Baboon envelope LVs efficiently transduced human adult, fetal, and progenitor T cells and corrected SCID-X1 T-cell deficiency

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Key Points

• BaEV-LVs efficiently transduce progenitor T cells, providing accelerated T-cell reconstitution in vivo.
• BaEV-LVs efficiently correct human SCID-X1 progenitor T cells.

T cells represent a valuable tool for treating cancers and infectious and inherited diseases; however, they are mainly short-lived in vivo. T-cell therapies would strongly benefit from gene transfer into long-lived persisting naive T cells or T-cell progenitors. Here we demonstrate that baboon envelope glycoprotein pseudotyped lentiviral vectors (BaEV-LVs) far outperformed other LV pseudotypes for transduction of naive adult and fetal interleukin-7–stimulated T cells. Remarkably, BaEV-LVs efficiently transduced thymocytes and T-cell progenitors generated by culture of CD34+ cells on Delta-like ligand 4 (Dll4). Upon NOD/SCIDγC−/− engraftment, high transduction levels (80%-90%) were maintained in all T-cell subpopulations. Moreover, T-cell lineage reconstitution was accelerated in NOD/SCIDγC−/− recipients after T-cell progenitor injection compared with hematopoietic stem cell transplantation. Furthermore, γC-encoding BaEV-LVs very efficiently transduced Dll4-generated T-cell precursors from a patient with X-linked severe combined immunodeficiency (SCID-X1), which fully rescued T-cell development in vitro. These results indicate that BaEV-LVs are valuable tools for the genetic modification of naive T cells, which are important targets for gene therapy. Moreover, they allowed for the generation of gene-corrected T-cell progenitors that rescued SCID-X1 T-cell development in vitro. Ultimately, the coinjection of LV-corrected T-cell progenitors and hematopoietic stem cells might accelerate T-cell reconstitution in immunodeficient patients.

Introduction

Gene transfer into T lymphocytes is a crucial step in the development of therapeutic strategies for the treatment of genetic dysfunctions of the hematopoietic system, such as severe combined immunodeficiency (SCID1–3) as well as cancers4,4 and acquired diseases.5 More than 15 years ago, children suffering from monogenic diseases such as adenosine deaminase SCID (ADA-SCID) and SCID-X1 were successfully treated with T-cell gene therapy (ADA-SCID)6 or hematopoietic stem cells (HSCs) (SCID-X16,7; ADA-SCID)6. T-cell gene therapy might also become an important treatment option for HIV-infected patients because several new combinatorial strategies have been proposed.9–11 To avoid graft-versus-host
The clinical efficacy of adoptively transferred T lymphocytes is correlated with their ability to persist in vivo,59 which is correlated with a less differentiated T-cell phenotype.10,16-18 Naive T cells are especially important as gene therapy target cells because they maintain the capacity to respond to novel antigens and can generate the entire spectrum of immunologic memory.31,32 Indeed, upon infusion, less differentiated naive and central memory T cells display superior proliferation, persistence, and antitumor responses when compared with the effector memory subset.15,31,33 Accordingly, naive T cells might constitute the best T-cell target population for gene therapy. Human cord blood (CB) T cells are mostly naive and differ from their adult naive counterparts in that the former represent mainly recent thymocyte emigrants,33,34 which express CD31+.35-36 Naive CB T cells proliferate significantly more than their adult counterparts in response to interleukin-7 (IL-7).37,38 Interestingly, IL-7 maintains naive CD31+CD4+ T cells during adult life.39 These characteristics prompted the development of the first CAR-based CB T-cell strategies for the treatment of cancer and infectious diseases.16,40-42

Other even more immature targets for gene modification are the T-cell progenitors, which are normally found in the thymus and are important for treatment of autoimmune disorders.43-46 Early T-cell development depends on the interaction between thymocytes and Notch ligand signaling pathways. Delta-like ligand 4 (Dll4) has been identified as the essential Notch1 activator in the T-cell development of HSCs.47-49 We have previously demonstrated that T-cell progenitors can be generated from CD34+ hematopoietic stem and progenitor cells (HSPCs) in a feeder-cell–free culture system based on Dll4.50,51 They displayed the phenotypic and molecular signatures of immature thymic precursors and were capable of differentiating into T cells and accelerating T-cell reconstitution in vivo compared with HSPCs.52

It is now generally accepted that resting T cells cannot readily be transduced by classical vesicular stomatitis virus G (VSV-G) protein pseudotyped lentiviral vectors (LVs)52-54 because of the absence of the VSV receptor.55 The latter is upregulated by TCR stimulation and is correlated with high-level transduction by VSV-G-LVs.55 However, TCR stimulation of T cells alters their half-life and immune competence and is associated with the loss of naive T-cell subsets and a skewed TCR repertoire.56,57 Exposure of T cells to cytokines such as IL-7 renders them permissive to transduction by VSV-G-LVs.55 However, TCR stimulation of T cells alters their half-life and immune competence and is associated with the loss of naive T-cell subsets and a skewed TCR repertoire.56,57 Exposure of T cells to cytokines such as IL-7 renders them permissive to transduction by VSV-G-LVs.55

