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High-efficiency retroviral-mediated gene transfer into human and nonhuman primate peripheral blood lymphocytes

(gene therapy/retroviral vector/adenosine deaminase)

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ABSTRACT **Peripheral blood lymphocytes (PBLs) are primary targets for gene therapy of inherited and acquired disorders of the immune system. We describe the development of an optimized transduction system that provides for high-efficiency retrovirus-mediated gene transfer into primary PBLs. This optimized transduction protocol combines centrifugation of the lymphocytes (1000 × *g*) at the inception of transduction with phosphate depletion, low-temperature incubation (32°C), and the use of the packaging cell line PG13. Gene marking studies of human and primate PBLs using these optimized transduction conditions demonstrated that the transduction efficiency exceeded 50% of the total lymphocyte population. The optimized transduction efficiency of PBLs with amphotropic retroviral vectors was in excess of 25%. The transduction procedure does not alter phenotype, viability, or expansion of the transduced cells. Our data indicate that this optimized transduction system leads to high-efficiency gene transfer into primary human lymphocytes, which obviates the requirement for selection of transduced cells prior to gene-therapy procedures. Thus, large quantities of healthy retrovirally transduced lymphocytes containing a broad immunological repertoire can be generated for use in clinical protocols. Our results represent a significant improvement in the methodology for the transduction of lymphocytes for gene therapy.**

Peripheral blood lymphocytes (PBLs) have become a primary target for gene therapy of disorders of the immune system. Lymphocytes have successfully been utilized for gene transfer and expression of therapeutic genes in tissue culture, *in vivo* for preclinical studies in animal models, and in clinical protocols for disorders of the immune system, including adenosine deaminase (ADA) deficiency and AIDS (1–3). Retroviral vectors are currently the most effective approach for the introduction of genes into PBLs. All retroviral vectors presently in use in clinical gene therapy trials are based on a Moloney murine leukemia virus backbone. Retroviral vectors are packaged into infectious particles after introduction into a retroviral packaging cell line. The species and cell type specificity (host range) of the retroviral vector particle is mainly determined by the type of envelope (*env*) gene contained within the packaging cell line. Murine amphotropic virus-based retroviral vector particles are capable of infecting human cells, but the transduction of human PBLs with amphotropic retroviral vectors generally results in low levels of gene transfer (1, 4–8).

In an attempt to improve the efficiency of retroviral-mediated gene transfer into human lymphocytes, we investigated the use of retroviral supernatants generated in a packaging cell line derived from the gibbon ape leukemia virus (GALV) envelope (PG13) (9). The PG13 packaging cells express a hybrid retroviral genome in which the Moloney murine leukemia virus *gag* and *pol* genes are expressed on one expression vector, while the GALV *env* gene is expressed from another expression vector. The GALV envelope-containing retroviruses generated in this packaging cell line are capable of transducing a wide range of cell types, including human cells.

This report describes the development of a procedure that permits high-efficiency gene transfer into human and nonhuman primate PBLs. This transduction protocol combines the use of PG13-derived retroviral supernatants with metabolic induction of the GALV receptor, low-temperature incubation, and centrifugation. The high levels of gene transfer obtained with this methodology will greatly improve the prospect of using PBLs to successfully treat inherited and acquired immune deficiencies.

MATERIALS AND METHODS

Cell Culture and Retroviral Vectors. The cell lines used include PG13 (ATCC CRL-10686), PG13-LN (ATCC CRL-10685), and PA317 (10). The PA317 and PG13 cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with L-glutamine at 0.30 mg/ml and 10% (vol/vol) heat-inactivated fetal bovine serum. Cells were cultured at 37°C in a 5% CO_2/95% air humidified incubator. In the majority of these studies, two similar retroviral vectors were utilized for transduction/marking experiments, LN (11) and G3Na. Both vectors are simple long terminal repeat-driven neomycin phosphotransferase II gene-containing vectors that differ by the presence of multiple cloning sites in G3Na. The LN vector was produced in the PG13 GALV packaging cell line and had a titer of 6×10^5 G418-resistant colony-forming units per ml measured on HeLa cells. The G3Na vector was produced by the PA317 packaging cell line and had a titer of 8×10^5 G418-resistant colony-forming units per ml measured on HeLa cells. Additional retroviral vectors used include LXSN (11) and LASN (12).

