Retroviral-Mediated Gene Expression in Human Myelomonocytic Cells: A Comparison of Hematopoietic Cell Promoters to Viral Promoters

By Punam Malik, Wanda J. Krall, Xiao-Jin Yu, Chen Zhou, and Donald B. Kohn

Gene transfer into human hematopoietic stem cells with expression targeted to the maturing myelomonocytic progeny has applications for gene therapy of genetic diseases affecting granulocytes and macrophages. We hypothesized that promoters of myeloid-specific genes that are upregulated with myelomonocytic differentiation would also upregulate expression of an exogenous gene in a retroviral vector. Moloney murine leukemia virus (MoMuLV)-based retroviral vectors using promoters from hematopoietic genes (CD11b, CD18, and CD34) were compared with vectors with viral promoters (MoMuLV long terminal repeat [LTR], cytomegalovirus [CMV], and simian virus 40 [SV40]). Human glucocerebrosidase (GC) cDNA was the reporter gene. HL-60 cells were transduced with these vectors and vector-derived GC activity was compared in undifferentiated HL-60 cells and the same cells differentiated into granulocytes using dimethyl sulfoxide or monocyte/macrophages using phorbol myristate acetate. In undifferentiated HL-60 cells, vector-derived GC activity was the highest when it was controlled by the MoMuLV LTR. In HL-60 cells differentiated into granulocytes, vector-derived GC activity transcribed from the CD11b, MoMuLV LTR, and CMV promoters was equivalent to 1.7, 1.5, and 1.5 times the normal endogenous GC activity, respectively, and 0.8, 2.0, and 3.6 times the normal GC activity, respectively, in those differentiated into macrophages. With granulocytic differentiation, the CD11b promoter showed maximal induction in GC activity (8-fold); with macrophage differentiation, the CD11b promoter showed a fourfold induction in GC expression. The CD11b promoter also generated significant levels of GC activity in the myelomonocytic progeny of transduced CD34+ cells. Expression from the CD11b promoter, unlike that from the CMV or the MoMuLV LTR promoters, was relatively myelomonocyte-specific, with minimal expression observed in Jurkat T cells or HeLa carcinoma cells. The induction of expression from the CD11b promoter with differentiation in HL-60 cells correlates with the developmental regulation of the CD11b gene. Retroviral vectors using the CD11b promoter have potential utility for gene therapy of disorders affecting the myelomonocytic lineage.

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Hematopoietic stem cells (HSCs) have the capacity to proliferate, self-renew, and differentiate into all of the various mature hematopoietic cell types, making them ideal targets for gene therapy of disorders affecting any of their progeny. Retroviral vectors are currently the most efficient means to stably transduce genes into human hematopoietic cells. However, expression of the inserted gene in a specifically affected lineage, rather than in all progeny of the HSCs, may be desirable in treating certain genetic disorders.

Myelomonocyte-specific expression of exogenous genes has potential for gene therapy of genetic diseases such as lysosomal storage diseases (eg, Gaucher disease), chronic granulomatous disease (CGD), and leukocyte adhesion defects.

Gaucher disease is due to deficiency of the lysosomal enzyme glucocerebrosidase (GC). It results in accumulation of unmetabolized glycolipid substrate in cells of the monocyte/macrophage lineage in the spleen, liver, bone marrow, and, in the neuromonophagocyte subtypes, the central nervous system microglia. We have been studying retroviral-mediated gene transfer of the human GC cDNA in mouse and human bone marrow as preclinical studies for the application of gene therapy to Gaucher disease. In previous reports, we have shown expression of human GC from retroviral vectors under the transcriptional control of the Moloney murine leukemia virus long terminal repeat (MoMuLV LTR) and the simian virus 40 (SV40) promoters. However, expression of the exogenous gene under the control of viral promoters is lineage nonspecific and may be transcriptionally silent in very primitive cells, as we have observed in a murine bone marrow transplant model.

Retrovirally mediated erythroid-specific gene expression has been previously reported when expression of the inserted gene was under transcriptional control of the β-globin promoter/enhancer. Myelomonocytic-specific gene expression at clinically relevant levels after retroviral-mediated transduction has not been previously shown. Preliminary reports from transient transfections of cell lines have shown that promoters from myeloid- and monocytic-specific genes are relatively more active in myelomonocytic cell lines than in other cell types. However, few investigators have compared the relative strength of these promoters in retroviral vectors with that of conventionally used viral promoters for applications in gene therapy. Use of promoters endogenously active in a particular lineage may circumvent the in vivo silencing of gene expression that has been observed with viral promoters.

Promoters from leukocyte integrin genes that are solely expressed in cells of the myelomonocytic lineage have the potential for directing expression of retrovirally transduced genes specifically into the myelomonocytic lineage. Human β-2 integrins are leukocyte-specific heterodimeric molecules consisting of a common β-chain (CD18) with varying lineage-specific α-chains (CD11a, CD11b, and CD11c). Expression of CD11b and CD11c is predominantly restricted to cells of the myelomonocytic lineage and is developmentally regulated, with the highest expression seen in differentiated granulocytes and macrophages.
are pan-leukocytic and also show upregulated expression with myelomonocytic maturation.10,16,17 The promoters of CD11a,13,15 CD11b,10,11 CD11c,14 and CD1818,19 have been cloned and transient transfections using the CD11b and CD18 promoters have shown tissue-specific gene expression.10,11,15,16,18,19

CD34, a molecule present on hematopoietic progenitors, can lead to signal transduction and enhanced adhesiveness of CD34+ hematopoietic cells20 and its expression is downregulated with differentiation.21 The promoter of the CD34 gene has also been cloned and shown to be functional in hematopoietic cell lines.22

We hypothesized that promoters of myeloid-specific genes that are upregulated with myelomonocytic differentiation would also upregulate expression of an exogenous gene in a retroviral vector. In this study, we quantitatively compared the strength, inducibility with differentiation, and myelomonocytic specificity of the human CD11b, CD18, and CD34 promoters to that from commonly used viral promoters. The aim of the study was to direct retroviral vectors to express the human GC cDNA in maturing myelomonocytic cells at clinically relevant levels and to study the regulation of these promoters with differentiation. We used HL-60 cells, a human acute myeloid leukemia cell line, as a model of myelomonocytic differentiation. HL-60 cells were transduced with retroviral vectors that used either viral promoters (the MoMuLV retroviralLTR, cytomegalovirus [CMV] promoter, or the SV40 promoter) or hematopoietic cell promoters (CD11b, CD18, or CD34) controlling the human GC cDNA as a reporter gene. We also transduced primary human CD34+ cells with the vector using the CD11b promoter. Our results showed that GC expression directed by the CD11b promoter produced GC activity at levels that would be clinically useful, with differentiation of both the transduced HL-60 cells and human CD34+ cells. GC expression from the CD11b promoter in HL-60 cells was upregulated with myelomonocytic differentiation and was relatively myelomonocytic-specific, correlating with the expression pattern of the CD11b gene.