We engineered LVs that displayed the feline retrovirus GP RD114, which conferred efficient transduction of hT cells upon TCR stimulation.64 The baboon envelope retrovirus (BaEV) belongs to the same betaretrovirus family as RD114. Both viruses use the neutral alanine-serine/cysteine amino acid transporter-2 (ASC-2) present on T cells.65,66 Interestingly, BaEV-LVs also bind to ASC-267 and far outperform VSV-G- and RD114-LVs for human HSC and B-cell transduction.68-70 Therefore, we hypothesized that BaEV-LVs might be excellent candidates for gene delivery into naive hT cells and T-cell progenitors.

Materials and methods

Plasmids

RD114TR, BaEVTR, BaEVRLess, and H+24 and F+30 envelope GPs were previously described.64-69,71 All chimeric envelope GPs were expressed in the pCMV-G expression plasmid.72 The SIN-HIVSFFVGF vector was described previously.73 The native IL-2RγC complementary DNA (cDNA) fragment from pRRL-PPT.SF91.II2Rγpre (a gift from A. Shambach, Medizinische Hochschule Hannover, Hannover, Germany) was cloned into the pHEF1-MCS-ires-GFP vector by ligation of BarnHI-Sall digested fragments.

Self-inactivating HIV-1-derived vectors encoding green fluorescent protein (GFP) or γc under the control of an SFFV or EF1 α promoter, respectively, were generated by transfection of 293T cells as described in detail elsewhere.71 Titration of LVs was described in detail elsewhere, and isolation of human primary T cells, thymocytes, and CD34+ cells is described in the supplemental Methods.

Transduction of primary cells

Peripheral blood (PB) T lymphocytes were prestimulated for 24 hours with anti-human CD3 (hCD3/anti-hCD28 (1 μg/mL, BD Pharmingen) in the presence of hIL-2 (1 ng/mL), and CB and PB T cells were prestimulated with recombinant human IL-7 (rhIL-7; 10 ng/mL; BD Biosciences) for 2 and 3 days, respectively.54 Next, 5 × 10⁶ CB or PB T cells were seeded in 48-well plates upon TCR stimulation or cytokines and transduced with LVs at the indicated multiplicity of infection (MOI). Cell cultures were replenished with cytokines every 3 days. At 3 and 6 days after transduction, the percentage of GFP+ cells was determined by FACS. Where indicated, transductions were performed on RetroNectin-coated plates (Takara Bio, Shiga, Japan).

In all, 5 × 10⁶ freshly isolated thymocytes were seeded in RetroNectin-coated 48-well plates in RPMI-1640 and 10% fetal calf serum in the presence of rIL-7 (20 ng/mL) and transduced with LVs at the indicated MOIs. Cell cultures were replenished with cytokines after 3 days. Four days after transduction, the percentage of GFP+ cells was determined by FACS. CB CD34+ cells were cultured in 48-well plates coated with a Dll4 Fab fusion protein (Dll4-Fc, 5 μg/mL; PX Therapeutics, Grenoble, France). Cultures were initiated at 1 × 10⁶ CD34+ cells per well in X-VIVO-20 medium (Lonza, Basel, Switzerland) and supplemented with 20% defined fetal calf serum (HyClone); Thermo Fisher Scientific, Ilkirch, France) and cytokines hIL-7, human FMS-like tyrosine kinase 3 ligand (hFlt3-L), human stem cell factor (hSCF), and human thrombopoietin (hTPO) (each 100 ng/mL; Miltenyi Biotech). The vectors were pre-incubated with Vectofusin.
(12 μg/mL; Miltenyi Biotech) for 10 minutes. After 24 hours of culture, the LVs plus Vectofusin were added to the cells at the indicated MOIs. After 7 days of culture on DI4, cells were transferred to 24-well plates freshly coated with DI4-Fc at a concentration of 1 × 10^5 cells per well. The percentage of GFP^+ cells was determined in the thymic subpopulations by FACS.

