RAPID COMMUNICATION

Transduction of Primitive Human Hematopoietic Cells With Recombinant Adenovirus Vectors

By Sarah J. Neering, Stephen F. Hardy, David Minamoto, S. Kaye Spratt, and Craig T. Jordan

We have examined the ability of recombinant adenoviral vectors to transduce human hematopoietic cells. Our findings indicate that adenovirus readily infects a large proportion of CD34⁺ cells. Using adenovirus vectors that transduce either a lacZ or an alkaline phosphatase reporter gene, we observed up to 45% of total CD34⁺ cells infected. Upon more detailed analysis, we observed comparable levels of transduction for CD34⁺/CD38⁻ cells and for CD34⁺ cells in G₀ phase of the cell cycle. Importantly, exposure to adenovirus resulted in negligible levels of toxicity as assayed by propidium iodide staining and colony-forming ability. Using adenovirus vectors, we also describe a model system for regulated gene expression in early hematopoietic tissues. CD34⁺ cells were simultaneously infected with two viruses, one carrying a TetR/VP16 transactivator (tTA) and the second carrying a TTA-dependent lacZ reporter gene. Using this approach, β-gal expression was only observed upon coinfection with the transactivator vector. In addition, as shown previously (Gossen and Bujard, Proc Natl Acad Sci USA 89:5547, 1992), tetracycline was able to inhibit TTA mediated induction, thereby providing an effective means to regulate expression of the reporter gene. We conclude that recombinant adenovirus is an effective vehicle for transiently expressing genes in primitive human hematopoietic cells.

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GENE TRANSFER into primitive hematopoietic cells has been a very active area of research for many years.¹⁻³ Numerous strategies have been evaluated for both basic research and clinical applications.⁴⁻⁷ To date, the most prevalent vehicle for stem cell gene transfer has been the retroviral vector. Studies in animal systems have shown that retrovirally marked stem cells can contribute to all major hematopoietic lineages and that a small number of pluripotent clones can effectively repopulate an entire hematopoietic system for extended periods of time.⁸⁻¹¹ Furthermore, infection of stem cells with therapeutically relevant genes has been modeled for a variety of applications.¹²⁻¹⁶ These studies have achieved levels of gene transfer that suggest that stem cell transduction may have clinical utility. Human gene transfer trials have shown that it is possible to achieve significant levels of retrovirally marked hematopoietic tissue¹⁷,¹⁸; however, most studies have also indicated the technical difficulty of transducing primitive human stem cells.¹⁹,²⁰ The most likely impediment to efficient gene transfer may be the quiescent nature of stem cells. Moreover, in addition to their predominantly noncycling status, primitive cells have proven difficult to manipulate in culture. Typically, conditions that stimulate growth of stem/progenitor cells also incur a rapid loss of developmental potential. Thus, although recent results appear more encouraging, it is perhaps not surprising that traditional techniques for retroviral gene transfer have often been less than optimal when applied to human stem cell gene transfer.

To investigate alternative strategies for the transduction of human stem cells, we have examined the use of adenoviral vectors. One previous study has suggested that human bone marrow (BM) may be permissive to adenovirus infection; however, primitive cell types were not specifically examined.²¹ Although standard recombinant adenovirus does not usually allow stable integration, it has been extremely successful for transient gene expression in many systems. Furthermore, adenoviral vectors frequently express transgenes at high levels (not subject to integration site-specific effects), have a broad host range, and have been shown to infect nondividing cells.²²,²³ Clearly, this approach is not currently suited for correction of genetic deficiencies or other situations in which stable gene transfer is required. However, for situations in which transient expression is sufficient, adenovirus has the potential to be an effective gene transfer vehicle.

We have found that adenoviral vectors readily infect primitive human hematopoietic tissue. High levels of expression can be detected in up to 45% of total CD34⁺ cells. In addition, expression can be seen at similar levels in CD34⁺/CD38⁻ cells as well as CD34⁺ cells in G₀. Consequently, we propose that adenoviral vector transduction is a useful means of transferring and expressing genes in primitive human hematopoietic cells.