MATERIALS AND METHODS

Retroviral vectors. The CD11b promoter used in the vector construct is a 404-bp HindIII/BamHI fragment provided by D.D. Hickstein (Fred Hutchinson Center, Seattle WA, Seattle, WA) from bp -312 to +92 from the transcriptional start site.11 The CD18 promoter used is a 947-bp BumHUHindIII fragment provided by D.D. Hickstein (Fred Hutchinson Center, Seattle VA, Seattle, WA) from bp -312 to bp +92 from the transcriptional start site. The CD18 promoter is a 404-bp HindIIUBamHI fragment provided by D.D. Hickstein (Fred Hutchinson Center, Seattle VA, Seattle, WA). The CD34 promoter, which was provided by D.G. Tenen, (Beth-Israel Hospital, Boston, MA), is a 391-bp fragment prepared by Exonuclease III digestion from the 5′ end of the 2 kb CD34-luciferase promoter plasmid.22

All retroviral vectors were based on the LN vector backbone23 or the GIna vector backbone (provided by Genetic Therapy Inc, Gaithersburg, MD; Table 1). The LN vector, in which the MoMuLV LTR drives the bacterial neomycin resistance gene, was constructed by cloning the I-GC-BS plasmid into the SalI sites of the LmGLa plasmid. The LGCSN and the LNCGC vectors were constructed by cloning the human GC cDNA from the I-GC-BS plasmid into pLXSN and pLNCX.22 In the N2SVGC vector, the SV40 early region promoter/enhancer was used to drive the expression of the human GC cDNA in the N2 vector backbone.22 The LN111bGC, LN18GC, and LNC34GC vectors were made by combining the respective promoter fragments and the human GC cDNA from the I-GC-BS plasmid into the SalI/Xc1 I sites of the GIna plasmid.

Each plasmid construct was transfected into the PE501 ecotropic packaging cells23 using DOTAP Transfection Reagent (Boehringer Mannheim, Indianapolis, IN). The transiently produced virus, which was collected after 48 hours, was used to transduce PA317 amphotropic packaging cells. The transduced PA317 cells were selected in 0.5 mg/mL active G418 (Geneticin; GIBCO, Grand Island, NY). Individual colonies were isolated, expanded, and screened for viral titer. Vector titers were measured on NIH 3T3 cells with serial dilutions of viral supernatant from the PA317 clones. PA317 vector packaging clones producing high viral titer were expanded and used for gene transfer. The vector titers were between 0.5 and 2 × 10⁸ G418-resistant cfu/mL. The PA317 clones were negative for helper virus when 3T3 cells transduced with viral supernatants from these clones were tested by polymerase chain reaction for amphotropic env sequences, as described.26

Transduction and differentiation of HL-60 cells. HL-60 cells, a human acute myeloid leukemia cell line, were acquired from American Type Culture Collection (ATCC; Rockville, MD) and grown in R-10 (RPMI-1640 medium [GIBCO-BRL; Bethesda, MD] with 10% fetal bovine serum [FBS; Irvine Scientific, Santa Ana, CA]), 2 mmol/L L-glutamine, and 100 U/mL penicillin-streptomycin). PA317 vector producing fibroblast cells were irradiated (40 Gy) and plated at subconfluency (1 × 10⁶ cells/25-mm² flask) for 24 hours in 10 mL D-10 (Dulbecco’s modified Eagle’s medium [DMEM] high glucose [GIBCO-BRL] with 10% FBS, 2 mmol/L L-glutamine, and 100 U/mL penicillin-streptomycin). Each 24-hour culture was transduced with PAN10 cells suspended in R-10 at a concentration of 2.5 × 10⁶ cells/mL and polybrene (Sigma, St Louis, MO) was added to a final concentration of 8 μg/mL. HL-60 cells were cocultivated on the vector-producing PA317 cells for 48 hours and washed once with Hank’s Buffered Saline Solution (HBSS) and these pools were bulk-selected in R-10 with 1 mg/mL of active G418 for 2 to 4 weeks. Each of these transductions was treated as a separate experiment and transduced G418-selected pools were either assayed in the undifferentiated state or after differentiation along the granulocytic lineage for 5 days with

<table>
<thead>
<tr>
<th>Vector Name</th>
<th>Retroviral Construct (gene order)</th>
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<tbody>
<tr>
<td>LN</td>
<td>LTR--NEO----LTR</td>
</tr>
<tr>
<td>LN-GC</td>
<td>LTR--NEO------GC----LTR</td>
</tr>
<tr>
<td>LGCSN</td>
<td>LTR--GC--SV40--NEO----LTR</td>
</tr>
<tr>
<td>LNC11bGC</td>
<td>LTR--NEO--CD11b--GC---LTR</td>
</tr>
<tr>
<td>LNC18GC</td>
<td>LTR--NEO--CD18--GC---LTR</td>
</tr>
<tr>
<td>LNC34GC</td>
<td>LTR--NEO----CD34--GC---LTR</td>
</tr>
</tbody>
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MoMuLV-based retroviral constructs with human GC cDNA as the reporter gene. The promoters driving expression of the human GC cDNA (italics) were the MoMuLV LTR or internal promoters CMV, SV40, CD11b, CD18, and CD34. All vectors had the bacterial neomycin resistance gene (NEO), which was controlled either by an internal SV40 promoter (in LGCSN) or the LTR (in all other vectors), to allow for selection of transduced cells. The LN vector, lacking the human GC cDNA, was the negative control.

The 1-GC-BS plasmid1 into the SalI/Xc1 I sites of the GIna plasmid. The LGCSSN and the LNCGC vectors were constructed by cloning the human GC cDNA from the I-GC-BS plasmid into pLXSN and pLNCX.22 In the N2SVGC vector, the SV40 early region promoter/enhancer was used to drive the expression of the human GC cDNA in the N2 vector backbone.22 The LN111bGC, LN18GC, and LNC34GC vectors were made by combining the respective promoter fragments and the human GC cDNA from the I-GC-BS plasmid into the SalI/Xc1 I sites of the GIna plasmid.
1.25% dimethyl sulfoxide (DMSO; Research Industries Corp, Salt Lake City, UT) or 10^-4 mol/L retinoic acid (Sigma) and, along the monocytic lineage for 3 days with 50 ng/mL phorbol myristate acetate (PMA; Sigma). Differentiation in the initial experiments was shown by morphology using Wright-Giemsa staining and nonspecific esterase staining. Fluorescence-activated cell analysis (FACS) for expression of differentiation antigens CD11b, CD11c, CD13, and CD18 was also performed in the same pool of transduced, G418-selected pools of cells that were assayed for GC activity in the initial experiments.

