Increased Gene Transfer Into Human Hematopoietic Progenitor Cells by Extended In Vitro Exposure to a Pseudotyped Retroviral Vector

By Christof von Kalle, Hans-Peter Kiem, Sondra Goehle, Boris Darovsky, Shelly Heimfeld, Beverly Torok-Storb, Rainer Storb, and Friedrich G. Schuening

Retroviral-mediated gene transfer is the most attractive modality for gene transfer into hematopoietic stem cells. However, transduction efficiency has been low using amphotropic Moloney murine leukemia virus (MoMLV) vectors. In this study, we investigated modifications of gene transfer using amphotropic MoMLV vectors in cell-free supernatant for their ability to increase the current low transduction of both committed hematopoietic progenitors, granulocyte-macrophage colony-forming units (CFU-GMs), and their precursors, long-term culture-initiating cells (LTC-IC). First, based on the observation that bone marrow cells express more gibbon ape leukemia virus (GALV) receptor (G1rv-1) than amphotropic receptor (Ram-1), PG13/LN, which is a MoMLV vector pseudotyped with the GALV envelope, was compared with the analogous amphotropic envelope vector (PA317/LN). Second, progenitor cell transduction efficiency was compared between CD34 enriched and nonenriched progenitor populations. Third, the duration of transduction in vitro was extended to increase the proportion of progenitor cells that entered cell cycle and could thereby integrate vector cDNA. In 20 experiments, \( 1 \times 10^6 \) marrow or peripheral blood mononuclear cells (PBMCs)/mL were exposed to identical titers of pseudotyped PG13/LN vector or PA317/LN vector in the presence of recombinant human interleukin.

**Retroviral Vectors** based on Moloney murine leukemia virus (MoMLV) are widely used to introduce foreign genes into hematopoietic cells and other somatic tissues. Transduction of murine hematopoietic stem cells with ecotropic retroviral vectors\(^2\) is more efficient than gene transfer with amphotropic vectors into hematopoietic stem cells of large animals and humans. Limited success by marking less than 1% to 5% of stem cells has been reported using feline, simian, or canine marrow cells\(^5\),\(^8\) and, more recently, canine peripheral blood mononuclear cells (PBMC)\(^13\). In vitro gene transfer into human committed hematopoietic progenitor cells (granulocyte-macrophage colony-forming unit, CFU-GM) and more primitive long-term culture-initiating cells (LTC-IC) achieved efficiencies of 10% to 40%.\(^14\)\(^-\)\(^18\) The first clinical gene-transfer studies suggest that short-term as well long-term hematopoietic repopulating cells engrafted at a frequency below 1% after ex vivo transduction.\(^19\)\(^,\)\(^20\)

Several reasons may account for this low transduction efficiency. The range of human cells that can be infected with a retroviral vector is determined by the envelope protein of the virus and the presence of the appropriate receptor on the target cell surface.\(^21\) Bone marrow cells poorly express the amphotropic retrovirus receptor (Ram-1),\(^22\) and may explain why amphotropic vectors infect primate hematopoietic stem cells via that receptor far less efficiently than murine ecotropic vectors infect murine hematopoietic stem cells via the ecotropic murine leukemia virus receptor.\(^23\)\(^,\)\(^24\) In addition, MoMLV-derived retroviral vectors require cell division to cross the nuclear membrane and integrate into the host cell genome.\(^25\)\(^,\)\(^26\) However, hematopoietic stem cells rarely divide spontaneously in culture\(^27\)\(^-\)\(^29\) unless induced to cycle with growth factor or chemotherapy treatment.\(^30\)\(^,\)\(^31\) Based on these observations, we hypothesized first that a receptor expressed at higher density on human hematopoietic cells than Ram-1 might be capable of mediating retroviral infection of hematopoietic progenitor cells more efficiently, second that by removing differentiated cells through CD34 enrichment, progenitor cycling and transduction might increase, and third that by extending the exposure to retroviral vector in the presence of cytokines, immature progenitor cells may divide at least once and allow integration of vector cDNA.

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Submitted July 19, 1994; accepted July 29, 1994.

Supported by Grants No. CA15704, CA18105, CA18221, CA31787, CA47748, DK42716, DK47754, and HL36444 awarded by the National Institutes of Health, Departments of Health and Human Services; and Ka 976/1-1 awarded by the Deutsche Forschungsgemeinschaft.