Isolation and Culture of Lymphocytes. A population of CD4-enriched lymphocytes was generated from CD8 magnetic bead-depleted (Dynal, Great Neck, NY) peripheral blood mononuclear cells. The human CD4-enriched cell population was resuspended in AIM-V modification 302 medium (Life Technologies, Grand Island, NY) containing 5% fetal bovine serum, OKT-3 (Ortho Diagnostics) at 10 ng/ml, and recombinant human interleukin 2 (rIL-2) at 200 units/ml and plated at 1×10^6 cells per ml in 6-well plates at 2 ml per well (Costar).

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Abbreviations: PBL, peripheral blood lymphocyte; GALV, gibbon ape leukemia virus; ADA, adenosine deaminase; moi, multiplicity of infection; rIL-2, recombinant human interleukin 2; Neo^R, neomycin resistance.

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The ADA patient whole blood lymphocytes were cultured in AIM-V medium supplemented with 5% fetal bovine serum and rIL-2 at 100 units/ml at a concentration of 2.5×10^5/ml in 24-well plates. Rhesus peripheral blood mononuclear cells were isolated by an apheresis protocol utilizing a modified CS 3000+ blood cell separator (Baxter Healthcare, Deerfield, IL). Rhesus $CD4^+$ lymphocytes were plated at a density of 1×10^6 cells per ml in 6-well plates at 2 ml per well in AIM-V modification 302 medium containing 5% fetal bovine serum, rIL-2 at 200 units/ml, phytohemagglutinin at 1 μg/ml, and FN-18 (monoclonal antibody to primate CD3, obtained from D. Neville, National Institute of Mental Health, Bethesda) at 50 ng/ml.

Transduction of Lymphocytes. Human and rhesus $CD4^+$ lymphocytes were transduced under different conditions denoted as standard, intermediate, and optimized. All transductions were performed at a multiplicity of infection (moi) of 1.0, unless otherwise indicated, using an equal volume of vector supernatant. The *standard transduction* was performed by direct exposure of the stimulated lymphocytes to retroviral supernatants supplemented with protamine sulfate at 8 μg/ml plus rIL-2 at 200 units/ml (e.g., to each well of the 6-well plate, 2×10^6 G418-resistant colony-forming units of vector supernatant, about 3.5 ml, was added, and the plate was then returned to the incubator). The standard transduction was performed for 12 hr at 37°C in a 5% CO_2/95% air incubator. The *intermediate transduction* protocol involved exposure of stimulated cells to supplemented retroviral supernatants, but, in addition, the tissue culture plates containing the lymphocytes were centrifuged in the retroviral supernatant at 1000 × *g* at 32°C for 60 min followed by incubation for 12 hr at 32°C in a 5% CO_2 incubator. The *optimized transduction* procedure was initiated by performing a phosphate depletion step for 3–12 hr at 37°C prior to the transduction start point by pelleting the cells, washing the cells in the RPMI 1640 phosphate-free medium, and resuspending the cells at 1×10^6/ml (2 ml per well, in 6-well plates) in RPMI 1640 phosphate-free medium supplemented with 5% heat-inactivated fetal bovine serum and rIL-2 at 200 units/ml for human cells or rIL-2 at 200 units/ml and phytohemagglutinin at 1 μg/ml for the rhesus cells. The phosphate-starved cells were subsequently exposed to the retroviral vector by the addition of supplemented supernatant, followed by centrifugation at 32°C for 60 min and incubation for 12 hr at 32°C in a 5% CO_2 incubator. At the end of the transduction, the lymphocytes were pelleted, washed, and cultured at 1×10^6 cells per ml in AIM-V modification 302 supplemented with 5% fetal bovine serum and rIL-2 at 200 units/ml. For multiple transductions, the process was repeated immediately after the conclusion of the initial transduction. Lymphocyte viability and number were determined using trypan blue exclusion. The ADA patient whole blood lymphocytes were transduced with either PA317/LASN (moi = 2) or PG13/LXSN (moi = 4) retroviral vector supernatants 72 hr after plating. The transduction was performed using the intermediate protocol as above.