**Transduction of Jurkat and HeLa cells.** Jurkat (E6.1) cells, a human T-cell leukemia cell line, and HeLa cells, a human cervical carcinoma cell line, were acquired from ATCC. Jurkat cells were maintained in R-10 and transduced by cocultivation on PA317 cells by the same procedure as described for the HL-60 cells. HeLa cells were grown in M-10 (Modified Eagle’s Medium [MEM; Gibco]) with 10% FBS, 2 mM L-glutamine, and 100 U/mL penicillin-streptomycin. They were transduced by adding cell-free viral supernatant four times, 12 hours apart, with polybrene to a final concentration of 8 μg/mL. Transduced Jurkat and HeLa cells were selected in 1 mg/mL and 0.9 mg/mL, respectively, of active G418 for 2 weeks. GC activity was measured in unstimulated Jurkat cells and in Jurkat cells stimulated with 10 μg/mL of phyohemagglutinin (PHA M/P; DIFCO, Detroit, MI) and 25 ng/mL PMA for 24 hours.

**Transduction of primary human cells.** CD34+ enriched progenitor cells, from non-Gaucher patients, were obtained either from cord blood, collected after normal deliveries (generously provided by Kaiser Permanante, LA, CA), or taken from granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood cells. Use of these samples was approved by the Committee on Clinical Investigations at Children’s Hospital Los Angeles. Light-density mononuclear cells were obtained by centrifugation on ficoll-hyphaque (Pharmacia, Piscataway, NJ), as previously described. The cells were then used to sort CD34+ cells using the 9C5 monoclonal antibody (a gift from Dr Ping Law, Baxter, Inc, Irvine, CA) and immunomagnetic beads, as previously described. CD34+ cells were plated on an irradiated (20 Gy) monolayer of allogeneic stromal cells obtained from a Gaucher patient to minimize contributions to background GC enzyme activity from stromal cells. Gaucher stromal cells were derived by culturing the adherent fraction of bone marrow cells in stromal medium (Iscove’s Modified Dulbecco’s Medium [IMDM; Gibco]) with 15% FBS, 15% Horse serum [Gemini Bioproducts, Calabasas, CA], 10^-4 mol/L 2-mercaptoethanol, 10^-4 mol/L hydrocortisone [Sigma], 2 mM L-glutamine, and 100 U/mL of penicillin-streptomycin) and passed 3 to 4 times before cocultivation with CD34+ progenitors, as described earlier. CD34+ cells, plated at a density of 10^6 cells/mL on monolayers of Gaucher stroma, were prestimulated for 48-hours in basal bone marrow medium (BBMM; IMDM with 30% FBS, 1% BSA [Sigma], 10^-4 mol/L 2-mercaptoethanol, 10^-4 mol/L hydrocortisone, 100 U/mL penicillin-streptomycin, and 2 mM L-glutamine containing the cytokines rHIL-6 [50 U/mL; R&D Systems, Minneapolis, MN] and rHIL-3 [10 mg/mL; Immunex Corp, Seattle, WA], and 50 ng/mL rH mast cell growth factor (MFG; a c-kit ligand; 50 ng/mL; Immunex). Cells were transduced on the stromal monolayer by replacing half the volume of BBMM with filtered viral supernatant from LN-and LNI IbGC-vector producing PA317 cells 12 hours apart 6 times over the next 72 hours, with the addition of polybrene to a final concentration of 4 μg/mL. Cytokine concentrations were maintained as stated previously throughout the transduction. One set of CD34+ cells was sham-transduced using D-IO medium instead of viral supernatants, with all the other transduction conditions remaining the same. After this procedure, the nonadherent progenitor cells were removed by vigorous pipetting, washed to remove the polybrene, and plated for 2 to 4 hours to remove the adherent stromal cells that may have been collected with the hematopoietic cells.

A portion of these cells was then plated in methylcellulose progenitor colony-forming assay, with and without G418 (0.9 mg/mL), to measure gene transfer efficiency, as described. Colony-forming units (CFUs) were scored on day 14 after plating. The rest of the cells were transferred to fresh irradiated Gaucher-derived stromal monolayers and cultured in BBMM with IL-3, IL-6, and MGF with the addition of GM-CSF (50 ng/mL), to hasten differentiation. On days 7 and 14 after transduction, nonadherent cells were harvested, washed twice in HBSS, and assayed for GC activity, as described below.

**Quantitation of GC expression.** GC enzyme activity was measured using a fluorometric assay, as previously described. Briefly, HL-60 cells were washed twice in HBSS at 4°C. Cell lysates were made by suspending the cells in a lysis buffer (50 mmol/L potassium phosphate, 0.25% Triton X-100, pH 6.5) and sonicating for four 10-second pulses at 40 watt seconds at 4°C. Lysates were spun at 16,000g at 4°C for 2 minutes and protein concentrations in the supernatants were quantitated using the Pierce BCA assay (Pierce, Rockford, IL). Aliquots of supernatants containing 10 μg protein were then incubated for 1 hour at 37°C with the fluorogenic substrate 4-methylumbelliferyl-β-glucoside (10 μmol/L) dissolved in a buffer containing 0.1 mol/L citric acid, 0.2 mol/L sodium phosphate, 0.25% sodium taurocholate, and 0.2% (wt/vol) Triton X-100. The reaction was stopped using glycine carbonate buffer (0.85 mol/L, pH 10.4) at 4°C. The cleaved fluorescent 4-methyl umbellifere was quantitated with a Fluoromet 2000 fluorescent spectrophotometer (Hitachi, Fremont, CA) at 390 nm by comparison to a standard curve. GC activity was expressed as nanomoles per minute per milligram of protein and all values reported are means of duplicate samples.

Because the cells tested for GC activity were not derived from persons with Gaucher disease, they have normal endogenous levels of the GC enzyme. The assay of the cell lysates therefore measured the total GC activity, which is the sum of the endogenous GC activity present in these cells and any vector-derived GC activity. Endogenous GC activity was measured in the LN-transduced cells. Vector-derived GC activity in the GC-transduced cells was calculated by subtracting the endogenous activity from the total GC activity. Because the background GC activity in HL-60 cells varied widely among different experiments, vector-derived GC activity was calculated in each experiment using background levels from that particular experiment. Fold induction of GC activity with differentiation was calculated by dividing the vector-derived GC activity in differentiated cells by that in the respective undifferentiated cells transduced by the same vector.