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Fig 1. PCR analysis of DNA extracted from G418-resistant CFU-GM colonies grown in semisolid agar medium after 5 days of transduction in long-term marrow culture is shown. The primers are specific for the bacterial neomycin phosphotransferase gene (note absence of signal in the nontransduced human DNA negative control lane). Positive (+) control amplified from 0.2 μg of TK'/LN cell line transduced with a single copy of PA317/LN vector. Lanes 1 through 8: PCR analysis of DNA extracted from marrow-derived G418 resistant CFU-GM colonies (experiments 1 through 4, 2 colonies each). Lanes 9 through 16: PCR analysis of DNA extracted from PBMC-derived G418-resistant CFU-GM colonies (experiments 13 through 16, 2 colonies each). Varying band intensities in different lanes result from variations in colony size and agar volume harvested.

In the present study, we analyzed human hematopoietic progenitor cell transduction using the PG13/LN retroviral vector, a pseudotyped MoMLV vector that carries the gibbon ape leukemia virus (GALV) envelope protein. We observed that gene transfer into hematopoietic progenitor cells was more efficient with the GALV pseudotyped vector PG13/LN than with the amphotropic envelope vector PA317/LN. Gene transfer also increased after CD34 enrichment. By extending the duration of the transduction culture to three weeks in the presence of recombinant human interleukin-1 (rhIL-1), IL-3, IL-6, stem cell factor (rh SCF, c-kit ligand) and cell-free high-titer PG13/LN vector supernatant, we obtained efficient gene transfer into most committed and immature hematopoietic progenitor cells.

MATERIALS AND METHODS

Target cells. Bone marrow (BM) or PBMC samples were obtained from patients and healthy volunteers who were undergoing stem cell harvests for marrow or peripheral blood stem cell transplants after informed consent as approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. BM cells were harvested by needle aspiration from the posterior iliac crest under general anaesthesia. PBMCs were obtained by apheresis on a Fenwal CS3000 Blood Cell Separator after 4 days of treatment with subcutaneous recombinant human granulocyte colony-stimulating factor (rh G-CSF, Amgen, Thousand Oaks, CA) at 10 μg/kg/d. Mononuclear cells were purified by Ficoll-gradient centrifugation (Lymphocyte Separation Media [LSM]; Organon Teknika-Cappel, Durham, NC) at 400g and 25°C for 30 minutes after 1:3 dilution in Hank’s balanced salt solution (HBSS) supplemented with 1% bovine serum (PBS; Gibco BRL, Bethesda, MD) and 10 U/mL of preservative-free heparin (Fujisawa USA Inc, Deerfield, IL). Samples of mononuclear cells were resuspended in HBSS/1% FBS for CD34 cell enrichment or set up in long-term culture for retroviral transduction. Long-term marrow culture medium consisted of Iscove’s modified Dulbecco’s medium (GIBCO BRL) supplemented with 12.5% of horse serum (GIBCO BRL), 12.5% of FCS (GIBCO BRL), 10^-6 mol/L hydrocortisone (Sigma Chemical Co, St Louis, MO), 10^-4 mol/L 2-mercaptoethanol (Sigma), 1% L-glutamine, and 10 U/mL penicillin/streptomycin (GIBCO BRL). Cultures were maintained at 37°C in humidified air at 8% CO₂.

Enrichment of CD34⁺ cells. Marrow and PBMCs were enriched for CD34—antigen-positive cells with the Ceprate LC stem cell concentrator (CellPro, Bothell, WA). Mononuclear cells were incubated for 25 minutes at room temperature with 20 μg/mL of biotinylated mouse IgM antihuman CD34 antibody 12.8 in phosphate-buffered saline (PBS; Gibco BRL, Grand Island, NY) with 1% bovine serum albumin (BSA; CellPro). Then washed over a sterile column of streptavidin-coated polyacrylamide beads (CellPro). After washing with PBS, CD34⁺ cells were resuspended by gentle mechanical agitation and eluted from the column with PBS/1% BSA with 10 U/mL of heparin (Fujisawa). Results were controlled by flow-cytometric analysis using a FACSscan (Becton Dickinson, San Jose, CA) after re-staining with fluorescein isothiocyanate (FITC)-conjugated HPCA-2 antibody (Becton Dickinson) compared to an FITC-conjugated isotypic control (Caltag, San Francisco, CA).

