1.25, 2.5, 5, and 10, both $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors had a 60%, 85%, 95%, 98%, and 99% transduction rate, respectively (Fig. 1G). In contrast, in CEMss-CCR5-rhTRIM5 α /GFP cells, the transduction efficiency by $\Delta 8.9$ WT-packaged HIV-1 vector was much lower than that of $\Delta 8.9$ LNEIE-packaged HIV-1 vectors. At a MOI of 0.625, 1.25, 2.5, 5, and 10, $\Delta 8.9$ WT-packaged HIV-1 vectors had a 6%, 25%, 34%, 38%, and 55% transduction rate, respectively, whereas $\Delta 8.9$ LNEIE-packaged HIV-1 vectors had a 24%, 55%, 68%, 91%, and 97% transduction rate, respectively (Fig. 1G), indicating that while it maintains efficient transduction of human T-cell lines, the $\Delta 8.9$ LNEIE-packaged HIV-1 vector overcomes rhesus TRIM5 α restriction.

$\Delta 8.9$ LNEIE-packaged HIV-1 vector is resistant to TRIM5 α -mediated capsid degradation resulting in high levels of proviral DNA

Rhesus TRIM5 α redirects incoming HIV-1 CA for proteasomal degradation by directly binding CA

Figure 3. The comparison of transduction efficiency between $\Delta 8.9$ WT and $\Delta 8.9$ LNEIE-packaged HIV-1 vectors. The rates of transduction to human (A) or rhesus (B) primary CD4 T cells from representative human and rhesus macaque donors by $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors in various indicated MOIs. (C–E) Preliminary testing of the transduction rates to rhesus primary T cells from a representative rhesus macaque donor with TFP/Q alleles of TRIM5 α by $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors. (C) and (D) Gating strategy of rhesus primary CD4 and CD8 T cells. (E) Percentage of GFP⁺ cells in rhesus primary CD4 or CD8 cell populations transduced with $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors at a MOI of 10. Color images available online at www.liebertpub.com/hum

with its B30.2/SPRY domain. Next, the study tested whether $\Delta 8.9$ LNEIE-packaged HIV-1 vector was able to avoid the disruptive effect of rhesus TRIM5 α . To accomplish this, equivalent amounts of $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors were applied to CEMss-CCR5-rhTRIM5 α /GFP

cells in a synchronized infection protocol. At 0, 1, 2, and 4 h post transduction, cells were harvested and lysed. The amounts of CA, rhesus TRIM5 α and actin were measured by Western blot analysis. Figure 2A and B shows that while similar amounts of rhesus TRIM5 α and actin were detected in cells

transduced with either $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors at all time points, the amounts of HIV-1 capsid protein were significantly higher and more stable in cells transduced with $\Delta 8.9$ LNEIE-packaged HIV-1 vector than with $\Delta 8.9$ WT-packaged HIV-1 vector. At 1 and 2 h post transduction, much higher amounts of CA were detected in cells transduced with $\Delta 8.9$ LNEIE-packaged HIV-1 vector than with $\Delta 8.9$ WT-packaged HIV-1 vector. Moreover, at 4 h post transduction, CA was undetectable in cells transduced with $\Delta 8.9$ WT-packaged HIV-1 vector, while the amount of CA remained relatively high in cells transduced with $\Delta 8.9$ LNEIE-packaged HIV-1 vector.

To compare proviral DNA between cells transduced with $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors, equivalent amounts of $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors were applied to CEMss-CCR5 or CEMss-CCR5-rhTRIM5 α /GFP cells in a synchronized infection protocol. At 0, 4, 8, and 16 h post transduction, genomic DNA was extracted. The amounts of proviral DNA were measured by quantitative PCR. Figure 2C shows that as expected, in CEMss-CCR5 cells transduced with $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors, similar amounts of proviral DNA were detected. In contrast, in CEMss-CCR5-rhTRIM5 α /GFP cells transduced with $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors, significantly higher amounts of proviral DNA were detected in cells transduced with $\Delta 8.9$ LNEIE-packaged HIV-1 vector than in cells transduced with $\Delta 8.9$ WT-packaged HIV-1 vector. Thus, taken together, these results indicate that $\Delta 8.9$ LNEIE-packaged HIV-1 vector is resistant to rhesus TRIM5 α -mediated CA degradation, resulting in higher levels of proviral DNA.