A total of 1.8 × 10^5 bone marrow (BM) SCID-X1 CD34^+ cells were prestimulated for 16 hours in X-VIVO-20 (Lonza) supplemented with 20% fetal bovine serum and 300 ng/mL hSCF, 100 ng/mL hTPO, 300 ng/mL hFlt3-L, and 20 ng/mL hIL-7 on RetroNectin (25 μg/mL) alone or on RetroNectin and DI4-Fc ligand (9 μg/mL; PX’ Therapeutics)—coated wells. In addition, 100 ng/mL of hIL-7 was added for prestimulation on DI4—coated wells. The prestimulated cells were incubated with BaEVI-L-2RγC-LVs at an MOI of 10 in the presence of 4 μg/mL protamine sulfate for 6 hours. For cells transduced for 6 hours on DI4-coated wells, the medium was replaced by Minimum Essential Medium Eagle—Alpha Modification (Gibco, Life Technologies) with 20% fetal bovine serum, 2 mM glutamine (Gibco BRL/Invitrogen), 100 ng/mL hSCF, 100 ng/mL hTPO, 100 ng/mL hFlt3-L, and 100 ng/mL hIL-7. Viability after transduction was determined after exclusion of 7-amino-actinomycin-D^+ cells analyzed by FACS. The in vitro T-cell differentiation assay was performed on OP9-hDL1 stroma; 8 × 10^4 cells per well were cocultured in 6-well plates with OP9-hDL1.73 From week 2, the medium was changed once per week, and cells were analyzed by FACS (see supplemental Data).

**qPCR analysis**

IL-2RγC messenger RNA (mRNA) expression was assessed by using semi-quantitative real-time polymerase chain reaction (RT-PCR). RT-PCR was performed with equal amounts of cDNA using IL-2RγC and gyceraldehyde-3-phosphate dehydrogenase (GAPDH) TaqMan Gene Expression Assay in an Applied Biosystems ViIA 7 Real-Time PCR System (Applied Biosystems Inc, Foster City, CA). The IL-2RγC mRNA expression was normalized with the GAPDH mRNA expression as endogenous control. The γC mRNA expression was calculated as 2^-ΔΔCt.

**Quantification of vector copy number and analysis of TCR repertoire**

Duplex quantitative RT-PCR was performed with 100 ng genomic DNA using primers and TaqMan probes specifically designed to amplify the HIV and albumin (ALB) sequences in the Applied Biosystems ViIA 7 Real-Time PCR System. Simultaneously, standard amplification curves were generated by serial dilutions of the plasmid pRRL-LTR-PSI-GK-GFP-WPRE-ALB ranging from 10^7 to 10^3 copies per well. All the reactions were performed in duplicate. The reactions were normalized to ALB. The vector copy number (VCN) per cell was determined from the linear standard curve. Analysis of the TCR repertoire was performed by multiplex PCR followed by GeneScan analysis as described previously.74,75

**Conditioning and reconstitution of NSG mice**

NOD/SCIDγC^−/− (NSG) mice were housed in our animal facility (Plateau de Biologie Experimentale de la Souris, Lyon, France). Experiments were performed in accordance with the European Union guidelines upon approval of the protocols by the local ethical committee. Two- to 3-day-old newborn NSG mice were irradiated at 1 Gy, and 5 × 10^5 transduced progenitor T cells derived from hCD34^+ cells or 2 × 10^5 transduced CD34^+ cells were injected intrahepatically. Six weeks after reconstitution, the thymocytes of these mice were analyzed by FACS. Statistical analysis was performed using a Student t test.

**Results**

**BaEV-LVs permit high-level transduction of both naive and memory hT cells**

We previously obtained efficient LV pseudotyping with 2 cytolytic tail mutants of the BaEV GP: BaEVTR, in which the cytolytic tail has been replaced by that of the murine leukemia retrovirus, and BaEVRless, which lacks the R-peptide.69 The new BaEV-LVs were compared with other reported LV pseudotypes that allow CD3^+ T-cell transduction with differing efficiencies: H/F-LVs,62 RD114TR-LVs,64 and VSV-G-LVs.54

After TCR stimulation, the BaEV-LVs reached 60% to 90% CD3^+ T-cell gene transfer in the presence of RetroNectin (MOI, 10; Figure 1A). VSV-G-LVs and H/F-LVs reached similar transduction levels (Figure 1A). Both BaEVTR- and BaEVRless-LVs enabled efficient transduction of hIL-7 prestimulated hT cells and were superior to VSV-G-LVs at an MOI of 10 in the presence of RetroNectin (percentage transduction rates: BaEVTR-LVs, 29%-43%; BaEVRless-LVs, 41%-60%; VSV-G-LVs, 2%-12%) (Figure 1B). They permitted efficient high-level transduction of IL-7—prestimulated naive and memory T cells (Figure 1B). The cell viability was not altered by the transduction process (supplemental Figure 1A-B). H/F-LVs resulted in more than 80% transduction of IL-7—stimulated T cells in the presence of RetroNectin. In this case, the H/F-LVs primarily targeted memory T cells for transduction, confirming previous results (Figure 1B).