Retroviral vectors. The retroviral vector LN, the PA317, and the PG13 pseudotyped producer cell lines were kindly provided by Dr A.D. Miller (Fred Hutchinson Cancer Research Center, Seattle, WA). In all experiments, we used the retroviral vector PA317/LN and PG13/LN to transduce bone marrow and PBMC samples.
The LN vector, the bacterial neomycin phosphotransferase (neo) gene is transcribed from the MoMLV long-terminal repeat. PG13/LN and PA317/LN cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 4.5 g/L of glucose, 10% FBS, 1% of L-glutamine and 1% of penicillin/streptomycin (all GIBCO BRL). To produce retrovirus-containing medium for hematopoietic progenitor cell transduction, long-term culture medium was incubated on a subconfluent retrovirus-producer cell layer (0.16 × 10^6 cells/cm^2) for 16 hours at 37°C, then centrifuged at 2,500g for 10 minutes to remove viable nonadherent cells. Retroviral vector titers were measured by a colony-formation assay with NIH 3T3 cells as targets for PA317/LN and HeLa cells as targets for PG13/LN as previously described. When PA317/LN and PG13/LN retroviral vectors were compared, both were used at a tier of 1 × 10^6 colony-forming units (CFU)/mL.

Transduction of marrow and PBMCs by exposure to retrovirus-containing medium. Nonenriched and CD34-enriched marrow cells or PBMCs were cultured in a 1:1 mixture of fresh and retrovirus containing long-term culture medium at a density of 1 × 10^6 cells/mL in vented 75-cm^2 tissue-culture flasks (Costar, Cambridge, MA). Unless indicated otherwise, these long-term cultures of hematopoietic progenitors were cultured on a confluent layer of allogeneic marrow stroma cells irradiated at 1,500 cGy 1 day before the initiation of culture. Polybrene (Sigma) was added to the cultures at a final concentration of 4 µg/mL. rhIL-1, rhIL-3 (Amgen), rhIL-6 (Immunex Corp, Seattle, WA), as well as stem cell factor (Steel factor, SCF, Immunex) were added on the first day at 50 ng/mL unless indicated otherwise. The supernatant was exchanged by pipetting off 50% of the culture media and centrifuging it at 400g for 5 minutes. The cell pellet was resuspended in an identical volume of fresh vector-containing supernatant supplemented with 4 µg/mL of Polybrene and added back to the culture. Cultures were maintained for 1, 2, and 3 weeks. Vector-containing supernatant in cultures fed 1 time/week was added 1 day after weekly demidepopulation according to the protocol of Carter et al. Mock-infection control experiments were conducted in each experimental arm with supernatant harvested from NIH 3T3 cell layers under the conditions described above.

**Clonogenic assays and detection of neo expression in CFU-GM.** Mononuclear cells were harvested from mock-transduced and LN vector–transduced cultures and assayed in triplicate for granulocytic (CFU-GM) colonies. Cells were plated at 5 × 10^4 for nonenriched populations or 1 × 10^4 for CD34-enriched populations in 2 mL of DMEM/0.3% agarose (FMC, Rockland, ME) supplemented with 500 µg/mL of G418.

**Table 2. Effect of Length of Vector Exposure on Efficiency of Gene Transfer Into CFU-GM**

<table>
<thead>
<tr>
<th></th>
<th>1 wk</th>
<th>2 wks</th>
<th>3 wks</th>
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<tbody>
<tr>
<td>1 Feeding/wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM 2*</td>
<td>ND</td>
<td>ND</td>
<td>26/276 (9)</td>
</tr>
<tr>
<td>BM 3*</td>
<td>1/190 (0.5)</td>
<td>61/243 (25)</td>
<td>12/238 (5)</td>
</tr>
<tr>
<td>5 Feedings/wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBMC</td>
<td>10/19 (53)</td>
<td>238/260 (91)</td>
<td></td>
</tr>
<tr>
<td>BM 1a†</td>
<td>90/1,070 (8)</td>
<td>ND</td>
<td>147/417 (35)</td>
</tr>
<tr>
<td>BM 1b†</td>
<td>33/1,098 (3)</td>
<td>ND</td>
<td>217/324 (67)</td>
</tr>
<tr>
<td>BM 1c†</td>
<td>20/1,200 (2)</td>
<td>ND</td>
<td>430/840 (67)</td>
</tr>
<tr>
<td>BM 2*</td>
<td>ND</td>
<td>162/221 (73)</td>
<td></td>
</tr>
<tr>
<td>BM 3</td>
<td>19/11 (11)</td>
<td>268/361 (74)</td>
<td>1,477/1,680 (99)</td>
</tr>
<tr>
<td>BM 4</td>
<td>2/11 (2)</td>
<td>23/50 (46)</td>
<td>ND</td>
</tr>
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</table>

