Improved Transfer of the Leukocyte Integrin CD18 Subunit Into Hematopoietic Cell Lines by Using Retroviral Vectors Having a Gibbon Ape Leukemia Virus Envelope

By Thomas R. Bauer Jr, A. Dusty Miller, and Dennis D. Hickstein

Leukocyte adherence deficiency (LAD) is an inherited immunodeficiency disease caused by defects in the CD18 leukocyte integrin subunit. Transduction of CD18 into hematopoietic cells from children with LAD represents a potential therapy for this disorder. In an attempt to maximize transfer and expression of CD18, we evaluated retrovectors with and without the neomycin selectable marker, with a modified tRNA primer binding site designed to prevent inhibition of gene expression, and with two different viral envelope proteins produced by using the amphotropic retrovirus packaging cell line PA317 or the gibbon ape leukemia virus packaging cell line PG13. The vectors were tested using transducing K562/CD11b cells and LAD Epstein-Barr virus (EBV) B cells and measuring levels of cell-surface CD11/CD18 expression by fluorescence-activated cell sorter analysis. The best results were obtained with vectors made using PG13 packaging cells, for which about 25% of the K562 cells exposed once to the vectors expressed surface CD11b/CD18 and about 25% of the LAD EBV B cells expressed three times over a 3-day period to the vectors expressed surface CD11a/CD18.

In contrast, transduction of cells under similar conditions with retrovectors produced using PA317 producer cells yielded less than 2% of the K562 cells and less than 4% of the LAD EBV B cells expressing the CD11/CD18 heterodimer on the cell surface. The presence or absence of the neomycin resistance gene or the modified tRNA primer had no effect on CD18 gene transfer rate or expression level. The increase in transduction with PG13 vectors correlated with Northern blotting and reverse transcription-polymerase chain reaction studies that indicated that both K562 cells and the LAD EBV B cells express transcripts for the gibbon ape leukemia virus receptor at higher levels than for the amphotropic virus receptor. These findings indicate that the transduction efficiency of retroviral packaging cell line correlates with receptor gene expression in the target cells and that vectors made using PG13 cells may be efficacious for gene therapy for LAD and other diseases in which gene transfer to hematopoietic cells is required.

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CHILDREN WITH LEUKOCYTE adherence deficiency (LAD) experience recurrent, life-threatening bacterial infections due to the inability of their leukocytes to adhere to the blood vessel wall and migrate to the site of infection. The clinical manifestations of the disease stem from the inability of leukocytes from affected children to express the four heterodimeric members of the leukocyte integrin family: CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), CD11c/CD18 (p150,95), and CD11d/CD18. Previous experimental studies have shown that the CD11 subunits are intrinsically normal in LAD and that defects in the common CD18 subunit are responsible for the inability to express the CD11/CD18 complexes on the leukocyte surface. We and others have studied the feasibility of using retroviral vectors to restore CD18 expression in cells from LAD patients, with the ultimate goal of long-term disease correction by CD18 gene transfer into hematopoietic stem cells. However, previous studies on retrovirus-mediated CD18 gene transfer have shown relatively low CD18 gene transfer rates and low expression levels in cultured cells. In our studies using the LCI18SN retroviral vector, produced by using PA317 amphotropic retrovirus packaging cells, gene transfer into Epstein-Barr virus (EBV)-immortalized LAD B cells required cocultivation of the target cells with the vector-producing line. Additionally, despite several weeks of selection in G418, the LAD EBV B cells transduced with the retroviral vector expressed only 5% to 10% of the normal surface levels of CD11/CD18 heterodimer.

In an attempt to improve gene transfer efficiency and gene expression levels, we have tested two modifications to the previously used LCD18SN retroviral vector. First, we have replaced the Moloney murine leukemia virus (MoMuLV) proline tRNA binding site in the CD18 vector with a glutamine tRNA binding site from an endogenous mouse virus. The tRNA binding site is required during the first steps in reverse transcription of the retroviral vector. Unrelated to this function, the normal MoMuLV tRNA binding site also can bind a cellular protein that causes repression of transcription from the retroviral long terminal repeat (LTR) in embryonic carcinoma and other cell types. Substitution of the glutamine site for the normal proline tRNA binding site abolishes this inhibition while still allowing proper reverse transcription of the vector. Second, we have investigated whether removal of the neomycin resistance (neo) gene present in the LCD18SN vector would result in increased CD18 expression. This approach was based on reports that neo gene sequences can inhibit expression from nearby promoters and that the presence of two transcriptional units within a retroviral vector may cause mutual interference. In addition, the neo gene product might stimulate immune responses.