Strict gating on CD45RA^−, CD62L^−, and CCR7—expressing CD4^+ and CD8^+ naive T cells confirmed the highly efficient transduction of these subpopulations by BaEV-LVs (Figure 1C). The RD114TR-LVs resulted in much lower transduction efficiency of hIL-7—prestimulated naive and memory T cells compared with BaEV-LVs (RD114TR-LVs, 5%-12%).

We have previously shown that H/F-LVs allow gene transfer into resting CD3^+ T cells.62,76 The RD114TR- as well as BaEVTR- and BaEVRless-LVs performed poorly on these quiescent target cells (supplemental Figure 1C). Importantly, mRNA levels of both BaEV receptors (ASCT-1 and ASCT-2) were upregulated upon IL-7 stimulation, and the upregulation was even more pronounced after TCR activation of T cells (Figure 1D). This coincided with higher transduction levels obtained in these prestimulated T cells compared with their resting counterparts (Figure 1C vs supplemental Figure 1C). Overall, our results show that the novel BaEVTR- and BaEVRless-LVs are promising gene transfer tools for adult T-cell gene therapy because they can target long-lived naive T cells at low vector doses.

**BaEV-LVs GPs allow high-level transduction of recent thymic emigrants**

T-cell gene therapy or immunotherapy would benefit from genetically modified naive T cells that are able to persist for the individual's entire life span. Here, we tested the different LV pseudotypes for their ability to efficiently transduce CB CD4^+ T cells, which are phenotypically similar to recent thymic emigrants (RTEs).
To conserve the naive T-cell phenotype, CB T cells were prestimulated with IL-7 and subsequently transduced with the different LV pseudotypes encoding GFP at MOI 10 (Figure 2A). BaEVTR- and BaEVRless-LVs permitted 40% to 55% transduction of the target cells at MOI 10 and thus outperformed VSV-G-LVs (<20%) and RD114TR-LVs (<10%; Figure 2A). In contrast, RTEs and mature naive CB T cells were poorly permissive for H/F-LVs$^{62}$ (Figure 2A). Importantly, BaEVTR- and BaEVRless-LVs easily reached 55% to 70% transduction when the MOI was doubled to 20 (Figure 2A vs supplemental Figure 2). The RTEs (CD31$^+$) were transduced to the same extent as the mature naive CB CD4$^+$ T cells (CD31$^-$; Figure 2A) without an effect on the distribution of these 2 naive CB T-cell populations (Figure 2B).

**BaEV-LVs promote highly efficient transduction of human thymocytes**

It would be of major interest to correct T cells or genetically change T-cell functions as early as possible, ideally during thymic development, which is a tightly regulated process (Figure 3A). We transduced human thymocytes with RD114TR-, BaEVTR-, BaEVRless-, and H/F-LVs (MOI, 10) in the presence of RetroNectin and IL-7. VSV-G-LVs were used at an MOI of 50. Four days later, we determined the transduction levels by FACS in the thymic subsets using CD4/CD8/CD3 staining. VSV-G-LVs transduced only 10% to 20% of the different thymic subpopulations (Figure 3B). RD114TR-, BaEVTR-, and BaEVRless-LVs preferentially transduced early thymocytes (ie, double-negative [DN] and immature single-positive cells; Figure 3A-B). The efficiency of RD114TR-LV transduction of all thymic subpopulations was lower compared with that of BaEV-LVs. Interestingly, H/F-LVs did not show a subpopulation preference and ensured an efficient transduction of all thymic subsets (Figure 3B).

The DN CD4$^+$CD8$^+$ population can be subdivided into early lymphoid progenitors (ELPs) and early thymocyte progenitors (ETPs), which then differentiate into proT and preT cells (see Figure 3A and supplemental Figure 3 for the gating strategy). BaEV-LVs targeted transduction...
to the most immature DN thymocytes; BaEVReless-LVs reached 100% of the ETPs and 90% of proT cells (Figure 3C). VSV-Gand RD114TR-LVs resulted in much lower transduction levels. H/F-LVsmediated equivalent high levels of transduction in ETP, proT, andpreT cells (Figure 3C).

Our results indicate that BaEV-LVs are better tools than VSV-G-and RD114TR-LVs for the transduction of ETPs and proT cells withoutpopulation skewing (supplemental Figure 4). This prompted us to test these vectors for transduction of in vitro–generated thymicprogenitors.