Summary of nine experiments analyzing neo gene transfer into CFU-GM after 1, 2, or 3 weeks of long-term transduction. PG13/LN vector containing supernatant was replaced either 1 or 5 times/wk. Colony assays were conducted in triplicate. Colony numbers are quoted as number of G418-resistant colonies/total colonies per 10^5 mononuclear cells plated. The transduction efficiency, expressed as the percentage of G418-resistant to total colonies, is quoted in parentheses.

Abbreviations: ND, not done; †, technical failure.
*No growth factors added.
† Mononuclear marrow cells that had been cryopreserved after enrichment to 68% CD34+ cells.
‡ Long-term transduction culture initiated on irradiated allogeneic stroma cell feeder layer. All other transduction cultures were initiated without stromal support.
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Table 3. Effect of Length of Vector Exposure on the Efficiency of Gene Transfer Into LTC-IC-Derived Colonies

<table>
<thead>
<tr>
<th>LTC-IC ± G418 (%)</th>
<th>1 wk</th>
<th>2 wks</th>
<th>3 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM 2*</td>
<td>ND</td>
<td>ND</td>
<td>21/33 (84)</td>
</tr>
<tr>
<td>BM 3*</td>
<td>ND</td>
<td>11/51 (22)</td>
<td>0/2 (0)</td>
</tr>
</tbody>
</table>

5 Feedings/wk

| BM 1b†           | 214/311 (69) | ND     | ND     |
| BM 1c†           | 9/21 (43)    | ND     | ND     |
| BM 2*            | ND           | ND     | 233/182 (100) |
| BM 3             | 265/323 (88) | 245/216 (100) | 195/308 (83) |
| BM 4             | ND           | 1,073/1,207 (82) | ND     |

Summary of seven experiments analyzing neo gene transfer into colonies derived from LTC-IC after 1, 2, and 3 weeks of long-term transduction. Colony assays were conducted in triplicate after 5 weeks of additional long-term culture without vector on irradiated stroma layers. Results are quoted as number of G418-resistant colonies/total colonies per 10⁶ mononuclear cells plated. The transduction efficiency, expressed as the percentage of G418-resistant to total colonies, is quoted in parentheses.

Abbreviation: ND, not done.
† No growth factors added.

with 20% of prescreened, heat-inactivated FBS (GIBCO) and 50 ng/mL each of IL-1, IL-3, IL-6, SCF, granulocyte-macrophage colony-stimulating factor (GM-CSF, Immunex) and G-CSF in 35-mm Petri dishes (Costar, Cambridge, MA) with and without G418 (Sigma, St Louis, MO) at 3 mg/mL, twice the pretested inhibitory concentration. Cultures were maintained at a 100% humidified atmosphere at 37°C and 10% CO₂. After 14 days, CFU-GM colonies were counted. The ratio of G418-resistant colony formation to total colony formation was used to measure the transduction efficiency. Isolated colonies were picked in 20 μL of agar, washed twice in HBSS at 50°C, and submitted to polymerase chain reaction (PCR) analysis for the detection of neo cDNA. No colony growth was observed in the triplicate G418-containing dishes of mock-infected control cells from any sample.

To evaluate gene transfer into more immature progenitor cells, one 10⁶ nonenriched or 10⁶ CD34-enriched cells were transferred onto a second irradiated stromal feeder layer. This culture was maintained for 5 weeks by weekly replacing 50% of the long-term culture media. Cells retrieved from these cultures were then assayed for CFU-GM with and without G418 as described above, using 10⁶ viable mononuclear cells from nonenriched or 2.5 x 10⁶ from CD34-enriched progenitor cell cultures.