**Quantitative Southern blot analysis.** Genomic DNA was extracted from transduced cells and digested with EcoRI and BamHI, cutting at sites within the GC coding region. DNA from a cell line with a single copy of the human GC cDNA (PNT-17) was also cut with the same restriction enzymes and mixed at various ratios with DNA from untransduced cells to generate a standard curve for copy number. Digested DNA were run on a 1% agarose gel in tris-acetate-EDTA buffer, transferred onto nylon filters, and probed with a [32P]-labeled 1.5-kb EcoRI-BamHI fragment of the human GC cDNA. Filters were washed to a stringency of 0.3× SSC at 65°C and exposed to Kodak AR 5 films at -70°C. The relative copy number were calculated by scanning densitometry of the autoradiographs on a SciScan 5000 densitometer (US Biochemicals, Cleveland, OH). The endogenous genomic GC bands detected by the same probe served as an internal loading control.

Quantitative Southern blot analysis of GC-transduced HL-60 cells showed that each population of transduced cells had a single copy of the vector per cell. Jurkat cells had between 1 and 1.3 vector copy number per cell population, whereas HeLa cells had between 1 and 3.1 vector copy number per cell population. The GC activity data for Jurkat and HeLa cells were therefore normalized for a single copy per cell.
RNA analysis. Northern analysis was performed on HL-60 cells transduced with the non–GC-containing vector LN, the promotless GC vector LN-GC, and the CD11b promoter vector LN11bGC. Cells were harvested either in the undifferentiated state or the same cells were differentiated into granulocytes with DMSO or retinoic acid for 5 days or into macrophages using PMA for 3 days. Half the cells were used for extracting RNA and the other half were used to quantify GC activity. Total cellular RNA was extracted using the guanidine isothiocyanate acid-phenol technique. Fifteen-microgram samples of total RNA were size-fractionated on a 1.2% agarose-formaldehyde gel in MOPS buffer and transferred onto a nylon membrane. The blot was hybridized first to a [32P]-labeled EcoRI/BamHI fragment from the human GC cDNA, which detects the endogenous human GC mRNA and the vector-derived GC transcripts. The blot was then stripped and subsequently hybridized to a [32P]-labeled Cla I/KpnI fragment of the GNA plasmid that contains the U3 region of MoMuLV LTR. The latter probe is complementary to any mRNA transcripts ending at the 3′LTR region, hence detecting all vector-derived mRNA transcripts. The same filters were stripped by boiling in 1% glycerol and rehybridized with a [32P]-labeled human β-actin cDNA as a loading control. Filters were washed to a stringency of 0.3× SSC at 65°C and exposed to Kodak AR films at −70°C. All mRNA transcripts were quantitated by measuring the beta emission (cpm) on the Betascope (Betagen, Mountain View, CA).

Antibody staining and FACS analysis. Vector-transduced, G418-selected HL-60 cells (differentiated and undifferentiated) were washed twice with HBSS and suspended at a concentration of 10⁷ cells/ml. They were then stained with monoclonal antibodies to myeloid differentiation antigens and analyzed using FACS. All staining was performed at 4°C. One million cells were incubated with unconjugated mouse Ig (MlgG; Coulter, Hialeah, FL) for 30 minutes to block nonspecific antibody binding. Conjugated monoclonal antibodies to human CD11b (Mo-1/fluorescein isothiocyanate [FITC]), CD11c (LeuM5-phycocerythrin [PE]), Becton Dickinson, San Jose, CA), CD13 (My7-PE; Coulter), or CD18 (LFA-1, β chain-FITC; Dako, A/S, Glostrup, Denmark) were added to the cell samples for 30 to 60 minutes. Cells were then washed twice with saline to remove excess antibody and fixed in 2% paraformaldehyde. Samples were subjected to flow cytometry on a Becton Dickinson FACScan.

RESULTS

Differentiation of HL-60 cells. The HL-60 cell-line is a convenient model of myelomonocytic maturation to study retroviral-mediated gene expression in hematopoietic progenitor cells and their differentiated progeny. HL-60 cells are arrested in the promyelocytic stage of development but can be induced to differentiate into either granulocytes or macrophages using various chemical and biologic inducers such as DMSO, retinoic acid, vitamin D₃, γ-interferon, or phorbol esters. We differentiated HL-60 cells into granulocytic cells using DMSO (or retinoic acid in 1 experiment) and into monocyte/macrophages using PMA. Wright-Giemsa staining of these cells showed maximum morphologic differentiation into granulocytes on day 5 with DMSO and retinoic acid and into monocytes/macrophages on day 3 with PMA. The monocyte/macrophages stained brightly for nonspecific esterase using α-naphthol acetate as the substrate.

Cell surface expression of CD11b, CD11c, CD13, and CD18 in undifferentiated and DMSO- or PMA-differentiated HL-60 cells was analyzed using flow cytometry. Surface expression of CD11b or CD18 with differentiation was similar in untransduced HL-60 cells and HL-60 cells transduced by any of the above-mentioned vectors. Surface expression of CD11b was detected in 2% to 7% of undifferentiated HL-60 cells and in 70% to 95% of cells with granulocytic differentiation (DMSO or retinoic acid) or macrophage differentiation (PMA), with an increase in the mean fluorescent intensity by 20- to 35-fold. Although nearly all undifferentiated HL-60 cells expressed CD18 on their surface, macrophage or granulocytic differentiation caused a 2- to 2.5-fold increase in the mean fluorescent intensity. Surface expression of other hematopoietic differentiation antigens, CD11c and CD13, also increased with myelomonocytic differentiation of HL-60 cells (data not shown).

Total GC activity in HL-60 cells. Because gene therapy for Gaucher disease could be beneficial if GC gene expression were targeted to monocytes and macrophages, we chose GC as a reporter gene to transduce HL-60 cells. However, the HL-60 cell line was started from a donor who did not have Gaucher disease. Therefore, these cells possess normal endogenous levels of the GC enzyme. The total GC activity measured in transduced cells represented endogenous GC activity plus any vector-derived activity. The total GC activity from cells transduced with the negative control vector, LN, therefore represented endogenous GC activity. The total GC activity depicted in Table 2 is a mean of two to eight different experiments. Each experiment represents activity from separately transduced G418-selected pools of HL-60 cells.