MATERIALS AND METHODS

Cells. BM was obtained from normal human volunteers or allogeneic marrow donors from BM transplantation procedures. Umbilical cord blood was purchased from Advanced Bioscience Resources (Alameda, CA). Mobilized peripheral blood (MPB) was obtained from cancer patients undergoing autologous stem cell harvest. Mobilization was performed by administering 10 µg/kg/d of granulocyte colony-stimulating factor (G-CSF) for 5 consecutive days. For each type of tissue, mononuclear cells (MNCs) were isolated by density centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden) at 500g for 20 to 30 minutes. Cells were then washed twice with phosphate-buffered saline and, where appropriate, were further enriched for CD34⁺ cells using the Miltenyi MACS column (Miltenyi Biotec Inc, Sunnyvale, CA; used according to manufacturer's instructions).

Construction and purification of adenoviruses. To construct the Ad-MFG-AP vector, an expression cassette (MFG-s-MAP) was obtained from an MFG-s vector²⁴ containing the Moloney murine leukemia virus long terminal repeat and a membrane-bound human
The mAP transgene was constructed from the reporter plasmid placental-like alkaline phosphatase (mAP) transgene. To create Ad-MFG-AP, the MFG-s-mAP expression cassette was inserted between the 5' and 3' sites for insertion in the MFG-s retroviral vector, oligonucleotide primer pairs 5'-CCACATGTTGCTGCTGCTGCTGCTGCTGCTGGCC-3' (forward) and 5'-CCACATATGGCAAGCGGT-3' (reverse) were used to amplify a 270-bp fragment from the pSEAP template. The fragment containing the internal coding region of mAP was obtained by polymerase chain reaction (PCR) amplification of the MFG-s vector. The construction of Ad-tet-ogal and Ad-tTA from pUHC-13-3 and pUHD IS-l and pBsrHI. Finally, a 200-bp fragment containing the 3' membrane-bound AP segment was obtained by polymerase chain reaction (PCR) amplification of pSV2AP using primers 5'-TTCGCCGCGCCGCGCA-3' (forward) and 5'-CCGGATCTTTACGGAGACTGCGG-3' (reverse). The three fragments were ligated together and cloned into the MFG-s vector. The construction of Ad-tet-βgal and Ad-tTA from pUHC-13-3 and pUHD IS-l and pBsrHI is described elsewhere (S.F. Hardy and J. Forsayeth, manuscript in preparation). Ad-MCLacZ was a generous gift from Inder Verma (Salk Institute).

All viruses were plaque-purified and expanded on 293 cells using the standard technique. Each viral inoculum was purified by a CsCl step gradient followed by a CsCl equilibrium gradient. The final viral band was diluted 1:1 with sterile glycerol and stored at -20°C. Concentrations of the viruses were determined by measuring their optical density at 260 nm and converting this figure to particles per milliliter, assuming that 1 × 10^10 particles/mL equals 1.0 OD units. For Ad-MFG-AP and Ad-MCLacZ, a functional titer was obtained by infecting 293 cells for 24 hours and then quantifying the number of infected cells by flow cytometric analysis. Ad-MFG-AP and Ad-MCLacZ stocks had functional titers of 1.3 × 10^10/mL and 7.8 × 10^10/mL, respectively.

Infections. CD34+ cells were cultured in X-Vivo 10 medium (Biowhittaker, Walkersville, MD) supplemented with 5% fetal bovine serum, interleukin-3 (IL-3; 5 ng/mL), IL-6 (10 ng/mL), and stem cell factor (SCF; 25 ng/mL). All growth factors were purchased from R&D Systems (Minneapolis, MN). Adenovirus stocks were added directly to cultures of marrow cells at the indicated multiplicity of infection (MOI). For all data presented, MOI was calculated based on the ratio of functional virus (as assayed on 293 cells) to CD34+ cells. In most cases, the total number of viral particles is 20- to 50-fold higher than the number of functional viral particles. For all infections (except data shown in Fig 3, lower panel), adenovirus was added at 12 to 16 hours of culture. Cells were assayed by flow cytometry 2 days later. For the experiments in Fig 3 (lower panel), adenovirus was washed out at the indicated timepoints and cells were replated in fresh medium. Flow cytometric analysis was performed 48 hours later.