$\Delta 8.9$ LNEIE-packaged HIV-1 vector retains transduction efficiency in primary human CD4 T cells but significantly increases transduction efficiency of rhesus primary CD4 T cells

Next, the transduction efficiency was compared between $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors in human and rhesus primary CD4 T cells. To accomplish this, human and rhesus primary CD4 T cells were isolated from human and rhesus PBMCs stimulated with anti-CD3 and anti-CD28 antibody-coated beads or plates and transduced with $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors expressing GPI-scFv X5/2A/GFP. Figure 3A and B shows the transduction rates of $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors of representative human and rhesus primary CD4 T cells. Both $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors transduced human primary CD4 T cells very well, even though the transduction rates by $\Delta 8.9$ LNEIE-packaged HIV-1 vector were slightly lower than those by $\Delta 8.9$ WT-packaged HIV-1 vector. At a MOI of 2.5, 5, 10, 20, and 40, the transduction rates of $\Delta 8.9$ WT-packaged HIV-1 vector were 39.6%, 61.4%, 86.5%, 92.3%, and 93.1%, respectively, while the transduction levels of $\Delta 8.9$ LNEIE-packaged HIV-1 vector were 28%, 48.5%, 76.5%, 85.6%, and 86.2%, respectively (Fig. 3A). In contrast, although $\Delta 8.9$ WT-packaged HIV-1 vector transduced rhesus primary CD4 T cells poorly, $\Delta 8.9$ LNEIE-packaged HIV-1 vector significantly increased transduction efficiency. At a MOI of 2.5, 5, 10, 20, and 40, the transduction rates of $\Delta 8.9$ WT-packaged HIV-1 vector were 3.1%, 5.9%, 9.8%, 12.1%, and 12.3%, respectively, while those of $\Delta 8.9$ LNEIE-packaged HIV-1 vector were 28.1%, 37.9%, 43.2%, 53.9%, and 54.8%, respectively (Fig. 3B).

Figure 4. The transduction rates to rhesus primary CD4 T cells from a cohort of 37 Chinese rhesus macaque by $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors. (A) Individual transduction rates. (B) Statistical analysis of transduction rates between cells transduced with $\Delta 8.9$ WT-packaged HIV-1 vector and with $\Delta 8.9$ LNEIE-packaged HIV-1 vector.

In addition, preliminary testing was done on transduction efficiency in anti-CD3 and anti-CD28 antibody-stimulated rhesus PBMCs transduced with $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vector at a MOI of 10. Figure 3C–E shows that $\Delta 8.9$ LNEIE-packaged HIV-1 vector significantly increased transduction efficiency in both rhesus CD4 and CD8 T cells compared with $\Delta 8.9$ WT-packaged HIV-1 vector. With $\Delta 8.9$ LNEIE-packaged HIV-1 vector, 44% of CD4 T cells and 47.7% of CD8 T cells were GFP⁺, whereas with $\Delta 8.9$ WT-packaged HIV-1 vector, only 6.1% of CD4 T cells and 9.4% of CD8 T cells were GFP⁺.

