Multiply Attenuated, Self-Inactivating Lentiviral Vectors Efficiently Deliver and Express Genes for Extended Periods of Time in Adult Rat Cardiomyocytes In Vivo

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Background—Among retroviral vectors, lentiviral vectors are unique in that they transduce genes into both dividing and nondividing cells. However, their ability to provide sustained myocardial transgene expression has not been evaluated.

Methods and Results—Multiply attenuated, self-inactivating lentivectors based on human immunodeficiency virus-1 contained the enhanced green fluorescent protein (EGFP) gene under the transcriptional control of either the cytomegalovirus (CMV) immediate-early enhancer/promoter, the elongation factor-1α (EF-1α) promoter, or the phosphoglycerate-kinase (PGK) promoter. Lentivectors transduced adult rat cardiomyocytes in a dose-dependent manner (transduction rates, >90%; multiplicity of infection, ≈5). The CMV promoter achieved higher EGFP expression levels than the EF-1α and PGK promoters. Insertion of the central polypurine tract pol sequence improved gene transfer efficiency by ≈2-fold. In vivo gene transfer kinetics was studied by measuring the copy number of integrated lentivirus DNA and EGFP concentrations in cardiac extracts by real-time polymerase chain reaction and ELISA, respectively. With CMV promoter-containing lentivectors, vector DNA peaked at day 3, declined by ≈4-fold at day 14, but then remained stable up to week 10. Similarly, EGFP expression peaked at day 7, decreased by ≈7-fold at day 14, but was essentially stable thereafter. In contrast, vector DNA and EGFP expression declined rapidly with EF-1α promoter–containing lentivectors. Peak EGFP expression with titer-matched adenovectors was ≈35% higher than with CMV lentivectors but was lost rapidly over time.

Conclusions—Lentivectors efficiently transduce and express genes for extended periods of time in cardiomyocytes in vivo. Lentivectors provide a useful tool for studying myocardial biology and a potential system for gene heart therapy.

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Plasmid DNA and adenoviral vectors have been used in gene therapy trials involving cardiac delivery of angiogenic genes in patients with coronary artery disease.1,2 However, plasmid DNA and adenovectors are limited by poor DNA uptake and short-lived transgene expression, respectively. In addition, both vectors do not integrate into the cell chromatin. In contrast, vectors derived from retroviruses such as the Moloney murine leukemia virus, which belong to the Oncoretroviridae genus, integrate their genome into the host chromosomes, thus providing a potential for permanent gene modifications of target cells. However, these vectors require the breakdown of the nuclear envelope during mitosis for the nuclear translocation of the preintegration complex, and hence, they do not transduce nondividing cells efficiently.3

Among the Retroviridae, the genus Lentiviridae is unique in that these viruses, including human immunodeficiency virus (HIV)-1, are independent of cell division for completion of their replication cycle.3 Nuclear localization signals mediate the transport of the HIV-1 preintegration complex through the nucleopore during interphase.4 HIV-1–based vectors delivered genes effectively to several tissues, including the brain, liver, skeletal muscle, and retina, in vivo.5–7 However, they are not equally efficient in all cells. For instance, human hematopoietic stem cells and monocytes are susceptible to lentiviral gene transduction only on stimulation with cytokines and fetal serum, respectively.8,9 Previous reports have shown HIV-1–based gene transduction in fetal human cardiomyocytes and rat cardiomyocytes.10–13 However, lentivectors used in these studies were not optimized for cardiomyocyte transduction. Moreover, their ability to provide sustained transgene expression in the myocardium in vivo has not been evaluated.

The present study was designed both to optimize the efficiency of lentivirus-mediated gene transfer into adult...
cardiomyocytes and to evaluate the duration of transgene expression in adult myocardium in vivo.

Methods
Transfer Vector Constructs
Self-inactivating (SIN) vectors were produced from the SIN-18 vector, which contains a deletion in the U3 region of the 3′-long terminal repeat (LTR). The enhanced green fluorescent protein (EGFP) gene was subcloned into this vector, which is deleted in major HIV-1 pathogenicity factors (Anf, Swif, Δ3pr, Δ5pr) and the envelope protein (Δenv). The EGFP gene was under the transcriptional control of the human cytomegalovirus (CMV) immediate-early enhancer/promoter, the human elongation factor-1α (EF-1α), or the murine phosphoglycerate kinase (PKG) promoter. The posttranscriptional regulatory element of woodchuck hepatitis virus was inserted between the EGFP gene and the 3′-LTR. A 118-bp sequence from pol of HIV-1 encompassing the central polyuridine tract (cPPT) and termination sequences was subcloned upstream of the transgene promoter to generate SIN.cPPT.CMV-EGFP-W, SIN.cPPT.EF1α-EGFP-W, and SIN.cPPT.PKG-EGFP-W.16