Expression analysis. For phenotyping, cells were stained with the following antibodies from Becton Dickinson Immunocytometry Systems (BDIS; San Jose, CA): CD34-APC, CD38-phycocerythrin (PE), CD3-fluorescein isothiocyanate (FITC; T lymphocytes), CD15-FITC (neutrophils), and CD20-FITC (B lymphocytes). In addition, analysis of erythroid-lineage cells and monocytes was performed using antiglycophorin A-FITC and CD14-FITC from Immunotech (Westbrook, ME). AP was stained using the 3β-gal antibody from Serotec (Washington, DC) and goat anti-mouse IgG2a-FITC (Caltag, South San Francisco, CA). LacZ expression was monitored using fluorescein di-β-D-galactopyranoside (FDG) by the technique of Nolan et al. For the experiment shown in Fig 6, tetracycline (at the indicated concentrations) was added to the culture medium before the addition of virus. Both the tet-βgal and the tTA viruses were used at an approximate MOI of 200 to 300. For lacZ analysis, at 10 minutes after FDG loading, the β-gal inhibitor phenylethyl-β-D-thiogalactopyranoside (PETG) was added to 1.0 mmol/L final concentration. This step is necessary to observe differing levels of β-gal enzymatic activity via the FDG staining procedure. For cell sorting experiments (Table 2), cells were stained for CD34 and AP (propidium iodide was also included to distinguish dead cells), and 100 cells/well were deposited directly into 24-well plates containing 0.5 mL methocult (Stem Cell Technologies, Vancouver, British Columbia, Canada). Sort gates were set using appropriate isotype controls. To identify CD34+ cells in G1 (Fig 4), a novel technique was used that combines staining for cell surface antigens, nuclear antigens, and DNA simultaneously (Jordan et al, manuscript in preparation). Briefly, cells were stained for cell surface antigens (CD34 and CD38) using standard procedures. Next, samples were fixed in 0.4% formaldehyde (Polysciences, Warrington, PA; Ultrapure grade) for 30 minutes on ice, followed by permeabilization with 0.1% Triton X-100. Cell suspensions were then assayed on 293 cells.

Fig 1. Adenovirus infection of human CD34+ cells. CD34+ cells from BM were infected at an MOI of 500 and analyzed 48 hours later for expression of AP. All analysis was performed by staining for both CD34 and AP simultaneously. Data shown are gated on CD34+ cells only. M1, percentage of cells positive for AP expression. (A) Uninfected control. (B) Ad-MCLacZ infected. (C) Ad-MFG-AP infected.
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X-100 for 30 minutes on ice. Cells were then stained with the MIB-1 antibody (anti-Ki-67-FITC; Immunotech) and finally stained with 0.5 μg/mL 7-aminoactinomycin D (7-AAD) overnight. For characterization of primitive tissue in G0, cells were identified that were positive for CD34, yet failed to stain for nuclear antigen Ki-67. Previous studies have shown that lack of expression of Ki-67, combined with staining for DNA content, can be used to identify cells in G0. All analyses and sorting were performed on a FACStar Plus flow cytometer (Becton Dickinson) equipped with 488 nm argon and 633 nm HeNe lasers. Viability was determined by standard propidium iodide staining.

In vitro colony assays. For analysis of progenitor colonies, 1,000 cells were plated in 1.0 mL of methylcellulose medium (Methocult GF+ H4534; Stem Cell Technologies) in a 35-mm dish (supplemented with 3 U/mL erythropoietin; Amgen, Thousand Oaks, CA). Colony-forming units-cell (CFU-C) were scored after 14 days of culture at 37°C.