Analysis of Transduction Efficiency. The transduction efficiency of the retroviral vector preparations was analyzed using Southern blots of genomic DNA and semiquantitative PCR. Genomic DNA was isolated from populations of transduced lymphocytes by extraction using a TurboGen DNA isolation kit (Invitrogen). Ten micrograms of DNA was digested with the *Sst* I restriction enzyme, followed by Southern blot analysis using a neomycin-resistance (Neo^R)-specific radiolabeled DNA probe. PCR for the Neo^R or β-actin gene was performed as described (13, 14), using the Neo^R primers (5′-GATAGAAGGCGATGCGCTGCGAATCG-3′, 5′-TCCATCATGGCTGATGCAATGCGGC-3′) or the β-actin primers (5′-CATTGTGATGGACTCCGGAGACGG-3′, 5′-CATCTCCTGCTCGAAGTCTAGAGC-3′). The transduction efficiency of the ADA patient cells was analyzed by semiquantitative PCR for the Neo^R gene using primers (5′-GGTGGAGAGGCTATTCGGCTATGA-3′, 5′-ATCCTGATCGACAAGACCGGCTTC-3′) in standard 100-μl reaction mixtures with the addition of 0.01 μCi (1 Ci = 37 GBq) of [^{32}P]dCTP. All quantitation was performed using a PhosphorImager (Molecular Dynamics).

RESULTS

Transduction Efficiency of Lymphocytes. Retroviral-mediated gene transfer into primary lymphocytes of human and nonhuman primate origin resulted in low levels (<10%) of gene transfer (1, 4–8). Transduced cells can be enriched for by culture in medium containing G418 to select for the Neo^R gene but at the expense of decreased overall viability (6). In this study, we sought to improve transduction conditions to optimize lymphocyte gene transfer efficiency.

Gene marking studies were initiated on rhesus and human $CD4^+$ lymphocytes using a Neo^R gene-containing retroviral vector produced in the GALV packaging cell line (PG13-LN) or a similar vector produced in an amphotropic packaging cell line (PA317-G3Na). Successful retroviral vector-mediated transduction of primary T lymphocytes requires the stimulation (e.g., using an antibody to CD3 plus rIL-2) of these quiescent cells to begin cell division. For human lymphocytes, we previously determined that 72 hr of stimulation yielded the highest amount of transduced cells (B.A.B. and R.A.M., unpublished observations). To determine this time for rhesus lymphocytes, a series of standard transduction experiments were initiated. Using standard transduction conditions (see *Materials and Methods*), the transduction efficiency for the rhesus lymphocytes was determined at 24 hr and 72 hr after stimulation. Semiquantitative PCR analysis of PA317-transduced rhesus lymphocytes showed a low level of transduction at 24 hr after stimulation but not at 72 hr after plating (Fig. 1*A*, lanes 1 and 6), but no significant gene transfer was detected at either time point using the PG13 packaged vector (Fig. 1*A*, lanes 7 and 13). As suggested by previous experience (15), we next attempted to improve the standard gene transfer protocol by decreasing the temperature of incubation after addition of the retroviral vector supernatant. Rhesus lymphocytes were again subjected to a 32°C standard supernatant transduction at both 24 hr and 72 hr after plating using the PA317 and PG13 packaged retroviral vectors. The PCR data (Fig. 1*A*, lanes 2, 5, 8, and 12) demonstrated better transduction at 24 hr after stimulation for both the PA317 and PG13 vector transductions and higher levels of gene transfer at 32°C versus 37°C (Fig. 1*A*; compare lanes 1 to 2, 7 to 8, and 12 to 13). The transduction efficiency of human lymphocytes was also increased with both PA317-G3Na and PG13-LN supernatants when lymphocytes were incubated at 32°C instead of 37°C (data not shown).

Using standard transduction conditions, between 1% and 12% transduction efficiencies can be obtained in $CD4^+$ lymphocytes (Fig. 1 and Table 1); the PG13-based retroviral vector supernatants are more efficient than PA317-derived retroviral vector supernatants. A further increase in transduction efficiency was obtained using intermediate transduction conditions. For intermediate conditions, a centrifugation step (1000 × *g*) was added to the 32°C incubation protocol. Centrifugation combined with low-temperature incubation increased the transduction efficiency of rhesus lymphocytes using the PG13-LN retroviral vector supernatant from 5% to 8% (Fig. 1*A*, lanes 8 and 9, respectively, normalized values). Centrifugation did not increase the transduction efficiency of the amphotropic retroviral vector on rhesus lymphocytes (Fig. 1*A*, lanes 2 and 3). Addition of the centrifugation step increased the transduction efficiency of human lymphocytes with both types of retrovirus vectors when compared to the standard transduction conditions (Fig. 1*C*, compare lanes 1 to 2, 5 to 6,