**BaEV-LVs mediate efficient transduction of hT-cellprogenitors generated in vitro**

We made use of our new culture system based on immobilized Dll4, as described previously. CB CD34+ cells were cultured on Dll4–coated plates in the presence of cytokines inducing T-cell differentiation. After 24 hours, the different LV pseudotypes were added at an MOI of 10. As observed for freshly isolated thymocytes, BaEV-LVs efficiently transduced ELPs (65%-75%) and ETPs (70%-80%) at high levels when generated in vitro (Figure 3C vs Figure 4A-B). As expected, VSV-G- and RD114TR-LVs permitted only poor transduction of ELPs (15%) and ETPs (10%), whereas H/F-LVs achieved transduction levels of 60% (Figure 4A-B). CD7+ CD34− proT cells appeared on day 7 of differentiation (supplemental Figure 5A). BaEV-LVs performed stable high-level gene transfer into both ELPs and ETPs but also into proT cells without skewing these T-cell populations, whereas VSV-G- and RD114TR-LVs were much less efficient (supplemental Figure 5B-C) in agreement with Hübner et al. In contrast, the H/F- and BaEV-LV transduction levels were equivalent at day 7 (supplemental Figure 5B). On day 14, a subset of CD34− CD7+ started to express CD5, allowing us to distinguish between proT1 (CD7+/CD5−) and proT2 (CD7+/CD5+) T-cell stages followed by CD1a expression (proT-cell stage; supplemental Figure 6A). As expected, the highly efficient transductions obtained with BaEV-LVs persisted at day 14 in the ELPs, ETPs, proT1 and proT2 thymocytes, and also in the preT cells (Figure 4C; supplemental Figure 6A). Importantly, differentiation into ETPs and proT cells was not perturbed by the transduction process (supplemental Figure 6B). Because the original CD34+ cells had expanded 50-fold, on average, after 14 days of T-cell differentiation, they went from 5 × 106 CD34+ cells to 2.5 × 108 T-cell progenitors (ELP, ETP, proT, and preT); these findings confirmed stable transduction in the different T-cell populations.

**BaEV- and H/F-LV–transduced T-cell progenitorsseed the thymus and enable accelerated in vivothymopoiesis compared with HSCs in NSG mice**