Southern analysis. At the indicated timepoints, 1 x 10⁶ cells were harvested and DNA was isolated by Hirt extraction. Briefly, cells were lysed in Hirt buffer (10 mmol/L Tris, 10 mmol/L EDTA, 0.6% sodium dodecyl sulfate [SDS], and 200 μg/mL proteinase K) and incubated at 37°C for 2 hours. NaCl was added to 1.0 mol/L and samples were incubated at 4°C overnight. Samples were then centrifuged at full speed for 30 minutes in a Beckman microfuge (Beckman, Palo Alto, CA). Supernatant was extracted twice with phenol/chloroform and DNA was precipitated with isopropanol. Finally, samples were resuspended in TE (10 mmol/L Tris, 1 mmol/L EDTA) + 20 μg/mL RNase A. Before Southern analysis, 10% of each sample was subjected to 10 rounds of PCR using primers specific to the lacZ gene that yield a product of 692 bp (5'-CGATTCCCAGTGTGCACCTC-3’ and 5'-TACACTCGGTTGATTACGA-3’). This step was performed to amplify the samples such that they could be more easily detected by Southern blot. One-tenth of each sample was then electrophoresed on an agarose gel and analyzed by standard Southern blot procedure using a probe specific to the lacZ gene.

RESULTS

Adenovirus transduction of human hematopoietic cells. Initially, we used adenovirus vectors carrying both the bacterial lacZ gene (Ad-MCLacZ) and human placental alkaline phosphatase (Ad-MFG-AP) to monitor viral transduction. The AP reporter gene was engineered to retain the mem-

![Fig 2. Adenovirus infection of CB and MPB CD34+ cells. CD34+ cells from either human umbilical CB or MPB were infected with the Ad-MFG-AP virus at an MOI of 100 to 200 and analyzed 48 hours later for expression of AP. All analysis was performed by staining for both CD34 and AP simultaneously. Data shown are gated on CD34+ cells only. M1, percentage of cells positive for AP expression. (Top panels) CB; (bottom panels) MPB.](image-url)
brane-anchoring domain so that the gene product is maintained on the cell surface. Consequently, we can detect expression of this gene in live cells via standard flow cytometric analysis (see the Materials and Methods). Target cells for infection were derived from normal adult BM and were enriched for CD34+ cells (60% to 80% purity) using an immuno-affinity column. As shown in Fig 1, AP+ cells are readily evident when BM CD34+ cells were infected. In addition to uninfected cells, as a further negative control, cells infected with Ad-MCLacZ were analyzed for nonspecific AP staining. This sample was negative, showing that the observed staining is not due to exposure of CD34+ cells to adenoviral particles. Further analysis of CD34+ cells from both MPB and umbilical cord blood (CB) showed that these alternative sources of primitive tissue are also permissive for adenovirus infection (Fig 2). Titration experiments show that transduction usually saturates in the range of 25% to 35% of the CD34+ population at an MOI of approximately 500 (Fig 3, top panel). When the time of exposure to adenovirus was analyzed, it was found that approximately 50% of transduction (for a constant MOI) occurs during the first 2 hours of infection. The remaining 50% appeared to occur at a roughly constant rate over the subsequent 24 hours (Fig 3, bottom panel).

Infected cells were further characterized by examining additional surface markers and cell cycle status. We observed comparable levels of transduction between CD34+cells and the more primitive CD34+/CD38- subpopulation (Fig 4A). In addition, using a novel technique that allows delineation of CD34+ cells in G0, G1, or G2/S/M phases of the cell cycle (see the Materials and Methods), we observed that reporter gene expression was readily evident for CD34+ cells in G0 (Fig 4B).

When total mononuclear BM cells were examined for adenovirus infection, we found that, aside from cells of a CD34+ phenotype, only the erythroid lineage (glycophorin A-positive cells) showed appreciable levels of transduction (approximately 10% at an MOI of 100). T and B lymphocytes were almost entirely negative for expression of the reporter gene (1% to 2% positive T cells and 0% to 0.5% positive B cells). Granulocytes were only weakly positive, with 2% to 4% of CD15 cells being positive for AP staining. Monocytes (CD14+ cells) also showed very low levels of transgene expression (1% to 2%).