Packaging Constructs
pMDLg/pRRE is a CMV-driven expression plasmid that encodes the viral capsid. It contains the gag, pol coding sequences and a 374-bp Rev-responsive element (RRE)—containing sequence. pCMVDR-8.92 is another capsid-encoding plasmid derived from pCMVDR-8.91, which contains the tat coding sequence and a destruction of the BamHI restriction site in the coding region of rev. RSV-Rev is a rev-expressing plasmid in which the second and third exons of tat are under the control of the Rous sarcoma virus U3 promoter. pMD2.G is a CMV-driven expression plasmid that encodes the vescular stomatitis virus (VSV)-G envelope protein. Packaging plasmids were kindly provided by Luigi Naldini, University of Turin (Italy), and Didier Trono, University of Geneva (Switzerland).

Lentiviral Vectors
VSV-G—psuedotyped, HIV-1—based vector particles were produced by cotransfection of 4 plasmids (pMDL/gpRRE, 12 μg; pRSVrev, 3 μg; pMD2.G, 5 μg; SIN vector, 20 μg) onto 293T cells. The tat-deficient pMDL/gpRRE plasmid was used to make virus preparations for in vitro studies, whereas the tat-containing pCMVDR-8.92 plasmid was used to make higher-titer virus preparations for in vivo studies. Culture medium was replaced by serum-free SFM-II with 10% FCS and 2% FCS. To inhibit fibroblast outgrowth, 2.5 × 10⁵ cells were cultured in a 10-cm dish. Cells were cultured at 37°C in a humidified atmosphere of 5% CO₂ in air.

In Vitro Gene Transfer
Ventricular cardiomyocytes of 12- to 16-week-old male Wistar rats were isolated by retrograde heart perfusion with type II collagenase, as described. Six well-plates were coated with 2 μg/cm² laminin. Culture medium M-199 (Sigma) contained 5 mM/L creatine, 2 mM/L L-carnitine, 5 mM/L taurine, 1 μg/mL insulin, 1% penicillin/streptomycin, and 2% FCS. To inhibit fibroblast outgrowth, 20 μg/mL cytosine arabinoside was added daily. Fresh medium without cytosine arabinoside was added 24 hours before infection. Cardiomyocyte culture purity was >95% by immunocytochemistry with a mouse anti-rat α-sarcomeric actin monoclonal antibody (mAb) (Sigma). Unless stated differently, cardiomyocytes were infected at culture-day 5 with various multiplicities of infection (MOI ~1 to 50). They were harvested at day 5 after infection for EGFP expression analysis by fluorescence microscopy and FACS. To demonstrate lentivirus-specific gene transfer, myocytes were incubated with 0.1 to 5 μg/mL Efavirenz (Roche), a nonnucleoside viral reverse transcriptase inhibitor.

Histological and Immunohistochemical Analysis
At varying time intervals (1 to 10 weeks) after gene transfer, rats were killed by lethal pentobarbital injection and either perfusion-fixed with PBS/4% paraformaldehyde (for fluorescence microscopy) or perfused with PBS and snap-frozen in liquid nitrogen (for PCR, EGFP-ELISA, and immunohistochemistry). Two series of 8-μm sections (n = 40 each) per heart were cut at 200-μm steps parallel to the atrioventricular groove and analyzed by direct fluorescence microscopy and immunohistochemistry with the following mAbs: anti—ED1-like (IC7) for macrophage/microglia, anti—CD45RA on B cells (OX-33), anti—TCRαβ (R73), anti—CD8α (OX-8; all from Pharmingen), anti—CD4 (W3/25; Accurate Chemicals), irrelevant mouse mAb MOPC-31C (Pharmingen), and biotin-conjugated rabbit F(ab')2, anti-mouse Ig (Jackson ImmunoResearch Laboratories), followed by StreptABComplex/HRP and DAB/H₂O₂ (Dako). Morphometric analysis was performed in 7 microscopic fields (400 × 400 μm) per section (n = 5 per heart). Three fields covered the injected area (0 to 400 μm from the injection center) and 4 fields the adjacent areas (≈500 to 1000 μm from the injection center). Positive-staining areas were measured with the NIH Image-1.62 program.