Because in both human and rhesus primary CD4 T cells the transduction levels plateaued at a MOI of 20, a MOI of 20 was used to compare the transduction efficiency between $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors in rhesus primary

CD4 T cells from a cohort of 37 Chinese rhesus macaques tested. Figure 4A summarizes transduction rates between $\Delta 8.9$ WT or $\Delta 8.9$ LNEIE-packaged HIV-1 vectors in primary CD4 T cells of a cohort of 37 individual macaques. The average transduction rate by $\Delta 8.9$ WT-packaged HIV-1 vector was 8.4% (ranging from 1.1% to 18.4%), whereas the average transduction rate by $\Delta 8.9$ LNEIE-packaged HIV-1 vector was 26.5% (ranging from 10.5% to 57.8%). In each animal, $\Delta 8.9$ LNEIE-packaged HIV-1 vector outperformed $\Delta 8.9$ WT-packaged HIV-1 vector. The average fold increase in the percentage of GFP⁺ cells was 4.04 (ranging from 2.1% to 16.9%). Statistically, the difference in transduction rates between these two groups was extremely significant ($p = 5.2 \times 10^{-11}$; Fig. 4B). Taken together, these results clearly indicate that while the CA modified packaging vector maintains

Table 1. Age, sex, weight, *TRIM5 α* genotypes, and alleles and transduction rates of rhesus primary CD4 T cells by $\Delta 8.9$ WT- or $\Delta 8.9$ LNEIE-packaged HIV-1 vector among a cohort of 37 individual Chinese rhesus macaques

ID	Age	Sex	Weight (kg)	<i>TRIM5α</i>		% GFP	
				Genotypes	Alleles	$\Delta 8.9$ LNEIE	$\Delta 8.9$ WT
1	3.5	F	6.1	Mamu-4/-4	Q/Q	14.30	4.30
2	3.3	M	4.8	Mamu-3/-3	TFP/TFP	11.00	1.10
3	3.4	F	4.6	Mamu-3/-4	TFP/Q	20.10	5.20
4	3.5	F	5	Mamu-3/-3	TFP/TFP	25.70	6.40
5	2.6	M	5	Mamu-3/-4	TFP/Q	35.50	12.00
6	2.8	F	4.7	Mamu-3/-3	TFP/TFP	29.50	8.90
7	3.3	M	4.6	Mamu-3/-3	TFP/TFP	27.00	11.20
8	3.3	F	4.7	Mamu-3/-3	TFP/TFP	20.50	6.40
9	3.8	F	4.8	Mamu-3/-5	TFP/Q	34.00	15.90
10	3.2	M	6.1	Mamu-3/-3	TFP/TFP	21.50	8.40
11	2.7	F	4.7	Mamu-3/-3	TFP/TFP	29.50	10.50
12	2.7	F	5.4	Mamu-3/-5	TFP/Q	37.80	14.20
13	5.1	F	4.5	Mamu-3/-3	TFP/TFP	24.50	6.10
14	3.5	F	4.7	Mamu-3/-3	TFP/TFP	22.40	5.20
15	3.5	M	4.3	Mamu-3/-3	TFP/TFP	20.80	3.40
16	2.6	M	4.6	Mamu-3/-5	TFP/Q	38.60	11.90
17	3.5	M	5.1	Mamu-3/-3	TFP/TFP	19.80	8.90
18	2.9	M	4.5	Mamu-3/-5	TFP/Q	40.50	18.40
19	3.4	F	4.6	Mamu-3/-4	TFP/Q	25.60	10.20
20	2.9	M	4.5	Mamu-3/-3	TFP/TFP	28.90	11.00
21	3.5	M	5.4	Mamu-3/-3	TFP/TFP	25.60	12.50
22	2.7	F	5.2	Mamu-3/-3	TFP/TFP	27.50	13.20
23	2.6	M	4.9	Mamu-3/-3	TFP/TFP	34.60	14.30
24	3.5	M	6.1	Mamu-3/-4	TFP/Q	23.30	9.80
25	3.5	M	5.2	Mamu-3/-3	TFP/TFP	10.50	2.10
26	3.5	M	4.1	Mamu-3/-4	TFP/Q	18.90	5.60
27	3.4	M	4.1	Mamu-3/-3	TFP/TFP	20.40	7.30
28	3.4	F	4.4	Mamu-3/-3	TFP/TFP	19.20	8.40
29	3.5	F	4.3	Mamu-3/-3	TFP/TFP	24.20	10.20
30	3.4	F	4.2	Mamu-3/-3	TFP/TFP	18.90	9.30
31	3.4	F	4.5	Mamu-3/-3	TFP/TFP	35.30	2.09
32	3.4	M	5	Mamu-3/-4	TFP/Q	53.20	5.85
33	3.4	M	4.4	Mamu-1or2/-3	TFP/TFP	12.10	3.84
34	3.4	F	5.2	Mamu-1or2/-3	TFP/TFP	24.80	6.55
35	3.4	M	4.4	Mamu-3/-4	TFP/Q	57.80	12.25
36	3.4	F	5.2	Mamu-3/-3	TFP/TFP	12.40	4.56
37	3.3	M	4.8	Mamu-3/-3	TFP/TFP	32.50	2.75