**Functional ability of adenovirus infected CD34+ cells.** For the titration experiment shown in Fig 3, we also assayed both viability and functional ability of cells exposed to adenovirus. Propidium iodide staining of infected cells showed only a very slight degree of toxicity as a consequence of adenovirus infection (Table 1). The colony-forming ability of CD34+ cells after adenovirus infection was assayed by plating cells in methylcellulose. Table 2 shows the results of three experiments in which CFU-C were scored for virus infection ranging from an MOI of 1 to 500. Note that for experiments 1 and 3, no statistically significant reduction in myeloid or erythroid colonies is seen, even at the highest MOI tested. Experiment 2 did show a reduction in myeloid colonies of approximately 30% at an MOI of 500 and some loss of burst-forming units-erythroid (BFU-E) at all virus concentrations. Finally, to directly show that adenoviral vectors were transducing functional progenitors, we sorted infected cells into CD34+/AP+ versus CD34-/AP- populations and assayed for CFU-C. Table 3 shows that CD34+/AP+ cells clearly do have colony-forming ability, albeit at a somewhat lower frequency than the AP- cells.

**Molecular analysis of adenoviral transduction.** To corroborate data obtained from the expression studies, we also analyzed for the presence of the viral genome in transduced cells. CD34+ cells were infected with the Ad-MCLacZ virus and, at 2, 5, and 8 days after infection, cells were harvested. For Southern analysis, DNA was isolated by Hirt extraction and subjected to a limited PCR amplification (10 cycles). In addition, part of each sample was also analyzed for expression of lacZ by FDG staining. The presence of viral DNA was evident at all timepoints (Fig 5), with an apparent dim-
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Fig 4. Adenovirus infection of CD34+ subpopulations. (A) An enriched population of CD34+ cells was stained with antibodies to CD34 and CD38 (left dot plot). The box designated R3 shows cells of a CD34+/CD38- phenotype. The cells in R3 were analyzed for AP expression (right histogram). The M1 marker was drawn based on the position of the uninfected control. M1, percentage of CD34+/CD38- cells positive for AP expression.

(B) Staining for DNA content with 7AAD and cycle activity with Ki-67 allows delineation of cells in Go, G1, and G1/S/M (see the Materials and Methods). The left dot plot shows data from cells gated on CD34+. R3 designates CD34+ cells that are negative for Ki-67 and are thereby defined as being in Go. The histogram on the right shows analysis of cells in R3 for expression of AP. The M1 marker was drawn based on the position of the uninfected control. M1, percentage of CD34+/Go cells positive for AP expression.

Regulated gene expression using a coinfection strategy.

In an effort to achieve an inducible gene expression system, we tested the use of a two-virus transduction strategy. One virus carried a lacZ reporter gene under the transcriptional control of a minimal CMV promoter and flanked by seven tet operator sites (Ad-tet-βgal). The second virus vector carried a chimeric transcription factor gene composed of the tet repressor and the VP16 transactivator domain (Ad-tTA). Previous studies have shown that this hybrid transactivator molecule strongly activates expression of the tet-βgal gene. Figure 6 shows analysis of the coinfection experimental strategy. The Ad-tet-βgal virus alone (sample 2) fails to express β-gal, whereas cells coinfected with Ad-tet-βgal and the Ad-tTA virus (sample 3) show a strong induction of β-gal expression. Moreover, as shown previously in other systems, the interaction between tet-βgal and tTA is sensitive to the presence of tetracycline (samples 4 through 7). Furthermore, this inhibition shows a dose-dependent response.