ELISA
Hearts were excised, weighted, and homogenized. Total cytoplasmic proteins were extracted by use of the NE-PER kit (Pierce). Intact cell nuclei were kept separately for PCR. EGFP concentrations in cardiac extracts were measured by ELISA using React-bind anti-GFP coated plates (Pierce). Briefly, 100 μL of sample in Assay Diluent for OptEIA (Pharmingen) was coated for 1 hour at room temperature. Plates were washed, and rabbit anti-GFP serum (IgG fraction; Molecular Probes) was added (1 hour), followed by goat anti-rabbit biotinylated Ab (1 hour), streptavidin—horseradish peroxidase (45 minutes), and TMB substrate. The reaction was stopped with 100 μL H₂SO₄, 1.8N. Plate reading was done at 450 nm. A standard curve (0.8 to 4000 pg/mL) with recombinant GFP (Molecular Probes) was made. Background values in PBS-injected hearts were subtracted from measurements in gene-transduced hearts.
Insertion of the cPPT-sequence into SIN.CMV-EGFP-W

cPPT Sequence Enhances Gene Transfer

Quantitative PCR

High-molecular-weight genomic DNA was extracted (Qiagen) from nuclear pellets of cardiac extracts. To quantify integrated lentivirus DNA, real-time quantitative PCR (ABI Prism 7700; Perkin-Elmer Applied Biosystems) using the SYBR-green dye was made on \( \sim 200 \) ng genomic DNA. Primer sequences were as follows: lentivirus LTR, 5'-GTTAGACCAGATCTGAGCCTGGG-3' (sense), 5'-CAACAGACGGGCACACTTGG-3' (antisense); \( \beta \)-actin, 5'-GATACTCCAGCACACTTAACTTAGC-3' (sense), 5'-CAAGTCCTAAAGCCACAAGAAC-3' (antisense). The copy number of integrated lentivirus genome was normalized per 10^6 cell genome copies, as determined by \( \beta \)-actin PCR.

Statistical Analysis

Comparisons between lentivectors, adenovectors, and PBS were made by the Friedman test (a nonparametric test for matched groups).

Results

Lentivectors Efficiently Transduce Cultured Cardiomyocytes

Lentivectors transduced cultured adult rat cardiomyocytes in a dose-dependent manner. With SIN.cPPT.CMV-EGFP-W (MOI, \( \sim 5 \)), 94% of myocytes expressed EGFP (Figure 1A). Higher MOIs (\( \sim 10 \) to 50) resulted in increased EGFP expression (Figure 1B and C). Both freshly isolated rod-shaped and dedifferentiated myocytes (culture-day 5) were efficiently transduced (\( \geq 90\% \); not shown). Efavirenz prevented gene transfer in a dose-dependent manner (\( \geq 95\% \); not shown), demonstrating lentiviral transduction. Promoter activities in transduced myocytes were measured as mean fluorescence values (MFVs) by FACS. At an MOI of \( \sim 20 \), the CMV promoter achieved \( \sim 130 \)- and \( \sim 40 \)-fold higher MFVs than the EF-1\( \alpha \) and PGK promoters, respectively (Figure 2).

cPPT Sequence Enhances Gene Transfer

Insertion of the cPPT-pol sequence into SIN.CMV-EGFP-W (MOI, \( \sim 5 \)) increased the number of EGFP-positive myocytes (63% versus 36%) at day 2 and MFVs at day 3 after infection (Figure 3, A and B). At day 5, similar proportions of EGFP-positive cells were found with and without cPPT (97% versus 87%), but MFVs remained \( \sim 2 \)-fold higher with cPPT. Similar results were observed at an MOI of \( \sim 50 \) (Figure 3, C and D). Thus, the cPPT sequence improves the kinetics of lentiviral gene transfer into cardiomyocytes.

In Vitro Comparison of Lentiviral and Adenoviral Vectors

At day 3 after infection (MOI, \( \sim 2 \)), EGFP expression was detected microscopically in a majority of myocytes by use of adenovectors but not lentivectors (Figure 4C). At day 4, EGFP was detectable in a majority of cells with both lentivectors and adenovectors (77% versus 89%; Figure 4A), although MFVs were \( \sim 9 \)-fold lower with lentivectors (9 versus 79; Figure 4B). Differences in MFVs between the 2 vectors decreased by \( \sim 3 \)-fold when higher MOIs of \( \sim 20 \) were used (71 versus 190). Thus, both vectors transduce similar proportions of myocytes, but the kinetics of gene transfer is slightly slower with lentivectors.