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in the context of human gene therapy or cause inappropriate protein phosphorylation; thus, removal of the gene would be advantageous provided that gene transduction efficiencies were at least as high as vectors that encode selectable markers.

We have also tested whether substitution of the gibbon ape leukemia virus (GALV)-derived retrovirus packaging cell line PG13 for the amphotropic PA317 cell line that was previously used to produce the LCD18SN vector might result in improved transduction efficiency in hematopoietic cells. The key difference between these two packaging cell lines is the viral envelope protein incorporated into the retroviral vector particles. Cell-surface receptors that interact with retroviral envelope proteins to allow entry of GALV or amphotropic retroviruses have now been cloned,14-16 and the GALV receptor is highly expressed in bone marrow, while the amphotropic receptor is expressed at low levels in bone marrow.17

The results of these studies indicate that, although vector modifications involving the primer binding site and removal of the neo gene did not influence CD18 expression levels in vector-transduced hematopoietic cells in short-term in vitro assays, the use of PG13 GALV-derived retrovirus packaging cells allowed more efficient gene transfer into both K562 and LAD EBV B cells than did PA317 amphotropic packaging cells and allowed direct transduction of the target cells by exposure to vector-containing medium. The levels of CD18 expression in transduced LAD EBV-immortalized lymphocytes were similar to CD18 expression levels in normal lymphocytes. These studies support the use of retrovirus-mediated gene therapy for LAD by using retroviral vectors produced by PG13 cells.

MATERIALS AND METHODS

Cell culture. The retroviral packaging cell lines PG13,18 PA317,19 and PES0120; the chronic myelogenous leukemia (CML) cell line K562; and Cf2Th canine thymus cells21 were grown in Dulbecco’s modified Eagle medium (BioWhittaker, Walkersville, MD) with high glucose (4.5 g/L) and supplemented with 10% fetal bovine serum (FBS; Gibco-BRL, Grand Island, NY). The EBV-immortalized B cells derived from LAD patients (A, Gb, Gs, Z4) were grown in RPMI 1640 (BioWhittaker) supplemented with 10% FBS. All cells were grown at 37°C in a 10% CO2 atmosphere. Selection for neomycin phosphotransferase expression was performed with 1 mg/mL Geneticin (G418 Sulfate; Gibco-BRL; approximately 67% active product) as described.20

Retroviral vector construction. The retroviral vector LgCD18SN was constructed by cloning the 3.6-kb SpeI/HindIII fragment from LCD18SN containing the extended packaging region, CD18, and SV40 promoter into SpeI/HindIII-digested retroviral vector LgXSN (C. Halbert, T. Palmer, and A.D. Miller, unpublished observations). The retroviral vector LgCD18 was constructed by cloning the 1.0-kb SpeI/BamHI fragment from LCD18SN and the 2.3-kb BamHI/HindIII fragment from a CD18 cDNA clone2 into SpeI/HindIII-digested retroviral vector LgNSX (C. Halbert, T. Palmer, and A.D. Miller, unpublished observations). The LgXSN and LgNSX vectors were derived from LXSX and LNSX, respectively,20 by replacement of the KpnI to MacI region containing the proline tRNA binding site with the same region of dls587rev5 that contains a glutamine tRNA binding site derived from an endogenous mouse virus. Vector descriptions are based on the order of sequence elements within the vector, including promoters, coding regions, and viral transcription initiation sites.

Vector production and assay. To generate retroviral vectors for gene transduction, 10 μg of plasmid DNA from LgCD18SN was transfected into PES01 cells using calcium phosphate precipitation.20 Supernatant from the transfected PES01 cell line was used to transduce both PA317 and PG13 cells. Twelve G418-resistant clones were isolated from each of the transduced PA317 and PG13 packaging cell lines. DNA was extracted from the individual clones and examined for rearrangements on Southern blots. The LCD18SN vector was constructed and introduced into PA317 cells as previously described.2 Virus from these cells was used to transduce PG13 cells. The resulting G418-resistant colonies were pooled. Retroviral vectors from LgCD18 were produced by cotransfection of 10 μg of plasmid DNA from LgCD18 with 0.1 μg of pSV2neo into PG13 cells by calcium phosphate precipitation. Thirty-two G418-resistant clones were isolated.