The in vivo T-cell differentiation potential of LV-transduced progenitor T cells and HSCs was evaluated by transplantation into NSGmice. CD34+ cells were plated on Dll4-coated wells with cytokines and then incubated with BaEV- and H/F-LVs at MOI 10 or without vector in the presence of Vectofusin. On day 8 of T-cell differentiation, these transduced T-cell progenitors were injected intrahepatically into NSG neonates. In parallel, CD34+ cells from the same donor were briefly prestimulated with cytokines and were then transduced with BaEV- and H/F-LVs (MOI, 10) or without vector in the presence of RetroNectin. Twenty-four hours after transduction, these transduced CD34+ cells were transplanted into a second group of NSG recipients (Figure 5A). At 6 weeks after engraftment, high levels of transduction were detected by FACS in all thymic subpopulations for both NSG groups (Figure 5B). However, intrathymic T-cell development was accelerated in recipients of T-cell progenitors compared with recipients of hCD34+ cells, which was confirmed by higher percentages of mature CD3+ CD4+ and CD3+ CD8+ T cells (Figure 5C, left vs right panel). Indeed, the CD4+ single-positive (CD4-SP) cells in progenitor T-cell NSG recipients were all CD3+ (Figure 5C, left panel). For the mice that received CD34+ cells, CD4-SP cells did not express CD3 and still corresponded.
to the earlier immature single-positive CD4 stage (Figure 5C, right dot blots). Overall, mature CD3⁺ T-cell levels in the thymus and the periphery were significantly higher in progenitor T-cell recipients than in CD34⁺ recipients, and they expressed TCRαβ (Figure 5D-F; supplemental Figure 7). Importantly, this was true for both nontransduced and LV-transduced progenitor T cells, which emphasizes that transduction did not affect T-cell potential in vivo (Figure 5E-F). To summarize, the BaEV- and H/F-LVs performed similarly for transduction of T-cell progenitors, we selected BaEV-LVs encoding the γC receptor to correct the γC deficiency in BM SCID-X1 CD34⁺ cells under conventional conditions or in combination with the already established DLL4-based T-cell progenitor differentiation technique. Therefore, BM SCID-X1 CD34⁺ cells were cultured in the presence of RetroNectin alone (conventional) or DLL4 and RetroNectin (DLL4 culture; supplemental Figure 8A) and then transduced or not (mock) with BaEV-LVs encoding the γC receptor at an MOI of 10. FACS analysis did not show any major differences in the frequencies of gated lymphocytes between the transduced and mock groups, and 97% of the gated lymphocytes were viable (supplemental Figure 8B). Thus, BaEV-LV transduction had no toxic effect on the cells. After 4 days of culture, T-cell progenitor
Figure 4. BaEV-LV GPs confer efficient gene transfer into in vitro--generated early T-cell progenitors. CB CD34^+ cells were plated on Dll4-coated culture wells in the presence of a cytokine cocktail (IL-7, TPO, Flt3-L) for 24 hours, and they were subsequently transduced with VSV-G-, RD114TR-, BaEVTR-, BaEVRless- and H/F-LVs.
differentiation revealed higher frequencies and numbers of CD7+ early T-cell progenitors in the Dll4 culture than in conventional cultures (Figure 6A-B), suggesting that the Dll4 cultures were able to generate early T-cell progenitors. We then evaluated the efficiency of gene correction by testing the ability of transduced cells to differentiate into T cells. Transduced and nontransduced cells were cocultured with an OP9-hDL1 stromal cell layer, and their T-cell differentiation status was analyzed (supplemental Figure 8A). The VCN in CD7+ cells sorted after 5 weeks of coculture reached 4.04 in BaEV-LV transduced conventional cultures and 5.94 in BaEV-LV transduced Dll4 cultures (Figure 6C). IL-2RγC mRNA levels were 60-fold higher in the transduced groups than in the mock groups in CD45+ cells sorted at week 7 and, consistent with the high VCN, approximately threefold higher than in healthy CB CD34+ cells under Dll4 culture conditions (Figure 6D). CD4+ CD8+ (DP) cells and CD3+ cells were present as early as week 2 in the transduced Dll4 culture conditions and week 3 in the transduced conventional culture conditions and continued to be detected afterward in higher numbers in the Dll4 culture conditions until week 5. This suggested accelerated restoration of T-cell differentiation in the BaEV-LV transduced Dll4 culture conditions (Figure 6E-F; supplemental Figure 8C). Importantly, the CD3+ cells were found to express either TCRγδ or TCRαβ; this was especially true in the Dll4 culture conditions (supplemental Figure 6D-E). TCR repertoire analysis of the polyclonality in CD7+ cells sorted at week 5 revealed only diverse TCRγδ rearrangement but not TCRαβ rearrangement in nontransduced conditions (Figure 6G; supplemental Figure 8F); this was consistent with the appearance of TCRγδ+/CD3+ cells but not TCRαβ+/CD3+ cells in the later weeks (supplemental Figure 8D-E). This was also in line with the leaky mutation in the γC gene found in this particular patient. However, the DP and CD3+ cell counts remained very low in these mock groups (Figure 6E-F). Conversely, TCRγδ and TCRαβ rearrangements were polyclonal under BaEV-LV transduction conditions regardless of the presence or absence of Dll4; this confirmed the polyclonality of differentiated cells and the absence of any deleterious effect of Dll4 on the polyclonality of cells (Figure 6G; supplemental Figure 8F). Our data demonstrate the correction of γC deficiency in BM SCID-X1 CD34+ cells under both conventional and Dll4 culture conditions. However, T-cell differentiation was faster and more efficient under Dll4 conditions. Collectively, BaEV-LVs allowed efficient correction of the γC defect in SCID-X1 HSCs with high VCN, high levels of γC expression, and de novo T-cell differentiation from SCID-X1 HSPCs.

Discussion

Here we highlighted the exceptional capacity of BaEV-LVs to transduce 60% of IL-7–stimulated naive adult T cells and CB RTEs. In contrast, VSV-G- or RD114TR-LVs performed poorly. Remarkably, BaEV-LVs transduced up to 80% of early thymocytes such as ETPs, proT-cell, and preT-cell lineages differentiated from CD34+ cells using a feeder-cell–free Dll4 culture system.50 Hence, BaEV-LVs constitute a promising tool for the transduction of naive and early progenitor T cells, whereas H/F-LVs are complementary because they can efficiently transduce memory T cells and late-stage thymocytes: CD4+ CD8− and CD4− SP cells. Accordingly, we confirmed the upregulation of the BaEV receptors67,80 upon IL-7 and TCR T-cell stimulation coinciding with increased BaEV-LV–mediated gene transfer.

A successful T-cell immunotherapy consists of engineering autologous or allogeneic T cells to express CARs specific for either viruses or tumor antigens.23,28 Recently, this CAR T-cell technology has been translated to naive cells from CB.81 Micklethwaite et al16 transduced CB T cells with a CAR against CD19, which also coexpressed TCRs specific for 3 different viruses that can affect patients after a CB transplant. When compared with adult T cells, naive CB T cells showed a greater tolerance to HLA disparity82,83 and thus induced graft-versus-host disease less frequently. Therefore, naive CB T cells represent a source of allogenic lymphocytes for cell therapy of viral infections or/and cancer, particularly when patients do not have enough autologous T cells. Indeed, IL-7–stimulated CB T cells combined with BaEV-LVs resulted in gene delivery levels of 70%. This might constitute an important advantage for CAR therapy because naive CB T-cell subsets show a high capacity to expand and might provide longer-lasting antitumor responses in vivo than adult T cells, allowing protection against relapse after transplant.88,89