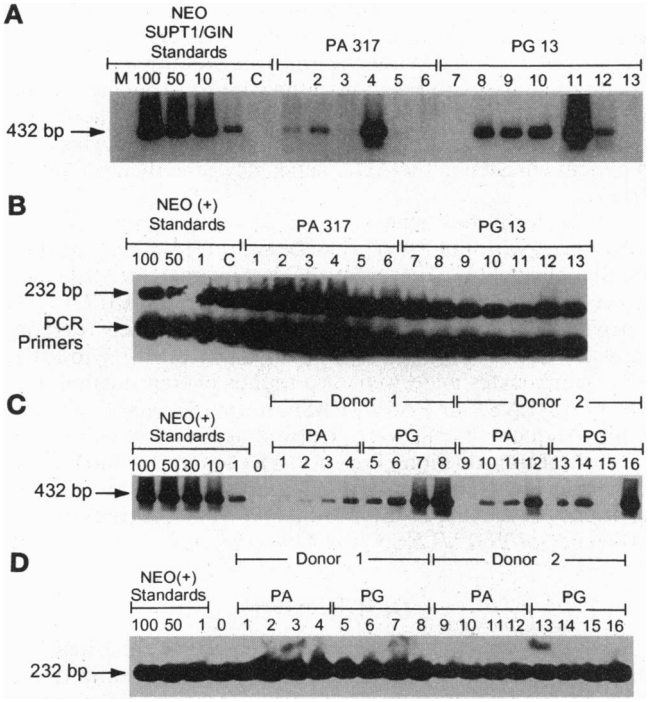

FIG. 1. PCR analysis of lymphocyte transduction efficiency. (*A*) Quantitation of the transduction efficiency of rhesus CD4$^+$ lymphocytes using primers specific for the Neo^R gene after retroviral-mediated transduction with supernatants generated in the PA317 or PG13 packaging cell lines as indicated. Lanes 1 and 7, transduction at 37°C, 24 hr after plating; lanes 2 and 8, transduction at 32°C, 24 hr post-plating; lanes 3 and 9, transduction at 32°C with centrifugation at 1000 × *g*, 24 hours after plating; lanes 4 and 11, P_i depletion for 12 hr followed by transduction at 32°C with centrifugation at 1000 × *g*, 24 hr after plating; lanes 5 and 12, transduction at 32°C, 72 hr after plating; lanes 6 and 13, transduction at 37°C, 72 hr after plating; lane 10, P_i depletion for 12 hr followed by transduction at 32°C, 24 hr after plating. The percent Neo^R-positive standards of 100%, 50%, 10%, and 1% are indicated. Lane C, control DNA; lane M, molecular size markers. (*B*) The results of PCR analysis for the β-actin gene in rhesus PBLs. The lanes are as described in *A*. (*C*) Quantitation of the transduction efficiency from two human donors after retroviral-mediated gene transfer. CD4$^+$ lymphocytes were transduced with either PA317 (PA) or PG13 (PG) retroviral vector supernatant as indicated. Cells were transduced at 72 hr after plating under the following conditions: 32°C incubation only (lanes 1, 5, 9, and 13), 32°C incubation and centrifugation at 1000 × *g* (lanes 2, 6, 10, and 15), P_i depletion followed by 32°C incubation (lanes 3, 7, 11, 14), P_i depletion followed by 32°C incubation with centrifugation at 1000 × *g* (lanes 4, 8, 12, and 16). (*D*) All human lymphocyte samples were normalized by performing PCR amplification of the β-actin gene. The lanes are as described in *C*.

and 9 to 10). The increase observed with the PG13-LN retrovirus was more than the increase seen with the PA317-G3Na retroviral vector preparation. The combined analysis of 14 separate transductions demonstrated that the intermediate transduction protocol yielded a 2- to 3-fold increase in transduction efficiency over standard transductions (Table 1).