Table 2. Total GC Activity in Undifferentiated and Differentiated HL-60 Cells

<table>
<thead>
<tr>
<th>Vectors</th>
<th>Undifferentiated</th>
<th>DMSO-Induced</th>
<th>PMA-Induced</th>
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<tbody>
<tr>
<td>LN</td>
<td>120.2 ± 11.67 (10)</td>
<td>142.5 ± 11.47 (8)</td>
<td>175.6 ± 13.96 (7)</td>
</tr>
<tr>
<td>LGCSN</td>
<td>243.6 ± 17.11 (8)</td>
<td>323.7 ± 25.84 (6)</td>
<td>486.7 ± 34.68 (6)</td>
</tr>
<tr>
<td>LNCGC</td>
<td>215.3 ± 36.14 (2)</td>
<td>480 ± 180 (2)</td>
<td>1,008.0 ± 488 (2)</td>
</tr>
<tr>
<td>N25VG</td>
<td>117.0 ± 10.37 (2)</td>
<td>208 ± 5.0 (2)</td>
<td>176.0 ± 17.44 (2)</td>
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<tr>
<td>LN11bGC</td>
<td>153.8 ± 11.72 (8)</td>
<td>386.0 ± 23.81 (8)</td>
<td>310.7 ± 23.77 (7)</td>
</tr>
<tr>
<td>LN18GC</td>
<td>126.5 ± 14.29 (2)</td>
<td>162.7 ± 9.70 (2)</td>
<td>151.0 ± 33.94 (2)</td>
</tr>
<tr>
<td>LN34GC</td>
<td>111.8 ± 5.12 (3)</td>
<td>169.70 ± 8.80 (2)</td>
<td>170.3 ± 33.31 (2)</td>
</tr>
</tbody>
</table>

Mean total GC activity ± SEM in undifferentiated, DMSO-induced (granulocytes), and PMA-induced (monocyte/macrophages) HL-60 cells. Parentheses next to total activity denote the number of experiments (n) and those below denote the range of total activity measured.
Fig 1. GC activity in HL-60 cells. GC activity was measured in undifferentiated HL-60 cells (A) and the same HL-60 cells differentiated into granulocytes with DMSO (B) or into macrophages with PMA (C). The top unshaded area of each bar represents the mean vector-derived GC activity (vector-derived GC activity = total GC activity - endogenous activity in that experiment) ± SEM, whereas the lower hatched bar represents the mean endogenous GC activity from the LN-transduced cells in those experiments. Each bar represents means of 2 to 8 different transduction (n = 2 to 8 experiments) and each experiment was performed on a G-418-selected pool of HL-60 cells. Endogenous equivalents of GC activity were calculated (ratio of vector-derived activity to endogenous activity) in vectors that confer significant vector-derived activity (P < .05) and are depicted as numbers above the bars. The same scale was used in all three diagrams to show increases in activity with differentiation.
Fig 2. Fold-induction in vector-derived GC activity with myelomonocytic differentiation. HL-60 cells were induced to granulocytic differentiation using DMSO (A) and monocyte/macrophage differentiation using PMA (B). Mean fold-induction in activity was calculated by dividing vector-derived GC activity in differentiated cells by that in the undifferentiated cells transduced by the same vector (n = 2 to 8 experiments). The numbers above the bars denote the average fold-induction.

Fig 3. RNA analysis and enzyme activity of CD11b promoter-derived GC expression in HL-60 cells. HL-60 cells harboring the LN11bGC vector were compared with those transduced by the non/GC-containing LN vector or the promoterless GC-containing LN-GC vector by Northern analysis and GC enzyme assay. (A) depicts the vector maps displaying the expected mRNA transcripts with the probes used (not to scale).
by the LGCSN, LNCGC, and the LN11bGC vectors; cells transduced by the N2SVGC vector showed moderately high GC activity; in cells transduced by the LN18GC and LN34GC vectors, the total GC activity was similar to the background activity in control cells (Table 2).

Differentiation of the HL-60 cells into macrophages led to high total GC activity in cells transduced by the LGCSN, LNCGC, and LN11bGC vectors; total GC activity was highest when GC was transcribed by the CMV promoter. In cells transduced by the N2SVGC, LN18GC, and LN34GC vectors, total GC activity was similar to the background activity in LN transduced cells.

Vector-derived GC activity. Because the endogenous GC activity varied widely among experiments, we calculated vector-derived GC activity in each experiment by subtracting the corresponding endogenous activity in that experiment. The vector-derived GC activity along with the respective endogenous GC activity (in LN-transduced cells in those experiments) in undifferentiated HL-60 cells and the same cells differentiated into granulocytes and macrophages is

**Fig 3.** (Cont’d)(B), (C), and (D) depict a Northern blot from HL-60 cells transduced with the above vectors. Total cellular RNA was extracted from transduced HL-60 cells that were either undifferentiated (lanes 1 through 3) or treated with PMA for 3 days (lanes 4 through 6) or DMSO for 5 days (lanes 6 through 9) or retinoic acid (lanes 10 through 12) for 5 days. Northern blotting was performed as described in the Materials and Methods and the same blot was probed with the human GC cDNA (B) followed by the 3’ LTR probe (C) and then for human β-actin cDNA (D), as a loading control. The transcripts derived from the 5’ MoMuLV LTR are labeled a or a’ (unspliced) and b or b’ (spliced). Transcripts c are derived from the internal CD11b promoter and/or endogenous GC. (E) depicts the total GC enzyme activity measured in the same set of cells.
depicted in Fig 1. The numbers above the bars represent the ratio of vector-derived activity to endogenous activity (endogenous equivalents) to facilitate comparisons of activity conferred by the vectors to normal endogenous levels.

In the undifferentiated HL-60 cells (Fig 1A), the vector-derived GC activity was the highest when GC was transcribed from the MoMuLV LTR (1.1 endogenous equivalents), followed by that transcribed from the CMV and CD11b promoters (0.3 endogenous equivalents). There was no significant vector-derived GC activity when GC was transcribed from the SV40, CD18, or CD34 promoters.

Differentiation of HL-60 cells induced by DMSO and PMA led to an increase in endogenous GC activity, even in the control cells (transduced by the LN vector). Therefore, vector-derived GC activity was compared with the higher endogenous GC level in the differentiated control cells.

With induction of granulocytic differentiation using DMSO (Fig 1B), the vector-derived GC activity was the highest from cells transduced with the LN11bGC vector (1.7 endogenous equivalents), followed by those transduced with the LNCGC vector or the LGCSN vectors (1.5 endogenous equivalents). The N2SVGc vector produced only 0.3 endogenous equivalents of GC activity and the remainder of the promoters were minimally active. Thus, GC activity from the LN11bGC vector, although low in undifferentiated cells, was comparable to that derived from strong viral promoters (the LTR and the CMV promoters) in granulocytic cells.

With the induction of monocytic/macrophage differentiation using PMA (Fig 1C), vector-derived GC activity was the highest when GC was transcribed from the CMV promoter (3.6 endogenous equivalents), followed by that transcribed from the MoMuLV LTR (2 endogenous equivalents) and the CD11b promoter in the LN11bGC vector (0.8 endogenous equivalents). Vector-derived GC activity was not detected in cells transduced by the N2SVGc, LN18GC, and LN34GC vectors. Thus, the CMV promoter was most active in PMA-treated HL-60 cells. GC activity derived from the LN11bGC vector was comparable to endogenous GC activity.