For vector production, medium was harvested from confluent monolayers of the vector-producing cell lines 12 to 24 hours after the addition of fresh medium. The titer of vectors carrying the neo gene were determined using Cf2Th canine cells as targets for transduction, because these cells are susceptible to transduction by vectors produced by either PA317 or PG13 cells. The Cf2Th cells were seeded at 105 cells per 6-cm dish on day 1, infected with a range of virus dilutions in the presence of 4 μg/mL polybrene on day 2, exposed to 670 μg/mL G418 (active concentration) on day 3, and counted after staining on day 8.

Flow cytometry assay for CD18 expression from retroviral vectors. K562 cells were transfected with an episomal vector pREP4 (Invitrogen, San Diego, CA) and used in the retroviral vector. The K562 cells were transduced with a plasmid containing the CD18 gene and these transduced cells were then transduced with the retroviral vector. The resulting CD18 expressing cells were tested for the expression of CD18 using indirect immunofluorescence staining followed by flow cytometry. Monoclonal antibodies (MoAbs) directed against an irrelevant antigen (the MoMuLV env protein) were used as the primary antibodies in this assay. Cells were analyzed by flow cytometry on a Becton Dickinson FACScan (Becton Dickinson, Mountain View, CA).

Transduction of LAD EBV B cells. LAD EBV B cells derived from a patient (Z) with the severe deficiency form of LAD were transduced with 105 K562/CD18 cells produced in the presence of 4 μg/mL polybrene. Two and one half days after transduction, the cells were analyzed by FACS. Monoclonal antibodies (MoAbs) directed against an irrelevant antigen (the MoMuLV env protein) were used as the primary antibodies in this assay. Cells were analyzed by flow cytometry on a Becton Dickinson FACScan (Becton Dickinson, Mountain View, CA).

Transduction of LAD EBV B cells derived from a patient (Z) with the severe deficiency form of LAD (expressing no detectable CD18 RNA or protein) were used in the retroviral vector comparison studies. In these assays, 2 × 105 cells were transduced in a 24-well plate with 1 mL of viral supernatant from either PG13/LgCD18SN or PA317/LgCD18SN, c54, PA317/LgCD18SN c35, PG13/LCD18SN, or PG13/LgCD18 c1 cells in the presence of 4 μg/mL polybrene. After 24 and 48 hours, the cells were harvested and analyzed by FACS. Transduced LAD EBV B cells were immunostained with MoAbs 9E8, R3.1 (which recognizes CD11a27), and 60.3.

For studies examining the transduction capability of five different
LAD EBV B-cell lines, 5 x 10^5 cells from each cell line were transduced separately (day 0) in a 24-well plate with 1 mL of viral supernatant from PG13/LgCD18SN c54 cells and 4 µg/mL polybrene. On days 1, 3, and 4, the plate was centrifuged, the transduced cells' supernatant was carefully aspirated and discarded, and 1 mL of fresh viral supernatant was added along with 4 µg/mL polybrene. On day 5, 1 mL of RPMI 1640 medium containing 10% FBS was added to each well. Cells were expanded for 1 week and were examined by FACS analysis.