Lentivectors Transduce Genes Into Adult Cardiomyocytes In Vivo

After intramyocardial lentivector injection (10^5 TU), cardiomyocytes accounted for the vast majority of EGFP-expressing cells, as evidenced by striated appearance and \( \alpha \)-sarcinergic actin immunostaining (Figure 5E). At the injection site, up to \( \sim 5 \)% of myocytes were EGFP-positive by direct fluorescence microscopy at day 7 after infection (Figure 5, B and C). By comparison, titer-matched adenovectors achieved \( \sim 6 \)-fold higher numbers of EGFP-positive myocytes, which were distributed on larger myocardial areas (Figure 5A). At 10 weeks after transduction, EGFP-expressing myocytes were detected with lentivectors only (Figure 5D). No EGFP-positive myocytes and very low EGFP tissue concentrations were found after in vivo intrapericardial or ex vivo intracoronary instillation of lentivectors, unlike adenovectors (data not shown). In contrast, ex vivo intramyocardial lentivector injection (5 \times 10^7 TU in 300 \( \mu \)L) into donor hearts resulted in significant EGFP expression (649 pg/g tissue) 7 days after heart transplantation into syngeneic recipients.

Lentivirus DNA Decreases Early After Gene Transfer but Is Stable Thereafter

At day 3 after infection, real-time PCR showed peak median values of 1.85 \times 10^5 and 2.3 \times 10^5 copies of integrated lentivi-
rus DNA per $10^6$ cell genomes with CMV and EF-1α promoter-containing vectors, respectively (Figure 6, A and C). With CMV vectors, integrated lentivirus DNA declined by $\approx$3-fold ($5.4 \times 10^4$ copies/$10^6$ genomes) by day 14 but remained stable thereafter ($5.0 \times 10^4$ copies/$10^6$ genomes at 10 weeks). With EF-1α vectors, lentivirus DNA decreased by $\approx$5-fold by day 14 ($4.4 \times 10^4$ copies/$10^6$ genomes) and by $\approx$3-fold thereafter ($1.6 \times 10^4$ copies/$10^6$ genomes at 10 weeks).

**Lentiviral Vectors Achieve Prolonged Transgene Expression In Vivo**

With CMV lentivectors, EGFP was detectable by ELISA at day 3 (5200 pg; normalized per gram of tissue), peaked at day 7 (36 348 pg), and declined by $\approx$7-fold (5125 pg) by day 14 (5125 pg), then by $\approx$2-fold between days 14 and 28 (2427 pg), and by $\approx$60% between day 28 and 10 weeks (1490 pg; Figure 6B). EF-1α lentivectors achieved similar EGFP levels at day 7 ($42 \times 10^2$ pg; Figure 6D), followed by an $\approx$7-fold decline by day 14 (5820 pg) to barely detectable levels at 4 and 10 weeks (10 to 325 pg). Using adenoviral vectors, peak EGFP levels were $\approx$35% higher (55 250 pg) than with CMV lentivectors but were barely detectable at 10 weeks (154 pg; Figure 6E).

**Systemic Vector Dissemination**

After intramyocardial injection, lentivirus DNA was detected by PCR in spleen ($7 \times 10^3$ copies/$10^6$ genomes) and liver ($3 \times 10^5$ copies/$10^6$ genomes) but not in lung, testes, brain, and bone marrow extracts ($n=3$). Systemic virus dissemination was presumably a result of inadvertent virus injection into the LV cavity or the cardiac circulation. Migration of lentivirally infected cardiac leukocytes to remote organs may also have contributed to the observed PCR signals.

**Lentiviral Vectors Cause Myocardial Inflammation**

The integrity of tissue structure was essentially preserved after lentivector or PBS but not adenovector injection. At the injection site, significant tissue inflammation was observed with both lentivectors and adenovectors but not PBS (Figure 7). In adjacent areas, inflammatory cell infiltrates with
lentivectors were statistically smaller than with adenovectors (P<0.05). The spatial distribution of inflammatory cell infiltrates corresponded to the respective distribution of EGFP-positive cells with the 2 vectors. Inflammatory cell populations included monocytes/macrophages (recognized by anti-ED1-like and anti-CD4 mAbs), CD8\(^+\) T cells (recognized by anti-CD8\(^\alpha\) and anti-\(\alpha\)\(\beta\) TCR mAbs), and rare B cells (not shown).