**Induction of GC activity with myelomonocytic differentiation.** The same data were then analyzed to assess inducibility of the CD11b, CMV, and MoMuLV LTR promoters with granulocytic and macrophage differentiation. The mean fold-induction in GC activity was calculated by dividing the vector-derived GC activity in differentiated cells to the respective undifferentiated HL-60 cells transduced by the same vectors (Fig 2). The SV40, CD18, and CD34 promoters were not compared because they were minimally active in these cells.

Granulocytic differentiation with DMSO led to an eightfold induction of the vector-derived activity in cells transduced by the LN11bGC vector (Fig 2A); a sixfold induction was observed in cells transduced by the LNCGC vector and a twofold induction was observed in cells transduced by the LGCSN vector. Thus, the net fold-induction in vector-derived activity with granulocytic differentiation was the highest when GC expression was directed by the LN11bGC vector, followed by that by the LNCGC vector.

Monocyte/macrophage differentiation of HL-60 cells using PMA (Fig 2B) resulted in a 14-fold induction in vector-derived GC activity in cells transduced by the LNCGC vector; a fourfold induction in GC activity was seen in cells transduced by the LN11bGC and LGCSN vectors. Thus, the induction in vector-derived activity with PMA-induced monocyte/macrophage differentiation was the highest when GC expression was directed by the CMV promoter.

**RNA analysis.** We performed a Northern analysis on undifferentiated HL-60 cells and those differentiated with PMA, DMSO, and retinoic acid to determine if the increase in GC enzyme from the LN11bGC vector is reflected by the steady-state level of RNA. LN-transduced cells served as a control for the normal endogenous GC. We designed another control vector with the GC cDNA but lacking an internal promoter (LN-GC). Because eukaryotic mRNA translation is monocistronic, GC-containing transcripts from the LTR of LN-GC should only translate neo and should not translate any GC protein. We compared transcripts from LN-, LN-GC-, or LN11bGC–transduced cells and correlated them to the total GC enzyme activity assayed in the same cells (Fig 3).

Figure 3A is a map of the vectors with the expected transcripts. The LTR-derived transcripts, unspliced (a) and spliced (b), would be detected by both the GC and the 3’LTR probes. The CD11b promoter-directed GC transcript (c) is about 2 kb in size and is also detected by both GC and 3’LTR probe. Because the endogenous human GC mRNA (in the LN transduced cells) is the same size (~2 kb) as that transcribed from the internal CD11b promoter, the GC probe would detect endogenous GC and any CD11b-derived GC mRNA. However, the 3’LTR probe would only detect the CD11b-derived GC transcript from the vector.

Figure 3B shows the Northern blot probed with human GC cDNA. The relatively abundant vector-derived transcripts from the MoMuLV LTR, unspliced (a) and spliced (b) containing GC (approximately 4.5 and 4 kb, respectively), are present in cells transduced by either the LN-GC or LN11bGC vectors (lanes 2, 3, 5, 6, 8, 9, 11, and 12). The transcripts from the internal CD11b promoter (lanes 3, 6, 9, and 12) are similar in size (2 kb) and overlap the endogenous GC transcripts seen in LN- and LN-GC-transduced cells (lanes 1, 2, 4, 5, 7, 8, 9, and 10), depicted by the symbol c. Quantitation of transcripts with β-emission counting (normalized to β-actin, Fig 3D) showed that LTR-derived GC transcripts from both the LN-GC and LN11bGC vectors (a and b, Fig 3B) were increased by 1.5- to 2-fold with DMSO- and PMA-induced differentiation and markedly so (8-fold) with retinoic acid-induced differentiation. Endogenous GC transcripts (c, Fig 3B) in cells transduced with the LN and the LN-GC vectors showed minimal increases with myelomonocytic differentiation. In cells transduced with the LN11bGC vector, transcript c was 1.8-, 2.1-, and 5-fold higher with PMA-, DMSO-, and retinoic acid-induced differentiation, respectively, compared with control/undifferentiated cells.

To determine whether this increase in the 2-kb transcript c in the LN11bGC lanes was specifically from the CD11b promoter, this blot was stripped and reprobed with the 3’LTR probe, which detects transcripts from the vector but not the endogenous GC mRNA (Fig 3C). Again, there is an increase in all LTR transcripts with differentiation (a and b in LN-
GC and LN11bGC lanes and the smaller a' and b' in LN lanes) relative to β-actin. Transcripts c in lanes 3, 6, 9, and 12 specifically represent those derived from the CD11b promoter and, when normalized to β-actin, were 2.1-, 2.9-, and 4.4-fold higher with PMA-, DMSO-, and retinoic acid-induced differentiation, respectively. Figure 3D depicts the same blot probed with β-actin cDNA and was used to normalize for loading.

Figure 3E shows the GC activity measured in the same cells used for the Northern analysis. There was a 1.5-, 2.5-, and 2.6-fold induction in vector-derived GC activity with PMA-, DMSO-, and retinoic acid-induced differentiation in LN11bGC-transduced HL60 cells, with minimal induction in the negative control cells. The GC activity in the LN-GC–transduced cells was slightly greater than in LN-transduced cells but was not significantly induced with differentiation.

Thus, the above data show that the steady-state level of the GC transcripts derived from the CD11b promoter increased twofold to threefold with differentiation. This increase in GC mRNA level correlated with the increases in GC enzymatic activity.

**GC activity in the progeny of human CD34+ cells.** Because the CD11b promoter directed high levels of GC activity with myelomonocytic differentiation in HL-60 cells, we tested this promoter in primary human progenitor cells. We used CD34+ cells from umbilical cord blood or G-CSF–mobilized peripheral blood from persons with normal endogenous GC activity. CD34+ cells (sorted from cord blood or peripheral blood) were either sham-transduced or transduced with the LN vector, as a control for normal endogenous GC activity. CD34+ cells (sorted from umbilical cord blood or G-CSF–mobilized peripheral blood) were either sham-transduced or transduced with the LN vector, as a control for normal endogenous GC activity. CD34+ cells (sorted from cord blood or peripheral blood) were either sham-transduced or transduced with the LN vector, as a control for normal endogenous GC activity. CD34+ cells (sorted from umbilical cord blood or G-CSF–mobilized peripheral blood) were either sham-transduced or transduced with the LN vector, as a control for normal endogenous GC activity. CD34+ cells (sorted from cord blood or peripheral blood) were either sham-transduced or transduced with the LN vector, as a control for normal endogenous GC activity.

Table 3 shows the GC activity and expression in the progeny of transduced CD34+ cells, from umbilical cord blood (UCB) or G-CSF–mobilized peripheral blood cells (PBSC), for 3 days, as described in the Materials and Methods. The percentage of cells expressing the inserted gene was determined by scoring the percentage of neomycin-resistant colony-forming units (G418R-CFU). The percentage of cells expressing LN11bGC was 45.6% in experiment 1 and 32.7% in experiment 2.