efficient transduction of human primary CD4 T cells, it significantly increases transduction of rhesus primary CD4 T cells.

Influence of TRIM5 α variations in transduction efficiency of rhesus primary CD4 T cells by Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vector

Based on amino-acid variations at positions 307, 327, 333, and 339–340, rhesus TRIM5 α consists of six genotypes (Manu-1, 2, 3, 4, 5, and TRIMCyp), which cluster into three types: TFP (Manu-1, 2, and 3), Q (Manu-4 and 5), and TrimCyp.^{54,55} The rhesus TRIM5 α variations have been shown to affect SIV infectivity and clinical outcomes in rhesus macaques.^{56,57} It was hypothesized that rhesus TRIM5 α variations might influence the variable transduction rates by Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vectors in primary CD4 T cells of the cohort of 37 Chinese rhesus macaques shown in Fig. 4. To test this hypothesis, TRIM5 α alleles of these 37 Chinese rhesus macaques were evaluated using direct sequencing of 525 bp PCR product (see Materials and Methods). Among the 37 Chinese

Table 2. Statistical analyses of the correlation between age, sex, weight, or TRIM5 α alleles and transduction rates of rhesus primary CD4 T cells by Δ 8.9 WT- or Δ 8.9 LNEIE-packaged HIV-1 vector among a cohort of 37 Chinese rhesus macaques

Variables	Correlations
Age vs. Δ 8.9 LNEIE	0.153 ($R^2 = 0.081$)
Sex (male vs. female) vs. Δ 8.9 LNEIE	0.389 ($R^2 = 0.024$)
Weight (kg) vs. Δ 8.9 LNEIE	0.188 ($R^2 = 0.011$)
TFP/TFP vs. TFP/Q (Δ 8.9 LNEIE)	0.00089 ($R^2 = 0.281$)
TFP/TFP vs. TFP/Q (Δ 8.9 WT)	0.014 ($R^2 = 0.166$)

rhesus macaques, 25 had TFP/TFP (23 with Manu-3/Manu-3 and two with Manu-1 or 2/Manu-3) alleles, 11 had TFP/Q (seven with Manu-3/Manu-4 and four with Manu-3/Manu-5), and one has Q/Q (Manu-4/Manu-4) alleles. Not animals were found with a TRIMCyp genotype (Table 1). Next, the transduction rates were compared between TFP/TFP and TFP/Q groups. The average transduction rate by Δ 8.9 WT-packaged HIV-1 vector in the TFP/Q group was 11.0%, while in the TFP/TFP group it was 7.38%. The difference in transduction rates between these two groups was significant ($p = 0.014$; Fig. 5A and Table 2). The average transduction rate by Δ 8.9 LNEIE-packaged HIV-1 vector in the TFP/Q group was 35.0%, while in the TFP/TFP group it was 23.2%. The difference in transduction rates between these two groups was highly significant ($p = 0.00089$; Fig. 5B and Table 2). Thus, these results clearly indicate that rhesus CD4 T cells with TFP/Q alleles are more susceptible than rhesus CD4 T cells with TFP/TFP alleles to the transduction by both Δ 8.9 WT and Δ 8.9 LNEIE-packaged HIV-1 vectors.