Detection of neo cDNA in CFU-GM colonies by PCR. For direct PCR amplification of neo cDNA, a modification of a previously described protocol²⁷ was used. Colony cells obtained after washing were digested with proteinase K in lysis buffer (KCl 50 mmol/L, TRIS-HCL 10 mmol/L, MgCl₂ 2.5 mmol/L, 0.45% NP40, 0.45% Tween 20, Proteinase K 400 μg/mL) at 55°C for 3 hours. The following oligonucleotides specific for neo cDNA were used to generate an 821-bp product: sense primer neo 350: 5' AAG AGA CAG GAT GAA GGA TCG 3', antisense primer neo 1150: 5' CAG AAC TCG TCA AGA 3'. After 15 minutes at 95°C and addition of nucleotides (50 ng each), primers (50 pmol each), 10 x Taq buffer, water and 100 μL and 2.5 U of Taq polymerase (Perkin Elmer-Cetus, Norwalk, CT) neo cDNA was amplified for 35 cycles of denaturation (94°C, 15 seconds), annealing (58°C, 1 minute) and extension (72°C, 1 minute) in a thermal cycler (Perkin Elmer-Cetus). Amplified products were separated by electrophoresis in 1% agarose gels and transferred to nylon membranes (zeta-probe, Bio-Rad, Richmond, CA).²⁶ Hybridization was performed overnight at 42°C with a ³²P-labeled 598-bp internal fragment obtained by amplification with sense primer neo 450: 5' ACAACAGACAAATCGGCT-GCT 3' and antisense primer neo 1027: 5' GCCAACCCTATGTCCT-GTATA 3' under the amplification conditions described above.

Giemsa and Wright staining of slides. Cells from single colonies were mounted on slides with a Cytospin 3 centrifuge (Shandon, Pittsburgh, PA), air-dried, and stained with Wright-Giemsa hematology staining solution (Curtin Matheson Scientific Inc, Houston, TX).

Statistical evaluation. The mean, median, and standard deviation of colony scores were calculated for each experiment. Data were analyzed using Student's t-test. Significant differences are labeled with the appropriate P values.

RESULTS

Efficiency of gene transfer into hematopoietic progenitor cells with the GALV envelope PG13/LN vector. A series of 20 experiments compared the efficiency of gene transfer obtained in marrow and peripheral blood-derived CFU-GM with the GALV-pseudotyped PG13/LN vector with that of the amphotropic envelope vector PA317/LN. Both vectors were used at a titer of 1 x 10⁶ CFU/mL using an identical 5-day transduction protocol. As shown in Table 1, the transduction of hematopoietic progenitor cells as measured by G418-resistant CFU-GM colony formation was more efficient in PG13/LN than in PA317/LN in marrow (17.9% ± 10.6%, respectively, P = .019) and PBMCs (20.8% ± 12.4%, respectively, P = .064). Long-term hematopoietic culture initiating cells (LTC-IC) were analyzed in two experiments using marrow and PBMC. After 5-day transduction with virus from PG13/LN cells, 4 of 14 colonies (28%) grown from marrow-derived LTC-IC, and 7 of 20 colonies (35%) grown from PBMC-derived LTC-IC were G418 resistant. Using PA317/LN supernatant, 4 of 21 (19%) of marrow-derived and 4 of 22 (18%) PBMC-derived colonies grown from LTC-IC were G418 resistant.

Transduction of human nonenriched and CD34-enriched marrow and peripheral blood cells. To test the influence of CD34⁺ cell enrichment on progenitor cell transduction, nonenriched and CD34-enriched mononuclear cells from each of three marrow and eight peripheral blood donors were transduced with the PA317/LN vector in a 5-day protocol...
Efficient gene transfer into hematopoietic stem cells is important for successful clinical gene therapy. Transduction with amphotropic vectors is hampered by the low affinity of retroviral vectors to hematopoietic target cells. Crooks and Kohn could not observe quantitative binding of these vectors to immature CD34+/CD38 progenitor cells without growth factors. In line with their studies, which analyzed PA317/LN binding but not transduction, our results suggest that vector binding to the amphotropic receptor Ram-1 is limiting for the transduction of hematopoietic progenitor cells with cell-free retrovirus supernatant. Using two identical LN vectors with different envelope proteins, we observed a significant increase in the transduction of both marrow and peripheral blood–derived CFU-GM with the GALV-pseudotyped PG13/LN vector compared with the original PA317/LN vector. Similar results were observed in two experiments that evaluated LTC-IC. This finding is compatible with the observation of Kavanaugh et al that the GALV receptor Glvr-1, a sodium-dependent phosphate symporter, is expressed at much higher density on rat marrow cells compared with Ram-1. Our data suggest that the GALV-pseudotyped PG13 packaging system may allow more efficient ex vivo gene therapy of hematopoietic stem cells.