**GC activity in Jurkat cells.** Jurkat T cells were transduced by the vectors to test the myeloid specificity of the promoters. The endogenous GC activity in control Jurkat cells (transduced with the LN vector) was 192 nmol/min/mg protein. In unstimulated Jurkat cells, the LTR and CMV promoter-derived GC expression was high (3 to 6 endogenous equivalents); the CD11b promoter and the SV40 promoter produced minimal GC activity above background (Fig 4). When these cells were stimulated with PMA and PHA for 24 hours, there was minimal increase in GC activity in either the control Jurkat cells or Jurkat cells transduced with

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Days Posttransduction</th>
<th>Vector</th>
<th>Total GC Activity</th>
<th>Vector-Derived GC Activity</th>
<th>CFU+/CFU-G418 (%G418CFUs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (UCB)</td>
<td>7</td>
<td>Sham</td>
<td>258</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN</td>
<td>294</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN11bGC</td>
<td>421</td>
<td>145</td>
<td>0/168 (0%)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>Sham</td>
<td>247</td>
<td>—</td>
<td>19/136 (12.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN</td>
<td>279</td>
<td>—</td>
<td>53/116 (45.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN11bGC</td>
<td>387</td>
<td>124</td>
<td>12/2,244 (50.3%)</td>
</tr>
<tr>
<td>2 (PBSC)</td>
<td>7</td>
<td>Sham</td>
<td>ND</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN</td>
<td>301</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN11bGC</td>
<td>349</td>
<td>48</td>
<td>23/1,936 (0.01%)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>Sham</td>
<td>ND</td>
<td>201/1,870 (11.0%)</td>
<td>734/2,244 (30.3%)</td>
</tr>
</tbody>
</table>

GC activity in CD34+ cells transduced with the LN11bGC vectors. Umbilical cord blood (experiment 1) or leukopheresed peripheral blood cells (experiment 2) from non-Gaucher patients were sorted for CD34+ cells by immunomagnetic beads, prestimulated, and either sham-transduced or transduced with LN and LN11bGC viral supernatants for 3 days, as described in the Materials and Methods. They were then cultured for 2 weeks with stromal and cytokine support and assayed for GC activity (nmol/min/mg protein). Vector-derived activity from the CD11b promoter was calculated by subtracting the background activity from total activity measured in LN11bGC-transduced cells. The percentage of transduced cells expressing the neo gene was assessed by scoring G418-resistant colony-forming units (G418R-CFU) 2 weeks after transduction. The sham-transduced cells served as a negative control for G418R CFUs and as a control for nonspecific hematopoietic toxicity of the viral supernatants.
the N2SVGC or LN11bGC vectors. However, there was a marked induction in GC activity in cells transduced with the LGCSN and LNCGC vectors. Thus, the CMV and the LTR promoters were most active in Jurkat cells; the CD11b promoter was minimally active in these cells.

**GC activity in HeLa cells.** HeLa cervical carcinoma cells were transduced with the vectors to test the hematopoietic-specificity of the promoters. The endogenous GC activity in control HeLa cells was 244 nmol/min/mg protein. GC activity derived from the MoMuLV LTR, the CMV, and the CD11b promoter was 0.3, 2.8, and 0.5 endogenous equivalents, respectively (Fig 5). There was minimal or no vector-

derived activity in HeLa cells transduced with vectors in which GC was directed by the SV40, CD18, or the CD34 promoters. Thus, the CMV promoter was most active in HeLa cells.

**DISCUSSION**

In this study, we have compared expression of the human GC gene by hematopoietic cell promoters with that by viral promoters using stable retroviral-mediated transductions. We used HL-60 cells as a model of human hematopoietic progenitor cells. HL-60 cells were transduced with retroviral vectors containing the human GC cDNA under control of various
viral or hematopoietic cell promoters and differentiated along the granulocytic and monocyte/macrophage lineages. Eight separately LN1lbGC-transduced pools of HL-60 cells were analyzed to overcome biases by the positional effects on proviral integrants in individual clones. We also tested the CD11b promoter in primary human CD34+ cells. Expression of the GC cDNA from the CD11b promoter, a promoter active in myelomonocytic cells, produced levels of GC enzyme that are expected to be corrective for the genetic deficiency of Gaucher disease, both in the differentiated HL-60 cells and cells derived from primary human CD34+ cells. In HL-60 cells, GC expression from the CD11b promoter resulted in similar trends of inducibility and developmental upregulation as the endogenous CD11b gene. Furthermore, expression of GC directed by the CD11b promoter was relatively myelomonocytic specific, which correlates with the developmental pattern of expression of the CD11b integrin.

The order of the GC and neo genes in one of the vectors that used the retroviral LTR to direct GC expression (LGCSN) differed from the order in the vectors with the hematopoietic promoters. Such variations probably affect levels of expression of the individual genes. However, because these types of vector constructs are typically used for gene transfer, comparison of the net GC reporter activity produced by these different constructs provides a realistic analysis. Of the viral promoters studied, the MoMuLV LTR expressed GC at the highest levels in undifferentiated HL-60 cells. There was a twofold induction in vector-derived GC expression with granulocytic differentiation and a fourfold induction with monocyte/macrophage differentiation from the MoMuLV LTR. However, expression from the LTR was not lineage-specific. High levels of vector-derived GC expression were also seen in Jurkat T cells transduced by the LGCSN vector, and the expression was further enhanced with PHA/PMA stimulation of these cells. Increases in GC activity from the LTR in differentiated HL-60 cells and stimulated Jurkat cells may be due to upregulation of transcription factor synthesis in these cells caused by inducing agents or cellular differentiation.33 Whereas nonmyeloid expression of GC may not be harmful in Gaucher disease, gene therapy for other diseases such as CGD may require myeloid specific expression.