The cell surface receptor proteins for the GALV and amphotropic murine retrovirus have recently been cloned and characterized (16). Miller and coworkers (17) expressed both the GALV and amphotropic murine retrovirus receptor cDNAs in *Xenopus laevis* oocytes and determined that the receptors function as sodium-dependent phosphate symporters. They further reported that depletion of extracellular phosphate resulted in a 3- to 5-fold increase in receptor gene expression in rat 208F fibroblasts. Based on these data, we incorporated a phosphate depletion step into our transduction procedure to increase the expression of the GALV and amphotropic receptors on the surface of the cells.

The transduction efficiency of rhesus lymphocytes that underwent phosphate depletion and 32°C incubation during transduction with the PG13 retroviral vector supernatant was 10% (Fig. 1*A*, lane 10). To produce optimized transduction conditions, lymphocytes were subjected to transduction using phosphate depletion followed by exposure to supplemented retroviral supernatant, centrifugation at 1000 × *g*, and incubation at 32°C. These optimized conditions demonstrated a marked increase in the presence of the Neo^R gene in rhesus lymphocytes exposed to both amphotropic and PG13 retroviral supernatants (Fig. 1*A*, lanes 4 and 11). Under these transduction conditions, the PG13 supernatant transduced >70% of the rhesus lymphocytes, whereas the amphotropic supernatant transduced >40% of the rhesus lymphocytes.

The transduction efficiency of human lymphocytes was also markedly affected by manipulating the inorganic phosphate (P_i) concentration. Phosphate depletion for 12 hr prior to transduction at 32°C resulted in an increase from 2% transduction to 28% transduction using the PG13-LN supernatant (Fig. 1*C*, compare lanes 5 to 7 and 13 to 14). The increase in transduction efficiency using the PA317-G3Na vector supernatant was also significant (Fig. 1*C*, compare lanes 1 to 3 and 9 to 11). When the phosphate depletion step was used in combination with low-temperature incubation and centrifugation, the optimized transduction efficiency of human lymphocytes with the PG13-LN supernatant was >50% (Fig. 1*C*, compare lanes 7 to 8 and 14 to 16). Using the PA317-G3Na supernatant, the level of engineered cells obtained with the optimized protocol increased from <1% to between 2% and 18% of the cell population (Fig. 1*C*, compare lanes 1 to 4 and 9 to 12). Comparison of 17 independent transductions demonstrated that the optimal transduction protocol can yield a >10-fold increase in gene transfer efficiency in comparison to standard supernatant transductions (Table 1). All of the Neo^R PCR results have been normalized by amplification of the β-actin gene in the same samples (Fig. 1 *B* and *D*).

The transduction efficiency of lymphocyte populations was also determined by Southern analysis. In this experiment,

Table 1. Transduction efficiency of retroviral-mediated gene transfer into PBLs

		Lymphocyte sample					
		Rhesus			Human		
Protocol	Transduction conditions	PA317	PG13	*n**	PA317	PG13	*n**
Standard	Direct supernatant exposure at 37°C or 32°C	1–6	2–12	6	1–7	1–10	14
Intermediate	Centrifugation (1000 × *g*) and incubation at 32°C	5–15	15–33	6	1–34	10–40	8
Optimal	Phosphate depletion with centrifugation (1000 × *g*) and incubation at 32°C	15–47	41–77	9	25–46	35–65	8

Data are the percent transduction for CD4-enriched activated T cells transduced with PA317-G3NA or PG13-LN using an moi of 1. Data were determined by PCR analysis of the Neo^R gene and are normalized to the coamplified β-actin gene. All percents were rounded up to the next greatest whole number.

*The number of independent transductions at each condition.