For further statistical analysis, other host factors were also assessed that potentially affect transduction efficiency by Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vector, including the age, sex, and weight of these 37 rhesus macaques (Table 1). The results of univariate analysis show that there was no statistical difference in transduction efficiency between the different age and weight groups or between male and female animals (Table 2). Thus, taken together, it is concluded that while rhesus TRIM5 α alleles has a strong influence on transduction efficiency of rhesus primary CD4 T cells by either Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vector, the age, sex, or weight of the donor animal did not influence transduction efficiency.

DISCUSSION

Gene transfer to autologous HSCs and primary T cells utilizing HIV-1 vectors is a promising modality for the treatment of human diseases. Among nonhuman primates, the rhesus macaque has been proven to be a suitable preclinical model for safety

Figure 5. Statistical analysis of transduction rates between the TRIM5 α TFP/TFP group and the TFP/Q group. (A) Rhesus primary CD4 T cells transduced with Δ 8.9 WT-packaged HIV-1 vector. (B) Rhesus primary CD4 T cells transduced with Δ 8.9 LNEIE-packaged HIV-1 vector.

and efficacy studies. However, species-specific restriction factors such as TRIM5 α and TRIM5-CypA in rhesus macaques block HIV-1 infection, as well as significantly impair transduction efficiency of HIV-1 vectors.^{33–35} Rhesus TRIM5 α and TRIM5-CypA recognize the incoming HIV-1 core by binding HIV-1 capsid and mediate its degradation, whereas SIV capsid escapes rhesus TRIM5 α and TRIM5-CypA-mediated degradation.^{36,37} Previously, several strategies such as the CypA-binding mutant-modified HIV-1 vectors^{38,39} and a SIV capsid-packaged chimeric HIV-1 (χ HIV) vector⁴⁰ have been developed to improve transduction by HIV-1-based vectors. While these strategies significantly improved transduction efficiency of rhesus CD34⁺ HSCs, transduction rates for rhesus primary T cells by these modified HIV-1 vectors remain suboptimal. Therefore, the present study focused on testing whether HIV-1 vectors packaged by an HIV-1 Gag/Pol expression construct containing five LNEIE mutations in CA could efficiently transduce both human and rhesus primary CD4 T cells.

One interesting finding in the present study is that while it maintains efficient transduction to human T-cell lines and human primary CD4 T cells (Figs. 1F and G and 3A), Δ 8.9 LNEIE-packaged HIV-1 vector indeed overcomes rhesus TRIM5 α restriction (Fig. 1F and G), resulting in significantly higher transduction efficiency to rhesus primary CD4 T cells compared with Δ 8.9 WT-packaged HIV-1 vector (Figs. 3B and 4). Moreover, the preliminary data also show that Δ 8.9 LNEIE-packaged HIV-1 vector can also efficiently transduce both rhesus primary CD8 and CD4 T cells (Fig. 3C–E). Thus, taken together, it is concluded that Δ 8.9 LNEIE-packaged HIV-1 vector can indeed efficiently transduce human and rhesus primary T cells.

Several lines of evidence indicate that the HIV-1 capsid remains intact as it traverses the cytoplasm of newly infected cells. Prematurely uncoated incoming HIV-1 core triggers cytosolic sensors and nucleases,⁵⁸ assembled capsid proteins are required for properly engaging the nuclear pore complex,⁵⁹ and intact capsids have been observed at the nuclear envelope.⁶⁰ More recently, it has been shown that each capsid hexamer contains a size-selective pore bound by a ring of six arginine residues and a “molecular iris” formed by the N-terminal β -hairpin, which import nucleotides and fuel encapsidated DNA synthesis.⁶¹ Rhesus TRIM5 α and TRIM5-CypA recognize the incoming HIV-1 core by binding HIV-1 capsid with their C-terminal B30.2/SPRY and cyclophilin A (CypA) domains and then me-