Among the other viral promoters studied, the CMV promoter was also very active. The activity of the CMV promoter was further enhanced with PMA-induced differentiation of HL-60 cells and PMA/PHA stimulation of Jurkat cells. However, expression from this promoter, like that from the MoMuLV LTR, was also lineage nonspecific, with high levels of GC expression from the CMV promoter being observed in Hela and Jurkat cells. The high expression from the CMV promoter may be due to the numerous transcription factor binding domains present in the promoter, some of which are PMA responsive.32,33 Despite the high promoter strength observed in vitro, the CMV promoter has not shown consistent expression with in vivo retrovirally mediated gene transfer experiments.34,35

Of the hematopoietic cell promoters tested, the CD11b promoter showed the most favorable results in terms of levels of expression, inducibility with differentiation, and lineage-specificity. The CD11b promoter produced low levels of GC expression in undifferentiated HL-60 cells, in parallel with the low CD11b surface antigen expression. Expression from the CD11b promoter was increased by more than eightfold with granulocytic differentiation and by nearly fourfold with monocyte/macrophage differentiation of HL-60 cells, in parallel with induction of the CD11b surface antigen. The increase in GC activity from the CD11b promoter with differentiation correlated with increased steady-state GC mRNA transcribed from the CD11b promoter in HL-60 cells. This increase in GC enzyme activity may, in part, be derived from the LTR transcript, although the second reading frame (GC) of bicistronic messages is known to be poorly expressed.36 Possibly, the CD11b promoter may act on the LTR to enhance its expression on differentiation. This might be useful, even if unintended. The absolute levels of the GC enzyme produced by the CD11b promoter in mature myeloid cells were similar to those produced by the MoMuLV LTR.

In primary human CD34+ cells that were transduced and allowed to differentiate into myelomonocytic cells, the CD11b promoter consistently produced GC activity that was about one third to one half of normal GC levels in cells that were only partially transduced (35% to 46% vector positive). When normalized for the partial level of gene transfer, the activity from this promoter was essentially at the normal endogenous level.

The CD11b promoter, like other promoters active in myelomonocytic cells, contains neither the TATA nor CCATT boxes.10,12 However, unlike other TATA-less housekeeping promoters, it is tissue-specific and inducible.37 The CD11b promoter has binding sites for transcription factors PU.1 and Sp1, GATA-factor and ets binding domains, and several retinoic acid response elements.10,12,37,38 PU.1 is a myeloid- and B-cell-specific transcription factor that is upregulated with myelomonocytic maturation.9,39 The Sp1 transcription factor specifically binds to the CD11b promoter region in myeloid cells in vivo and in vitro but not in the nonmyeloid cells.37 Sequence analysis of the CD11b promoter also indicates the presence of consensus nucleotide sequences that are found in several other myelomonocytic cell promoters such as myeloperoxidase, cathepsin, and CD13.10,41 These transcription factor binding domains probably regulate the activity and specificity of CD11b expression in myeloid cells.

In addition to the contribution of specific transcriptional domains in the promoter, posttranscriptional mechanisms may also account for increased steady-state mRNA from the CD11b promoter in differentiated cells. Hickstein et al12 have shown that an increase in CD11b steady-state mRNA in differentiated HL-60 cells is primarily posttranscriptional. Therefore, the myelomonocytic specificity and upregulated expression with differentiation of the CD11b promoter may be due to presence of myeloid-specific transcriptional activators as well as the presence of a favorable posttranscriptional environment. The GC transcript from the CD11b promoter of the LN11bGC vector contains at its 3' end 92 bp of the untranslated region of the CD11b gene that may play a role in RNA stability. Previous reports on the transcriptional regulation of the CD11b promoter in HL-60 cells are controversial.11,42 The exact mechanism of upregulation of the GC transcript in the LN11bGC vector is currently unclear.
Previous studies in cell lines transiently transfected with CD11b promoter-reporter gene constructs have shown a relative myelomonocytic specificity.10,11 Dziennis et al43 and Back et al44 recently reported lineage specificity of the CD11b promoter in transgenic mice bearing the CD11b promoter driving a cell-surface reporter gene. Transgenic mice bearing the 1.7-kb CD11b promoter expressed the reporter gene in a lineage-specific pattern.35 The data presented here using a 0.4-kb promoter fragment show a relative myelomonocytic specificity in human cell lines using stable retrovirally mediated transductions. Recently, Bauer et al46 reported low levels of expression from the same CD11b promoter fragment in a retroviral vector that was not cell specific and not upregulated with differentiation. We cannot explain the reason for lack of activity from the promoter fragment reported by Bauer et al.45 Our studies show that the CD11b promoter fragment, found to be active and relatively myelomonocytic specific in HL-60 cells, is also active in the myelomonocytic progeny of human CD34+ cells. The level of GC enzyme produced by the CD11b promoter in the progeny of CD34+ cells was in the range present in normal myelomonocytic cells and would therefore be adequate for correction of the genetic defect in Gaucher disease. In addition, we have observed strong expression from this CD11b promoter fragment, in vivo, in the murine bone marrow transplant model (unpublished data). However, we did not determine whether the CD11b promoter-vector was active in cells of lineages of cells that do not normally express CD11b, eg, the erythroid and lymphoid cells derived from CD34+ cells.

The SV40 early promoter and the CD18 and the CD34 promoters were minimally active in all the three cell lines tested. Low expression from the SV40 and CD18 promoters was not due to a lack of trans-acting elements; previous studies have shown reporter gene expression from the SV40 promoter in HeLa cells,46 and we have observed surface expression of the CD18 antigen on HL-60 cells transcribed from the endogenous CD18 promoter. Our findings of low CD18 promoter activity in a retroviral vector concur with those recently reported by Bauer et al.45 Low CD34 promoter activity may be attributed to a lack of trans-acting elements because the HL-60 cells do not express the CD34 antigen. Although one group has reported CD34 promoter activity in the CD34+ myeloid leukemia cell line U937,22 HL-60 cells may lack the necessary trans-acting factors. An almost complete absence of SV40, CD18, and CD34 promoter activities in all the three cell lines tested also suggests that there may be some negative regulatory cis-acting elements in the vector constructs. Negatively acting cis elements in the LTR45 or the primer binding site46 in the vector backbone or the presence of the neo gene40 can interfere with the activity of internal promoters.

Lack of expression of inserted genes in vivo has been reported in the progeny of the pluripotent hematopoietic stem cells and in murine primary fibroblasts and myeloblasts transfected with MuMoLV-based vectors using viral promoters.8,34,35 Because the endogenous CD11b promoter is constitutively active in mature myelomonocytic cells, vectors using this promoter may circumvent the in vivo silencing of exogenous genes controlled by viral promoters. Retroviral vectors using the CD11b promoter hold potential for treating genetic diseases affecting cells of the granulocytic and monocyte/macrophage lineages.

ACKNOWLEDGMENT

We thank Dr Daniel G. Tenen for the CD34 promoter plasmid, Dr Dennis D. Hickstein for the CD11b promoter plasmid, and Dr E.D. Agura and S.J. Collins for the CD18 promoter plasmid. We thank Immunex Corp for providing us with rHMGF and rH IL-3. We also thank Parvin Lazi for helping with the morphologic staining and Drs Robert Parkman, Kenneth Weinberg, Guy M. Crooks, Jan A. Nolta, Paul B. Robbins, and Pia M. Challita for reviewing the manuscript.

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Retroviral-mediated gene expression in human myelomonocytic cells: a comparison of hematopoietic cell promoters to viral promoters

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