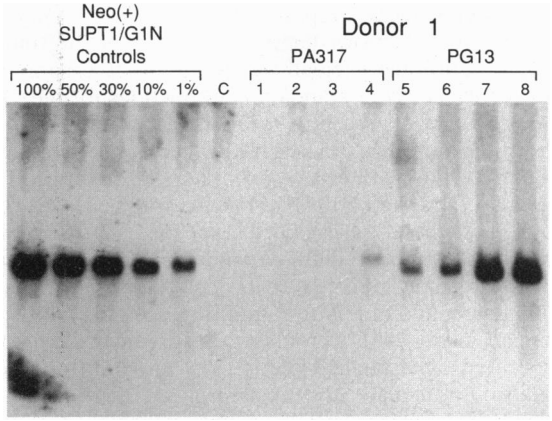

FIG. 2. Southern blot analysis to determine gene transfer efficiency of transduced lymphocytes. Total cellular DNA (10 μg) from the transduced lymphocytes of donor no. 1 was digested with *Sst* I and electrophoresed on a 0.8% agarose gel. The digested DNA was then transferred and hybridized with a ^{32}P-labeled Neo^R probe. *Sst* I cleaves the retroviral vector once in each long terminal repeat, excising a 2.3-kb Neo^R-containing proviral DNA fragment from the LN retroviral vector and a 2.4-kb Neo^R fragment from the G3Na retroviral vector. The Neo^R (+) standards are indicated. Lanes 1 and 5, transduced by standard transduction at 32°C. Lanes 2 and 6, transduced by centrifugation (1000 × *g*) followed by incubation at 32°C. Lanes 3 and 7, P_i-depleted for 12 hr and transduced at 32°C. Lanes 4 and 8, P_i-depleted for 12 hr and transduced with centrifugation (1000 × *g*) and 32°C incubation. Lane C, nontransduced control DNA.

DNA from the samples analyzed in Fig. 1*C* (donor no. 1) was subjected to Southern blot analysis; the resultant autoradiograph is shown in Fig. 2. For the amphotropic retrovirus-transduced samples, gene transfer efficiencies were <1% with only the optimized samples visualized in this assay (Fig. 2, lane 4). The number of lymphocytes engineered with PG13-LN was <1% using standard conditions (lane 5) and was increased to 50% using optimized methods (lane 8).

We also examined the effects of the optimized transduction system on the cell surface phenotype and long-term growth characteristics of transduced lymphocytes. Cell surface markers, cell viability, and cell numbers of lymphocytes were monitored for 3 weeks after transduction (12 hr of phosphate deprivation per round of transduction) with no differences observed versus lymphocytes transduced under the standard conditions (data not shown).

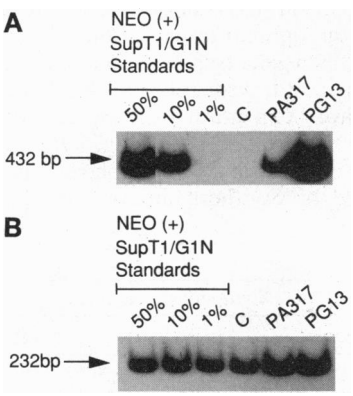

FIG. 3. PCR analysis of transduction efficiency in ADA patient lymphocytes. Quantitation of the transduction efficiency of ADA patient lymphocytes after retroviral-mediated gene transfer. Whole blood lymphocytes were transduced with either PA317 or PG13 retroviral vector supernatant as indicated. Cells were transduced 72 hr after plating by centrifugation at 1000 × *g* at 32°C. (*A*) Semiquantitative PCR for the Neo^R. (*B*) PCR amplification of the β-actin gene. The Neo(+) standards are indicated. Lane C, nontransduced control DNA.

Clinical Implications of the Optimized Transduction Procedure. To demonstrate that lymphocytes transduced with PG13-packaged retroviral vectors using optimal conditions retain their ability to function in the context of a gene therapy setting, we analyzed the transduction efficiency of the PA317 and PG13 retroviral vector supernatants on lymphocytes from a patient enrolled in the ADA deficiency gene therapy clinical trial.

To perform this study, whole blood lymphocytes were transduced with the PA317/LASN and PG13/LXSN retroviral vector supernatants using the intermediate transduction procedure. In previous transductions with the PA317/LASN retroviral supernatant, the cells from this patient demonstrated a transduction efficiency in a range from 0.1% to 1.0%. The lymphocytes underwent two rounds of transduction with the PG13/LXSN or PA317/LASN retroviral vector supernatants. Aliquots of cells were removed at 7 days of culture for analysis of transduction efficiency. The semiquantitative PCR results for the Neo^R gene demonstrated that the transduction efficiency of the PA317/LASN was 3%, while the transduction efficiency of PG13/LXSN was 42% (Fig. 3).