diates the degradation of HIV-1 core complex.^{36,37} The present study shows the significantly higher and more stable HIV-1 capsid, along with significantly higher proviral DNA detected in CEMss-CCR5-rhTRIM5 α /GFP cells transduced with Δ 8.9 LNEIE-packaged HIV-1 vector than with Δ 8.9 WT-packaged HIV-1 vector (Fig. 2A–C). Thus, the results not only indicate the resistance of LNEIE CA mutant to rhesus TRIM5 α /TRIM5-CypA-mediated degradation, but also suggest that the intact HIV-1 core complex of Δ 8.9 LNEIE-packaged HIV-1 vector leads to a higher amount of proviral DNA.

Another interesting finding in the present study is that allelic variation in TRIM5 α strongly influences transduction efficiency of rhesus primary CD4 T cells by both Δ 8.9 WT or Δ 8.9 LNEIE-packaged HIV-1 vectors. Among the cohort of 37 Chinese rhesus macaques tested in the present study, there were 25 TFP/TFP, 11 TFP/Q, one Q/Q, but no TrimCyp/TrimCyp, TrimCyp/TFP, or TrimCyp/Q alleles (Table 1). The allelic frequency found in these 37 Chinese rhesus macaques is consistent with two previous reports.^{54,62} When transduction efficiency was compared between TFP/TFP and TFP/Q groups, the transduction efficiency by both Δ 8.9 WT and Δ 8.9 LNEIE-packaged HIV-1 vectors was significantly higher in the TFP/Q group than it was in the TFP/TFP group ($p=0.014$ and 0.00089 , respectively; Fig. 5A and B and Table 2). Although at this time there is no explanation for this observation, the results are consistent with the study reported by Evans *et al.*⁴² They transduced cell lines expressing six different rhesus TRIM5 genotypes (Manu-1, -2, -3, -4, -5, and TRIM5-CypA) with chimeric χ HIV-1 vector expressing GFP and found that transduction efficiency is much higher in cell lines expressing Q (Manu-4 and -5) and TrimCyp alleles than in it is in cell lines expressing the TFP allele (Manu-1, -2, and -3). In addition, they also found higher transduction rates in CD34⁺ HSCs from TFP/Q (Manu-3/Manu-4) alleles than in TFP/TFP (Manu-3/Manu-3) alleles.

Finally, although significant improvement of transduction efficiency of rhesus primary CD4 T cells has been achieved with Δ 8.9 LNEIE-packaged HIV-1 vector, transduction rates of rhesus primary CD4 T cells are still lower than those of human primary CD4 T cells (Fig. 3). It is speculated that this could be due to the presence of other species-specific restriction factors in rhesus primary CD4 T cells that partially block the Δ 8.9 WT-packaged HIV-1 vector. Besides rhesus TRIM5 α /TRIM5-CypA, rhesus APOBEC3G and tetherin proteins have been shown to block HIV-1 infection in macaque cells, which can be antagonized by SIV Vif

and Vpu, respectively.^{32,63–65} Interestingly, Nomaguchi *et al.*⁶⁶ showed that a HIV-1 variant MN4/LSDQgtu, which combines LSDQ CA mutations and the substitution of the transmembrane domain of SIVgsn166 Vpu, grew comparably to SIVmac239 in rhesus PBMCs. Soll *et al.*⁴⁶ showed that a HIV-1 variant encoding the LNEIE CA substitutions and SIVmac239 Vif replicated efficiently in rhesus primary lymphocytes. Thus, it may be worthwhile to modify Δ8.9 WT-packaged HIV-1 vector further so as to improve transduction efficiency of rhesus primary T cells further. Nevertheless, the Δ8.9 LNEIE packaging construct provides improved transduction of rhesus cells, which should enhance the utility of HIV-1-based vectors in this nonhuman primate species.

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AUTHOR DISCLOSURE

None of the authors have any conflicts of interest to report.

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