**Assay of retrovirus receptor transcripts.** RNA samples from K562/CD11b cells and LAD EBV B cells were reverse transcribed with Superscript reverse transcriptase (GIBCO-BRL) according to the manufacturer's directions using oligo dT or random hexamers. Each CDNA was used in a polymerase chain reaction (PCR) containing 100 ng of forward primer (5'-TTCCAGTTCCTG-CAGGTCTCT-3') and reverse primer (5'-TCTTTCCCCTAGTGTCG-GAT-3'), 1× Vent buffer, 0.2 mmol/L dNTPs, and 2 U Vent polymerase (NEB, Beverly, MA). The reaction was performed in a Perkin Elmer GeneAmp PCR system 9600 thermocycler (Perkin Elmer, Foster City, CA) with cycling parameters of 94°C for 1 minute; 5 cycles of 94°C for 15 seconds, 55°C for 15 seconds, and 72°C for 15 seconds; 30 cycles of 94°C for 15 seconds and 60°C for 30 seconds; and a final extension of 60°C for 5 minutes. The forward primer differs by one base each from the Glvr-l (A to G at base 5) cDNAs, whereas the reverse primer is identical to Glvr-l and Ram-l. Reaction products were precipitated with ethanol in the presence of 20 µg glycercol and end-labeled with P^32 using T4 Polynucleotide kinase (Promega, Madison, WI). The labeled products were digested with Hae III for 2 hours, put through Nuctrap columns (Stratagene, La Jolla, CA), repurified, and resuspended in 10 µL H_2O. A total of 5 µL was added to 1 µL of loading dye, and 2.5 µL of the dye/sample mix was electrophoresed on a 6% nondenaturing acrylamide gel at 2,000 V, 50 W for 2 to 3 hours. The gel was fixed in a 12.5% methanol/12.5% acetic acid bath, transferred to Whatman paper (Whatman, Hillsboro, OR), dried, and exposed overnight to an x-ray film.

**Northern blots.** RNA samples from K562/CD11b cells and LAD EBV B cells were electrophoresed in denaturing formaldehyde gels, followed by positive pressure blotting onto a Zetaprobe GT (Bio-Rad, Hercules, CA) nylon membrane filter. The filter was hybridized to a P^32-labeled Glvr-l cDNA probe (a 227-bp fragment [bases 1913 to 2139 in O’Hara et al^14] specific for human Glvr-l), followed by stripping and rehybridization with a GAPDH probe as previously performed.26

**RESULTS**

**Construction of modified CD18 retroviral vectors.** The CD18 subunit was cloned into a retroviral vector containing a modified tRNA primer binding site to create the retroviral vector LgCD18SN. This vector is otherwise identical to the previously described LgCD18SN (Fig 1). The LgCD18SN vector was produced by using either the amphotropic packaging cell line PA31719 or the GALV packaging cell line PG13.18 Vector titers were determined using CF2Th canine fibroblast cells at targets for transduction (Table 1). Titers ranged from 4 x 10^3 to 4 x 10^6 colony-forming units (CFU)/mL. Of note, although PG13/LgCD18SN c49 had one of the highest titers, this clone was found to be rearranged on Southern blotting. The LgCD18 vector is essentially the LgCD18SN

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**Table 1. Characterization of Vectors From Clonal Vector-Producing Cell Lines**

<table>
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<tr>
<th>Packaging Line</th>
<th>Clone No.</th>
<th>Titer (PFU/ml)</th>
<th>CD11b DNA (%)</th>
<th>CD18 MoAb (%)</th>
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**K562 (control)**

|                | 0.00       | 0.00           | 0.00         | 0.00         |

[K562/CD11b cells were incubated with 1 mL of vector-containing medium from each producer clone. After 2.5 days, the cells were immunostained with anti-CD11b and CD18 MoAbs followed by fluorescein-conjugated secondary antibody (Cappel, Durham, NC) and analyzed by FACS. Values given for CD11b and CD18 MoAbs represent the percentage of gated cells above a fluorescence intensity of 200 (see Fig 2) and corrected by subtracting out the value given by an irrelevant MoAb.](www.bloodjournal.org) Titters reflect the number of colony-forming units on C2Th canine fibroblast cells. DNA indicates presence of unrearranged (+) or rearranged (-) integrant.)
vector without the SV40 promoter driving the neo gene and was packaged only in PG13 cells. Because neo was not present in this vector, relative vector titers were determined by the ability of the virus to transfer CD18 to K562/CD11b cells (as described below), and the best vector-producing clone (clone 11) of 32 clones tested was used for further experiments.

Development of a CD11/CD18 complementation assay. To functionally assay the CD18-containing retroviral vectors, we transduced K562/CD11b cells and measured CD11b/CD18 expression levels by FACS analysis. K562/CD11b cells will produce a functional CD11b/CD18 heterodimer on transduction with a CD18-containing retroviral vector. The transduction efficiency and expression of almost all of the PG13 producer clones (up to 24%) exceeded the levels achieved with the PA317 producer clones (all <2%; Table 1).