Enrichment for CD34+ cells reduces marrow to about 1% of its mononuclear cells while retaining most of the hematopoietic progenitor and stem cells, thus reducing the volume of cell culture for ex vivo gene transfer in primates and humans. Whereas efficient gene transfer into CD34-en-
riched cells\textsuperscript{9,42,43} and CD34-purified single cells\textsuperscript{44} has been described, this study first shows a significant improvement of progenitor cell transduction by CD34 enrichment as the only variable in a side-by-side comparison. Increased progenitor cell cycling after the removal of differentiated leukocytes\textsuperscript{45} and removal of cells that have a higher vector affinity than progenitor cells\textsuperscript{29} may be responsible for this observation.

MoMLV vectors require mitosis to integrate into the target cell genome.\textsuperscript{3,26} In vitro, cycling of immature hematopoietic progenitor cells can only be observed after long-term culture for days\textsuperscript{45} or weeks.\textsuperscript{3,46} It presumably requires the more mature leukocytes to differentiate or disappear.\textsuperscript{3,25} This may be the reason why short-term transduction of hematopoietic cells within 24 hours is neither efficient in murine stem cells\textsuperscript{42} nor in prestimulated human hematopoietic progenitor cells.\textsuperscript{47} Therefore, extending the culture duration in the presence of retrovirus vectors should increase the transduction efficiency in immature progenitor cells. Initial attempts to increase transduction by extending the cocultivation beyond 3 days have not been successful in large animal\textsuperscript{10} or human hematopoietic gene transfer,\textsuperscript{30} possibly because the horse serum in long-term marrow culture media interferes with the growth of vector producing cells (unpublished observation, June 1989). However, it has been shown by others that prolonged exposure of target cells to virus containing supernatant for 3 days\textsuperscript{41,43,49,50} or 5 days,\textsuperscript{10} or weekly exposure for three weeks\textsuperscript{12,51} improved retrovirus transduction into hematopoietic progenitor cells. Our data strongly suggest that most committed and immature hematopoietic progenitor cells can be transduced by extension of a long-term transduction culture for 3 weeks if fresh media with PG13/LN vector and growth factors is replaced on at least 5 of 7 days. The results obtained with human hematopoietic cells after one weekly feeding for 3 weeks suggest that, in contrast to observations in dogs by Carter and Bienzle,\textsuperscript{1,2} growth factor support is required to maintain immature human progenitor cells, and that daily exchange of vector-containing supernatant is more efficient than weekly feedings.

Transduction of immature progenitor cells\textsuperscript{3,25} at the time of their entry into cell cycle depends on the presence of retroviral vector. Retroviral vector particles have a half life of about 4 hours in culture medium at 37°C. After entering the cells, integration of the vector cDNA was not observed if cells did not divide within the next six hours.\textsuperscript{29} These data suggest that retroviral vector should be added at least every 24 hours to be available in significant titers over time.

The growth factors IL-3, IL-6,\textsuperscript{3,51} IL-1, and SCF\textsuperscript{30,54} enhance retroviral gene transfer into human hematopoietic progenitor cells and murine hematopoietic stem cells, presumably by the induction of stem cell cycling.\textsuperscript{18,31,55,48} Similar to reports about long-term perfusion cultures in the presence of IL-3, IL-6, and SCF,\textsuperscript{5,25} we observed an expansion of the number of CFU-GM and at least a maintenance of the number of LTC-IC. The frequent exchange of supernatant could explain the difference to static long-term cultures, in which LTC-IC numbers drop significantly after 10 days of culture.\textsuperscript{29,56,57} The number of LTC-IC we obtained was comparable with the yield of a fresh, noncultured marrow sample.\textsuperscript{20}

We have identified conditions that led to efficient transduction and expansion of CFU-GM as well as efficient transduction and maintenance of LTC-IC, the most immature human hematopoietic progenitor cells that can be assayed in vitro. Transduction with PG13-pseudotyped retroviral vector, after stem cell enrichment with the CD34 antigen and by extended culture in the presence of PG13 vector, IL-1, IL-3, IL-6, and SCF would be compatible with a clinical application.

**ACKNOWLEDGMENT**

We would like to thank Dr A.D. Miller for providing the retrovirus vectors and for helpful discussion of the manuscript.

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