Infections and relapse are the main causes of morbidity and mortality after allogenic HSC transplantation. This is undoubtedly related to delayed T-cell immune reconstitution and impaired de novo thymopoiesis.84-86 The same is true for autologous HSC-mediated gene therapy. Complete restoration of a polyclonal T-cell repertoire takes even longer, and patients can be immunocompromised for more than a year.87,88 In the case of gene therapy, the administration of autologous gene-corrected T-cell progenitors might be an option. We can genetically modify these T-cell progenitors derived from CD34+ cells at high levels using the BaEV- or H/F-LVs. Combination of the transduction with the Dll4 culture of hCD34+ cells, resulted in the expansion of gene-marked ETPs and proT1 cells, the most primitive T cells. Moreover, these cells retained their in vivo intrathymic T-cell differentiation capacity and displayed a conventional CD4, CD8, and TCRαβ/TCRγδ repertoire. Furthermore, T-cell reconstitution was faster than in hCD34−cell-engrafted NSG mice.50

These data suggest that BaEV- or H/F-LVs are valuable treatment options for SCIDs (eg, SCID-X1) and HIV, in which the thymic environment is not ideal for HSC differentiation into T-cell lineages. We have previously evidenced faster immune reconstitution by

Figure 4. (continued) encoding the GFP reporter at an MOI of 10, except for VSV-G-LVs for which a higher vector dose (MOI of 50) was applied. (A-B) At day 3 of culture, the cells were analyzed by FACS for surface expression of CD34 and CD7. The gating strategy to determine GFP expression in the subpopulations of ELP, ETP, and proT cells are shown for differentiation at day 3 in panel A for a representative experiment. The transduction levels for day 3 of differentiation toward T-cell lineage in these subpopulations are summarized in the histograms in panel B (mean ± SD; n = 3). (C) At day 14 of culture, the cells were analyzed by FACS for surface expression of CD34, CD7, CD5, and CD1a (for gating strategy, see supplemental Figure 6). GFP expression in the subpopulations ELP, ETP, proT1, proT2, and preT cells at day 14 of differentiation toward T-cell lineage is shown for the different pseudotypes (mean ± SD; n = 3; VSV-G vs other pseudotypes). **P < .01.
Figure 5. LV-transduced progenitor T cells allowed accelerated thymopoiesis in vivo in NSG mice compared with LV-transduced CD34+ cells. (A) CD34+ cells were plated on Dll4-coated wells in the presence of a cytokine cocktail (IL-7, TPO, Flt3-L) for 24 hours, and they were subsequently transduced with BaEV- and H/F-LVs encoding GFP at an MOI of 10 or without vector in the presence of Vectofusin, an agent that facilitates transduction. At 8 days of T-cell differentiation, these transduced T-cell progenitors were injected intrahepatically into NSG recipient mice. In parallel, CD34+ cells from the same donor were prestimulated briefly with cytokines that maintain HSC potential (SCF, TPO, Flt3-L) for 24 hours and were transduced with BaEV- and H/F-LVs (MOI, 10) or without vector in the presence of RetroNectin. At 24 hours after...
coinfusion of HSC-derived T-cell progenitors and CB HSPCs in the NSG mouse model. Infusion of autologous corrected T-cell progenitors together with corrected HSCs might accelerate immune reconstitution and reduce the gap in immune system recovery. In a clinical setting, part of the patient’s HSPCs would be gene-corrected with the classical protocol and another part would be gene-corrected with the Dll4-based protocol described herein. The 2 products could be transplanted simultaneously. We have shown that T-cell progenitors are able to directly seed the thymus without passing through the BM, thus overcoming 2 main obstacles of post-HSCT T-cell generation: the long time required for the first BM and thymic phases and the limited thymic supply by BM progenitors. We are currently testing this hypothesis in a clinical trial of cell therapy for SCID patients who received a transplantation in a haplo-identical setting.

This is supported by our BaEV-LV–based correction of γC-deficient CD34+ cells in the presence of Dll4, resulting in a large amount of T-cell progenitors. We previously confirmed that BaEV-LVs also allow high-level transduction of prestimulated and unstimulated hHSCs. Remarkably, γC-encoding BaEV-LVs efficiently rescued SCID-X1 CD34+–derived T-cell development in vitro, showing the clinical feasibility of this approach.