The transduction results obtained with viral supernatants from PA317/LgCD18SN c35 and PG13/LgCD18SN c54 are displayed (Fig 2). FACS analysis of cells transduced with supernatant from PA317/LgCD18SN c35 cells indicated a low (<2%) but detectable percentage of positive cells (Fig 2, row 3). In contrast, analysis of K562/CD11b cells transduced with virus from PG13/LgCD18SN c54 showed the presence of a discrete population of CD11b/CD18-positive cells (Fig 2, row 4). This population of cells constituted approximately 24% of the total number of cells. This system provides evidence for transfer of a CD18 protein capable of heterodimer formation and surface expression.

Transduction of EBV-immortalized LAD B cells. Previous studies indicated that CD11 complementation by the CD18 gene could occur in EBV-immortalized LAD B cells after transduction with a retroviral vector harboring the CD18 subunit. To examine for the expression of the CD18 gene with our new retroviral vectors, we transduced an LAD EBV B-cell line (Z) derived from a patient whose leukocytes do not express CD18 at the protein or RNA level. Transduction of these cells with virus from PA317/LgCD18SN c35 resulted in only 3% to 4% positive cells by FACS (Fig 3, row 2). Transduction and expression with virus from PG13/LgCD18SN c54 resulted in approximately 15% positive cells (Fig 3, row 3). The level of CD11a/CD18 expressed on the cell surface of the positive cells was similar to that present on EBV-immortalized normal B cells (Fig 3, row 4).

Comparison of LgCD18, LgCD18SN, and LCD18SN vectors. Our previous studies used the retroviral vector LCD18SN to correct the CD18 gene defect in LAD EBV B cells. Other studies have shown the presence of a negative
regulatory element within the proline tRNA primer binding site (PBS) of the MoMuLV virus that prevents expression from the 5' LTR in embryonic carcinoma cells. Embryonic carcinoma cells have characteristics of stem cells, the target of choice for retroviral gene therapy. Therefore, we replaced the endogenous proline tRNA PBS (in LCD18SN) with a glutamine PBS (LgCD18SN; see Materials and Methods) to abolish this potential repression. To test for improved CD18 expression in LAD EBV B cells, we compared the expression in LAD EBV B cells after transduction with the retroviral vectors LgCD18SN and LCD18SN. To ensure the conditions for transduction were comparable, both vectors were produced using PG13 packaging cells. FACS analysis showed that the cells transduced by each retroviral vector had approximately equal levels of surface expression of CD18 (Fig 4, row 3). These results indicate that neither the presence of the neo gene nor the modification of the tRNA PBS had a significant effect on CD18 expression from the retroviral LTR.

Comparison of Glvr-1 and Ram-1 expression. To investigate the basis for the improved transduction efficiency of the K562 and LAD EBV B-cell lines observed by using virus from PG13 producer cells as compared with that from PA317 producer cells, an assay was performed to assess the relative amounts of RNA for the amphotropic receptor for the PA317-derived vector, Ram-1, compared with the GALV receptor for the PG13-derived vector, Glvr-1. The relative amount of RNA present within a cell line should correspond with the amount of protein expressed on the cells' surface and hence the number of retroviral receptors present. Briefly, RNA from each cell line was reverse transcribed and amplified by PCR using primers designed to amplify both Glvr-1 and Ram-1. The PCR reactions were end-labeled and digested with Hae III, which cuts at different positions within each gene. The digested products were electrophoresed on 6% nondenaturing acrylamide gels to separate the fragments. The results shown in Fig 5 indicate that, using reverse transcription-PCR (RT-PCR), Glvr-1 RNA was present at much greater amounts than Ram-1 RNA in both K562 and LAD EBV B cells. The relative ratios ranged from 8 to 1 to 37 to 1 (by densitometric scanning), indicating higher levels of
Glvr-1 transcription. These results indicate that the increased transduction efficiency of PG13-derived vectors compared with PA317-derived vectors is due to increased expression of Glvr-1 compared with Ram-1.