Table 1. Viability of CD34+ Cells Exposed to Adenovirus

<table>
<thead>
<tr>
<th>MOI</th>
<th>Exp 1</th>
<th>Exp 2</th>
<th>Exp 3</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>5</td>
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<td>5</td>
</tr>
<tr>
<td>500</td>
<td>8</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

At the time of flow cytometric analysis (48 hours after infection), each sample was stained with propidium iodide (PI) to determine viability. %PI* indicates the percentage of dead cells in each sample (data derived from the same samples as shown in Fig 3, top panel).

Abbreviation: ND, not determined.


**Table 2. Colony-Forming Ability of CD34+ Cells Exposed to Adenovirus**

<table>
<thead>
<tr>
<th>Exp</th>
<th>Colony Type</th>
<th>MOI</th>
<th>CFU-GM</th>
<th>BFU-E</th>
</tr>
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<td>50 ± 3</td>
<td>24 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>52 ± 4</td>
<td>26 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>51 ± 3</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>CFU-GM</td>
<td>25</td>
<td>500</td>
<td>51 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>48 ± 2</td>
<td>45 ± 2</td>
</tr>
<tr>
<td>3</td>
<td>CFU-GM</td>
<td>100</td>
<td>500</td>
<td>48 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
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<td>45 ± 2</td>
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<tr>
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<tr>
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<tr>
<td>3</td>
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</table>

At the time of flow cytometric analysis (48 hours after infection), part of each sample was plated in methylcellulose medium to determine progenitor frequency (1,000 cells/35-mm plate). Each data point is derived from triplicate samples. Data are derived from the same samples as those analyzed in Fig 3 (top panel).

**Abbreviation:** ND, not determined.

with higher levels of tetracycline being sufficient to completely block lacZ expression.

**DISCUSSION**

Several studies have documented the presence of adenovirus in human lymphoid cells. Although reports vary considerably in their findings, it appears that some adenovirus serotypes can establish a latent or chronic infection in human lymphocytes. A more recent study has shown that adenovirus may replicate to some degree in human BM cell cultures.

Thus, there are precedents for at least a low level of viral infectivity for human hematopoietic cells. The studies described in this report are the first to specifically document adenovirus transduction of primitive human hematopoietic cells. This finding may have gone previously undocumented for several reasons. First, only a limited number of hematopoietic cell types show significant permissivity to adenovirus infection. Our analysis of the major hematopoietic lineages showed only low levels of infection for all phenotypes except CD34+ and glycoporphin A+. Thus, studies using unfractionated BM might easily have failed to note transduction in the CD34+ compartment. Second, the transcriptional control requirements of genes in primitive cells may differ substantially compared with other cell types. Consequently, earlier studies may have used promoter/enhancer combinations poorly suited for gene expression in early hematopoietic cells. Third, even for the more permissive cells described in this study (ie, CD34+ and glyA+), the virus concentration necessary to see a large percentage of positive cells was relatively high. Finally, all data presented in our experiments were generated using human tissue, whereas previous attempts may have used animal cells. We do not know the efficacy of adenoviral vectors for transduction of hematopoietic cells of any species other than human.

Within the CD34+ population, there appeared to be a subset of cells permissive for adenovirus infection. Thus far,
we have not been able to determine the cause of this apparent tropism. None of the various surface antigens we have examined (see the Materials and Methods) correlates with adenoviral transduction. Infection greater than an MOI of 500 to 1,000 was not tested, because this appeared to be the threshold beyond which significant levels of toxicity were evident. It is possible that virus infection does not plateau at the levels shown (see Fig 3); however, due to toxicity, it becomes difficult to accurately measure higher levels of transduction. It has also been shown that the αv integrins facilitate uptake of adenovirus for some cell types. We examined the expression of αv (CD51) by flow cytometric analysis and saw only very low levels of staining (2% to 3%) on CD34+ cells (data not shown).

Regardless of the above issues, a large proportion of cells with a primitive phenotype clearly are permissive to transduction. Thus, it seems likely that adenovirus can be useful for expressing genes of interest in at least some human stem cells. It has previously been shown that adenovirus transduction of retrovirus receptors can increase retroviral infectivity in various cultured cell lines. Given our findings with primary hematopoietic tissue, this strategy should also be feasible for CD34+ cells. In addition, for situations in which transient gene expression might be preferable to stable gene transfer, adenovirus could also be an effective vehicle.