In conclusion, H/F-LVs and, in particular, BaEV-LVs represent very useful new tools for applications in T-cell gene therapy and immunotherapy. In addition, injection of BaEV-LV corrected autologous HSCs and T-cell

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**Figure 5.** (continued) transduction, the transduced CD34+ cells were engrafted in a second group of NSG recipients. Human thymocyte reconstitution was assessed 6 weeks after injection into NSG mice by FACS. (B) Percentage of GFP+ thymocytes (CD4 SP, CD4+CD8–; DP, CD4+CD8+; DN, CD4-CD8–; CD8 SP, CD8–CD4–) for T-cell progenitor engraftment compared with CD34+ cell engraftment. (C) Distribution of CD4 SP, DP, DN, and CD8 SP thymic subpopulations for proT cells compared with CD34+ cell engraftment. CD4 SP cells were analyzed for CD3 surface expression for proT (left panels) and CD34+ (right panels) engrafted cells. ISP CD4, CD4–CD3– cells. (D) FACS analysis of the percentage of CD3+ T cells per total hCD45+ cells in the thymus. (E) The absolute numbers of mature CD3+ T cells per total human thymocytes for proT cells compared with CD34+ cell engraftment (mean ± SD; n = 3). (F) Percentage of mature CD3+ T cells in the blood per total hCD45+ cells for proT cells compared with CD34+ cell engraftment (mean ± SD; n = 3). *P < .05.
Figure 6. Efficient T-cell differentiation in vitro of SCID-X1 BM CD34+ HSPCs after transduction with BaEV-LVs encoding γC receptor. SCID-X1 BM CD34+ HSPCs were transduced (BaEV) or not (mock) with BaEV-LVs encoding IL-2RγC at an MOI of 10 for 6 hours after 16 hours of prestimulation with cytokines in either RetroNectin or Dll4 plus RetroNectin-coated wells (see supplemental Figure 6A). The X-SCID mutation is a breakpoint mutation in intron 3 of the IL-2RG gene, which is a γC leaky mutation. Flow cytometry analyses indicating the percentage (A) and absolute cell number (B) of CD34+CD7+ and CD34–CD7+ T-cell progenitors after 4 days of culture in the presence (+Dll4) or absence (−Dll4) of Dll4. After 4 days of culture, the cells were further cultured with OP9-hDL1 stromal cells to pursue the T-cell differentiation process. Analyses of VCN per cell in the genome by duplex quantitative PCR in sorted CD7+ cells at week 5 (C) and IL-2RγC mRNA expression by semi-quantitative RT-PCR in hCD45+ cells at week 7 (D) of differentiation (compared with CB CD34+ T-cell differentiation cultures in parallel). During coculture with OP9-hDL1 stromal cells, mock and BaEV-LV transduced cells were analyzed once per week by flow cytometry. Analyses of absolute cell numbers of CD4+CD8+ (E) and CD3+ cells (F) at the indicated weeks after OP9-hDL1 cocultures. (G) TCR repertoire analysis by multiplex PCR followed by GeneScan analysis in CD7+ cells sorted at week 5 after OP9-hDL1 cocultures. All the populations were gated on live 7-amino-actinomycin-D+ cells.
progenitors might produce both short-term recovery of T-cell immune response and long-term correction in the patients.

Acknowledgments

The authors thank the staff of the Plateau de Biologie Expérimentale de la Souris animal care facility at the Ecole Normale Supérieure de Lyon, the flow cytometry platform (SFR BioSciences Lyon [UMS3444/US8], Lyon, France, and Imagine Institute, Paris, France), and Gisèle Froment, Didier Nègre, and Caroline Costa from the lentivectors production facility/SFR BioSciences Lyon (UMS3444/US8, Lyon, France).

This work was supported by French grants from AFM, ANRS, ARC, and LABEX and grants from the European Community (FP7-HEALTH-2007-B/222878 “PERSIST” and “GENTHERTUAL-PLUS”), by INSERM, a European Union H2020 grant (SCIDNet 666908), an INCA-Plan Cancer grant (2009-2013), and a public grant overseen by the French National Research Agency (ANR) as part of the Investissements d’Avenir program (reference: ANR-10-IHU-01). R.M. was supported by a scholarship from the French ministry, and K.M. was supported by a scholarship from the China Scholarship Council.

Authorship

Contribution: E.V. coordinated the project, designed and performed experiments, analyzed and discussed the data, and wrote the manuscript; I.A. designed and performed experiments, analyzed and discussed the data, and wrote the manuscript; O.B., F.A., A.G.-G., C.C., C.L., D.N., and R.M. performed experiments, analyzed the data, and discussed results; R.D.M., H.S., C.L.-P., K.M., and M.C. performed T-cell differentiation of SCID-X1 CD34<sup>+</sup> cells, analyzed data, discussed results, and corrected the manuscript; V.A. and A.C. provided human thymocytes, critical discussion, and technical advice; C.R. provided technical advice and helped with design of experiments; D.F. provided Vectofusin and technical advice; and F.-L.C. provided critical discussion and read the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests

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