Analysis of Glvr-1 expression. LAD EBV B cells required multiple exposures to PG13-derived retroviral vectors to achieve transduction rates similar to those found in K562/CD11b cells after a single exposure. To investigate the basis for the increased transduction efficiency of K562/CD11b cells as compared with LAD EBV B cells (Z) obtained by using PG13-derived vectors, RNA was prepared from K562/CD11b cells and five different EBV B-cell lines and examined for transcription of the Glvr-1 gene, which encodes the retroviral receptor required for entry of PG13 virions. Northern blotting studies showed that K562 cells express 10-fold more Glvr-1 RNA than do LAD EBV B cells (Fig 6) and may explain the relative ease of K562 cell transduction by PG13-derived vectors. Glvr-1 RNA was present at detectable levels in all five LAD EBV B-cell lines examined, with about threefold variation between the cell lines (Fig 6). Additionally, very low levels of RNA encoding the amphotropic receptor Ram-1 were present in these cell lines using Northern analysis (not shown).

Expression of CD11a/CD18 in five different LAD EBV B-cell lines. We examined transduction efficiency in five LAD EBV B-cell lines after four exposures to LgCD18SN virus produced by PG13 cells. Cells were analyzed by FACS 1 week after transduction for surface expression of the CD11a/CD18 heterodimer. All five lines were transduced by the LgCD18SN vector (Table 2). The transduction efficiency of each cell line showed a weak correlation with Glvr-1 RNA amount. We also examined the relationship of cell division rate to transduction efficiency, because vector transduction is dependent on cell replication, 29 but found no relationship between doubling time and transduction efficiency (Table 2). These results indicate that other variables are responsible for the greater than 10-fold range in transduction rates observed in these cells.

DISCUSSION

In this report, we studied three different retroviral vectors (LgCD18SN, LgCD18, and LCD18SN) and packaged these vectors using two different retrovirus packaging cell lines (PA317 and PG13). The vectors were used to transduce K562/CD11b cells and LAD EBV B cells. The transduced cells were examined both for transduction efficiency and for levels of CD11/CD18 surface expression. Modification of the tRNA PBS had no effect on expression of the CD18 gene in the cell lines tested. Similarly, removal of the neomycin phosphotransferase gene driven by the SV40 promoter from the LgCD18SN vector (to produce LgCD18) did not affect expression of the transduced CD18 gene. In both K562/CD11b and LAD EBV B cells, the PG13 packaged vectors showed increased transduction efficiency compared with PA317 packaged vectors, as measured by the levels of surface expression of CD11/CD18 complexes.

LAD represents a candidate disease for human gene therapy for several reasons. First, previous studies of children with the moderate disease phenotype of LAD indicate that levels of CD11/CD18 expression of 5% to 10% of normal result in only minimal episodes of severe bacterial infection. Second, considerable clinical experience in patients with
neutropenia indicate that the presence of as few as 5% of normal numbers of neutrophils is sufficient to confer adequate host defense against common bacterial pathogens. Retroviral vectors may be useful in achieving levels of gene expression in hematopoietic cells capable of therapeutic benefit to children with LAD either by providing low-level gene expression in a large number of myeloid cells or high levels of expression in a small number of mature myeloid cells.

Three previous reports have examined conditions for optimizing cell transduction and gene expression using LAD EBV B cells as a model system. The first study by Wilson et al used a modified retroviral vector, pEMO-CD18, that contains a modified tRNA PBS using the B2 mutation to enhance viral expression of the CD18 gene. They also used an amphotropic cell line, psi-crip, to package their vector. After 72 hours of cocultivation of the EBV B cells on producer lines, they had a transduction efficiency of 2% to 4% by FACS analysis, with approximately 100% levels of CD18 expression. The second study by our lab used the LCD18SN vector packaged in PA317 cells to transduce LAD EBV B cells. Cocultivation of the cells for 72 hours on producer lines was performed, followed by selection of the cells for several weeks with G418. Although transduction efficiency was not measured, the level of CD18 expression was only about 5% of normal levels. This low level, in contrast to the results reported in this manuscript, may have been due to two differences in the experimental conditions: (1) a different EBV B-cell line was used in the previous study, and (2) the previous study the transduced cells were selected in G418 over 3 weeks. The third study by Wilson et al used the retroviral vector pΔN2-hCD18. This vector was packaged in GP+envAM12 cells. After two rounds of cocultivation of LAD EBV B cells on producer lines for 48 hours, between 55% and 60% of the cells were transduced, as determined by FACS analysis. The transduced cells expressed approximately 37% to 38% of normal CD18 expression. In summary, these reports show the variation in transduction efficiencies and CD18 gene expression that have been obtained. The work shown in this report shows that both high-level gene expression (nearly 100% of normal levels) and a high transduction efficiency (about 25%) can be achieved using the retroviral vectors and packaging cell lines described, without the need for cocultivation with packaging cells.