Fig 6. Coinfection of CD34+ cells with the Ad-tet-βgal and Ad-tTA viruses. CD34+ cells were infected with the Ad-tet-βgal virus (samples 2 through 7) and the Ad-tTA virus (samples 3 through 7). Forty-eight hours later, cells were analyzed for expression of CD34 and Lacz (see the Materials and Methods). The flow cytometric histogram shows the β-gal expression profile for CD34+ cells from samples 2 (Tet-βgal only), 3 (Tet-βgal + tTA), and 5 (Tet-βgal + tTA + 0.01 μg/mL tetracycline). The other samples were omitted from the flow cytometric profile for clarity. The table shows all samples, designating virus, percentage of β-gal+ cells, and tetracycline concentration.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Virus</th>
<th>% 34+/Lacz+</th>
<th>Tet conc.</th>
</tr>
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<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Tet-βgal</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Tet-βgal + tTA</td>
<td>5.7</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Tet-βgal + tTA + 0.01μg/mL</td>
<td>51</td>
<td>0.001μg/mL</td>
</tr>
<tr>
<td>5</td>
<td>Tet-βgal + tTA</td>
<td>17</td>
<td>0.01μg/mL</td>
</tr>
<tr>
<td>6</td>
<td>Tet-βgal + tTA + 0.1μg/mL</td>
<td>0.8</td>
<td>1.0ug/mL</td>
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<td>7</td>
<td>Tet-βgal + tTA + 1.0μg/mL</td>
<td>0.3</td>
<td>10ug/mL</td>
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</table>

Our data also show that tightly regulated gene expression can be achieved for primary hematopoietic cells. Using the tTA-dependent promoter/transactivator system and a two-virus coinfection strategy, we obtained gene expression that could easily be regulated by adding tetracycline to the culture medium. This approach might be particularly useful for studies of genes in which temporal regulation or dosage effects are of interest (e.g., cell-cycle or developmental stage-specific genes).

A recent study by Clarke et al has suggested that adenovirus could be useful clinically for purging of malignant cells from BM specimens. This strategy calls for the transduction of tumor cells with a gene predicted to cause apoptosis in neoplastic cells. Using the Bcl-xL gene, a functional inhibitor of Bcl-2, the investigators observed impressive tumor-specific cell death in the absence of any apparent marrow toxicity. In light of findings reported in this study, their data may indicate that expression of Bcl-xL in primary hematopoietic cells is benign. Alternatively, the Rous Sarcoma virus (RSV) promoter used to express Bcl-xL may not function well in stem and progenitor cells. Because our data suggest that hematopoietic cells are effectively transduced by adenovirus, we feel that, despite the success of Clarke et al, strategies for purging cancer cells from marrow should be approached with caution.

In conclusion, we have shown that recombinant adenovirus vectors can transduce human CD34+ cells. This was observed for human BM, MPB, and umbilical CB. Moreover, infection was evident at comparable levels in the more phenotypically primitive CD34+/CD38− subpopulation and in quiescent (G0) CD34+ cells. Finally, it was possible to show tightly regulated expression of an adenovirus transgene using a two-virus coinfection strategy with the tet/VP16 inducible system. Therefore, we suggest that adenovirus may have broad applicability as a gene transfer vehicle for studies of primitive human hematopoietic cells.

ACKNOWLEDGMENT

We gratefully acknowledge the expert assistance of the Somatix stem cell group (Glenn Yamasaki and Claudia Cabrera) for the isola-
tion and processing of human tissues; the adeno virus group (Marina Kitamura, Yumin Dai, and Lisa Phipps) for the production of adeno virus vectors; and the vector core group (Ya-li Lee and David Colvin) for the cloning and characterization of adeno virus vectors. We also thank Olivier Danos for critical review of the manuscript.

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