**Discussion**

The major findings of this study are that lentiviral vectors efficiently transduce genes into adult rat cardiomyocytes both in vitro and in vivo and that they achieve myocardial transgene expression for extended periods of time (>10 weeks) in vivo. These results were obtained with lentiviral vectors optimized both in the transgene promoter and in the vector backbone. Both freshly isolated rod-shaped cardiomyocytes and cultured, dedifferentiated cardiomyocytes were transduced efficiently (>90% at MOIs of ~5 to 10). The CMV immediate-early promoter achieved higher transgene expression levels than the EF-1\(\alpha\) and PGK promoters in vitro. Insertion of the cPPT sequence of HIV-pol into the vector backbone improved transgene expression and gene transfer kinetics. This effect may be a result of enhanced nuclear entry of the preintegration complex, presumably through formation of a triple-stranded DNA flap during reverse transcription.

After intramyocardial lentivector injection in vivo, a bright EGFP signal was detected by direct fluorescence microscopy in up to ~5% of cardiomyocytes within the injected region (Figure 5). By comparison, the number of EGFP-positive myocytes was ~6-fold higher and EGFP tissue concentrations were ~30% higher when titer-matched adenoviral vectors were used. The larger difference in the number of EGFP-positive myocytes compared with EGFP tissue content with the 2 vectors may be in part a result of suboptimal

**Figure 3.** Effects of the cPPT-pol sequence on lentiviral EGFP expression in cultured cardiomyocytes. Percentages of EGFP-expressing cardiomyocytes and mean fluorescence values at day 2, 3, and 5 after infection. A and B, MOI, ~5. C and D, MOI, ~50. SIN.cPPT.CMV-EGFP-W (gray columns); SIN.CMV-EGFP-W (white columns); SIN.cPPT.CMV-LacZ-W (black columns) (data from 1 representative experiment of 3).

**Figure 4.** In vitro comparison of lentivectors and adenovectors. A, Percentages of EGFP-expressing cardiomyocytes with SIN.cPPT.CMV-EGFP-W (white columns) and Ad.CMV-EGFP (hatched columns) at day 4 after infection (MOIs, ~2 and 20). B, Corresponding mean fluorescence values. C, Fluorescence photomicrographs of cardiomyocytes infected with SIN.cPPT.CMV-EGFP-W (left) or Ad.CMV-EGFP (right) at day 3 and 4 (top and middle: MOI, ~2; bottom: MOI, ~20; data from 1 representative experiment of 3).
sensitivity of direct fluorescence microscopy. This is supported by our in vitro data showing that multiple copies of lentivirus DNA per cell are necessary to elicit a fluorescence signal that can be readily detected under the microscope (Figure 1). Conversely, EGFP expression in cells containing just one or a few copies of the transgene may be below the microscopic detection threshold, yet may contribute to EGFP tissue levels measured by ELISA.

The usefulness of vector systems for gene therapy applications depends crucially on the time course of transgene expression. With lentiviral vectors, in vivo EGFP expression was detectable at day 3 after infection, albeit at a lower level than with adenovectors (Figure 6). The slower kinetics of lentiviral gene transfer may be accounted for by several factors, including reverse transcription, second-strand DNA synthesis, nuclear translocation of the preintegration complex, and virus genome integration. Nevertheless, demonstration of EGFP expression at day 3 implies that lentiviral vectors may be suitable for applications that require early-onset transgene expression. In this regard, lentivectors compare favorably to adeno-associated virus vectors, which are characterized by an ≈2-week delay in onset of expression.

Lentiviral EGFP expression peaked at day 7 but declined by ≈7-fold between days 7 and 14 regardless of the promoter used. The early decline in EGFP expression was paralleled by loss of vector DNA, probably because of tissue inflammation. With CMV promoter–containing vectors, both lentivirus DNA and EGFP levels were essentially stable thereafter (up to 10 weeks). EGFP-expressing myocytes were detectable by fluorescence microscopy at 10 weeks. With EF-1α promoter–containing vectors, however, lentivirus DNA and EGFP levels declined more rapidly than with CMV vectors after day 14, with barely detectable EGFP tissue levels at 10 weeks, despite residual vector DNA. The reason for the late loss of EGFP expression from EF-1α but not CMV promoter–containing vectors is unclear, but transcriptional silencing may be involved. With adenoviral vectors, EGFP was barely detectable at 10 weeks.