Treatment of LAD by gene therapy would be best achieved by CD18 gene transfer into hematopoietic stem cells followed by long-term expression of the gene in stem cell progeny. Hematopoietic stem cells have some properties in common with embryonic carcinoma or embryonic stem cells, which express a protein that inhibits expression from the MoMuLV-based retroviral vectors by binding to the viral tRNA primer site. Although we have found no difference in short-term assays of CD18 expression in K562 or LAD EBV B cells transduced with a retroviral vector with an altered tRNA primer binding site (LgCD18SN or LgCD18) compared with the unmodified vector (LCD18SN), the altered vectors may still provide better expression over the long term after gene transfer into hematopoietic stem cells. We have found that LAD EBV B cells transduced with the PG13/LgCD18SN vector continue to express CD18 over a 3-month period of growth in culture, with positive cells showing nearly 100% of the wild-type CD18 expression levels (data not shown), providing preliminary evidence for lack of vector inactivation in hematopoietic cells.

In this study, we investigated the effects of the retroviral envelope used to package retroviral vectors. Previous studies using amphotropic-derived retroviral vectors resulted in variable levels of transduction efficiency. We examined here gene transfer of amphotropic retroviral vectors packaged using PA317 cells to the same vectors packaged using PG13 cells. To compare the packaging cells, we packaged LgCD18SN in both the PA317 and PG13 lines. Producer lines from each packaging line were obtained and viral supernatant was used to transduce LAD EBV B cells or K562 cells containing the CD11b subunit. Transduction efficiency, as shown by FACS analysis, indicated that PG13 packaged lines were superior to PA317 packaged lines (Table 1 and Figs 2 and 3), in which about 25% of K562/CD11b cells and LAD EBV B cells were transduced, as compared to less than 4% transduction with PA317 producer lines. The superior transduction efficiency of PG13-derived vectors was also found in soft agar colony assays, in which G418-resistant colonies of K562 cells transduced with PG13/LgCD18SN were greater than threefold more abundant than PA317/LgCD18SN transduced cell colonies.

To assess the reason for the high transduction efficiency of
the K562 cells and LAD EBV B cells using PG13 packaged vectors, we investigated whether the levels of viral receptor expression correlated with transduction efficiency. Glvr-1 RNA was eightfold more abundant than Ram-1 in K562 cells and, on average, about 20-fold more abundant in five LAD EBV B-cell lines. A DNA probe derived from the Glvr-1 cDNA was used on Northern blots to examine RNA levels of this receptor in different cell lines. Northern blots of these cell lines indicated that the K562/CD11b cells, which were easily transduced, expressed more Glvr-1 RNA than LAD EBV B cells, which were not as easily transduced. These results indicate that receptor expression levels correlate with transduction efficiency.

Recent reports indicating that Glvr-1 RNA is more abundant than Ram-1 RNA in bone marrow cells led to a study by von Kalle et al., who examined the efficacy of PG13-derived vectors in comparison to PA317-derived vectors in transducing human progenitor cells. They used several transduction conditions and found that in both short-term and long-term cultures, transduction with a PG13-derived vector was superior to that of a PA317-derived vector. Their studies complement those presented in this report by indicating the effectiveness of the PG13-derived vectors in human progenitor cell transduction.

In conclusion, the results of this study indicate that, although retroviral vector modification to alter the viral tRNA binding site or to remove the neo selectable marker gene did not result in a significant increase in CD18 expression in hematopoietic cells in culture, the use of vectors produced by using PG13 GALV-derived retrovirus packaging cells resulted in much improved transduction efficiencies compared with the same vectors produced using the commonly used PA317 amphotropic retrovirus packaging cells. The relatively high rates of CD18 gene transfer and the expression of CD18 at near normal levels in cultured human hematopoietic cells indicate that the vectors developed here will be useful for treatment of LAD in humans and suggest that PG13 packaging cells will permit improved gene transfer into hematopoietic cells for treatment of other diseases.

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Improved transfer of the leukocyte integrin CD18 subunit into hematopoietic cell lines by using retroviral vectors having a gibbon ape leukemia virus envelope

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