DISCUSSION

The use of lymphocytes for gene therapy for inherited and acquired disorders of the immune system has been in development for several years (1–8). One problem in the use of lymphocytes for gene therapy has been the low level of gene transfer. In this report, we investigated several approaches to increase the levels of gene transfer into PBLs and describe a transduction system that results in high-efficiency retroviral-mediated gene transfer into primary lymphocytes. The results obtained were for both CD4-enriched T lymphocytes (Figs. 1 and 2, Table 1) as well as total T lymphocytes (Fig. 3).

High-efficiency gene transfer into human and rhesus PBLs was achieved by combining the use of PG13-derived retroviral supernatant with low-temperature incubation, centrifugation, and induction of the GALV receptor by phosphate depletion. By using this combined procedure, transduction efficiencies ranging from 45% to 80% were routinely observed in human and rhesus PBLs using PG13-packaged vectors and 20–40% with the PA317-packaged vectors. This is a significant improvement compared to the low levels of gene transfer (1–10%) in PBLs that have previously been reported (4–8). Transduction of lymphocytes with PG13-based supernatants under the optimized protocol generates a population of transduced cells that does not require selection to enrich for transduced cells. For lymphocyte-based gene therapy trials, these techniques permit reinfusion of large quantities of healthy lymphocytes that have a representative immunological repertoire.

The efficiency of gene transfer by both amphotropic and GALV envelope-containing supernatants was significantly affected by the individual components of the transduction system. Decreasing the temperature of incubation from 37°C to 32°C during the standard transduction protocol increased the transduction efficiency 2- to 4-fold. The increased levels of gene transfer observed at 32°C are most likely due to an increase in the half-life of the retrovirus at the lower temperature. The incorporation of a centrifugation step (1000 × *g*) along with decreased transduction temperature served to further increase (2- to 3-fold) the gene transfer efficiency in the intermediate transduction protocol. The mechanism by which centrifugation increases the transduction efficiency is unclear, but other investigators have observed similar results in other cell types (15). Infrequently, the centrifugation step used in the intermediate transduction protocol was associated with loss or inactivation of the vector supernatant, resulting in minimal transduction (see Fig. 1*A*, lane 3 and Fig. 1*C*, lane 15). The cause of these failures is unknown, but we have not observed

a similar loss in the optimized protocol, which also uses centrifugation.

Phosphate depletion of the lymphocytes was used in an attempt to increase the gene expression of the amphotropic and GALV receptors/phosphate symporters prior to transduction. Phosphate depletion dramatically increased the transduction efficiency. Lymphocytes that were starved and then transduced at the low temperature with the PG13-LN supernatant had levels of gene transfer >25% as opposed to the 2% observed without the phosphate deprivation. Miller and coworkers (17) previously observed that starvation of rat fibroblasts in phosphate-free medium increases the RNA levels for both the GALV and amphotropic receptors. It is unclear whether the increased gene expression leads to greater expression of the retroviral receptor proteins on the surface of the cell, but our transduction data strongly suggest that receptor protein levels must increase. Taken together, the combination of different transduction techniques was developed into an optimal transduction protocol that results in >50% lymphocyte transduction using PG13-packaged vectors and >25% transduction efficiencies with PA317-packaged retroviral vectors.

As a test of a potential clinical application, we demonstrated the improved effectiveness of the PG13 retroviral vector supernatant for transduction of ADA patient lymphocytes that had previously been demonstrated to transduce poorly with amphotropic retroviral vector supernatants. In our studies, the use of a PG13 retroviral vector supernatant transduced 42% of this patient's lymphocytes in comparison to an amphotropic retroviral vector that only transduced 3% of a total lymphocyte population. The different moi values used for this transduction study were not likely the cause of this increased rate, as varying the moi from 1 up to 10 with amphotropic retroviral vectors had previously not produced a difference in transduction efficiency with this patient (gene transfer efficiency was always ≤1%; data not shown).

In conclusion, we have developed a high-efficiency gene transduction system for human PBLs. The protocol is straightforward and easy to apply. The technique does not require the development of special vectors or packaging cell lines and may be used effectively with preexisting amphotropic retroviral vectors. The transduction approach discussed here could lead to a significant increase in the effectiveness of clinical trials for gene therapy of disorders of the immune system. It is also possible that this system could be widely applied to other target cells for gene therapy and may have applications for use in the development of gene therapies for a number of diseases.

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