Although tissue inflammation at the injection site was comparable with lentiviral and adenoviral vectors (Figure 7), it was significantly milder with lentivectors in adjacent areas, which paralleled the spatial distribution of EGFP-positive cells with the respective vectors. Monocyte/macrophages and T lymphocytes were the most abundant inflammatory cell populations, consistent with antigen-independent and -dependent immune responses, respectively. Although lentivectors are potentially less immunogenic than adenovectors because of the lack of viral open reading frames, immune responses to the VSV-G envelope protein, EGFP, and contaminants that copurify with vector particles may account for tissue inflammation. These responses may have been exacerbated by nonspecific inflammation caused by needle injury. In addition, limited systemic dissemination as a result of virus access to the circulation and/or migration of lentivirally infected cardiac leukocytes to remote organs may have

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**Figure 5.** Fluorescence photomicrographs of EGFP-transduced hearts in vivo. A, Ad.CMV-EGFP, day 7. B, SIN.cPPT.EF1α-EGFP-W, day 7. C, SIN.cPPT.CMV-EGFP-W, day 10. D, SIN.cPPT.CMV-EGFP-W, 10 weeks. E, Immunostaining for α-sarcomeric actin (red striations) showing EGFP-expressing cardiomyocytes (arrow) after lentivirus injection.

**Figure 6.** Time course of integrated lentivirus DNA and EGFP concentrations by real-time PCR and ELISA, respectively. DNA copy number normalized per 10^6 cell genomes. A, SIN.cPPT.CMV-EGFP-W. C, SIN.cPPT.EF1α-EGFP-W. EGFP content in the same cardiac extracts. B, SIN.cPPT.CMV-EGFP-W. D, SIN.cPPT.EF1α-EGFP-W. E, EGFP content with titer-matched adenovectors. Open symbols, individual values; closed squares, median values.
enhanced immune reactions via viral antigen presentation to immunocompetent cells in the spleen, lymph nodes, and liver.

In contrast, no EGFP-positive myocytes and very low EGFP tissue contents were observed after in vivo intrapericardial or ex vivo intracoronary lentivector administration. However, transplanted hearts could be gene-transduced by intramyocardial lentivector injection. These observations suggest that the endothelial barrier may prevent myocardial gene transfer after intracoronary infusion of lentivectors, unlike adenovectors. The ≈30% larger size of lentivirus (diameter, 100 to 120 nm) compared with adenovirus particles, as well as the high VSV-G affinity for the cell membrane, may limit transendothelial and transvascular diffusion of lentiviral vectors.

Biosafety is obviously a major concern when using HIV-1–based vectors. Third-generation vectors offer improved safety features, including (1) multiple attenuation (Δ nef, Δ vif, Δ vpr, Δ vpu, Δ env); (2) deletion of the transactivator-encoding gene in packaging constructs (Δ tat)14; (3) self-inactivating design that minimizes the risk of generating replication-competent retrovirus recombinants15; and (4) a split-genome packaging system with minimal sequence overlaps between different plasmids, further reducing the risk of plasmid recombination.14 However, the recent emergence of a lymphoproliferative syndrome in 2 patients treated by retrovirus-mediated γ-c gene transfer in a clinical gene therapy trial for inherited, X chromosome-linked, severe combined immune deficiency (X-SCID) has raised concerns about the safety of retroviral vectors.19 Vector insertion near the proto-oncogene Lmo2 was found in hyperproliferating lymphoblasts in both patients, suggesting insertional mutagenesis as a likely mechanism. It should be noted, however, that this complication was not observed in previous trials using retroviral vectors and thus, it might be due to the immunodeficient condition of the patients or the gene delivered in this trial. Thus, further studies in preclinical models will be needed to more precisely assess the biosafety of retroviral and lentiviral vectors. Moreover, tissue inflammation observed in the present study would be unacceptable for gene therapy applications. Improved techniques of virus pseudotyping (eg, with non–VSV-G proteins), production, and purification might minimize lentivector toxicity and prolong transgene expression.

In conclusion, lentiviral vectors efficiently deliver and express genes for extended periods of time in adult myocardium. These vectors are a useful tool for studying myocardial biology and a potential system for gene heart therapy, provided that safety issues can be definitively solved.

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