Transduction of Hematopoietic Cells by Foamy Virus Vectors

By Roli K. Hirata, A. Dusty Miller, Robert G. Andrews, and David W. Russell

Foamy viruses are retroviruses of the spumavirus family that are often isolated from primary cultures of primate cells. We previously constructed vectors based on human foamy virus (HFV) and found that they were able to transduce a wide variety of vertebrate cells by integration of the vector genome. Here we show that several types of hematopoietic cells are efficiently transduced by an HFV vector that encodes alkaline phosphatase (AP). These cell types include transformed cell lines and primary hematopoietic progenitors from mice, baboons, and humans. The transduction rates of HFV vectors compare favorably with those obtained by murine leukemia virus vectors, which suggests that HFV vectors may be effective in the treatment of hematologic diseases by gene therapy.

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MOST CURRENT ATTEMPTS to transfer genes into hematopoietic cells use retroviral vectors derived from murine leukemia viruses (MLV). Although these vectors are capable of efficiently transducing hematopoietic stem cells in mice, the results in larger animals have been disappointing. At best, approximately 10% of canine long-term repopulating cells have been transduced, and most dogs had lower transduction levels. In primate experiments, term repopulating cells have been transduced, and most dogs on cell division than oncoviruses such as MLV. The absence of appropriate retroviral receptors on the surface of hematopoietic cells. The ecotropic MLV vectors used in mouse experiments do not infect cells from the larger species studied, which necessitates the use of other, potentially less effective MLV envelope pseudotypes.

Foamy viruses are retroviruses of the spumavirus family that infect cells from many vertebrate species, and are not known to cause disease. Although foamy virus infection is decreased in nondividing cells, spumaviruses are less dependent on cell division than oncoviruses such as MLV. We recently developed retroviral vectors based on human foamy virus (HFV), which was originally isolated from a human nasopharyngeal carcinoma cell line, but subsequent studies indicate that it may be a chimpanzee virus variant. Foamy virus vectors transduce a wide variety of vertebrate cells by integration of the vector genome, and they transduce stationary cultures more efficiently than MLV vectors, which suggest that they may be more effective at transferring genes into hematopoietic stem cells.

Here we describe the transduction of hematopoietic cell lines and primary hematopoietic progenitors by foamy virus vectors that encode human placental alkaline phosphatase (AP). Using an in situ histochemical staining protocol for colonies grown in soft agar, we measured the transduction rates of mouse, baboon, and human hematopoietic colony-forming units (CFU). Cocultivation of cells that produce foamy virus vectors with hematopoietic progenitors led to transduction rates that compared favorably with those obtained by MLV vectors. Our results suggest that foamy virus vectors may be well suited for the transduction of hematopoietic cells.

MATERIALS AND METHODS

Cell culture. All cells were grown at 37°C, in 10% CO₂ and 100% humidity. Normal human fibroblasts, BHK21 cells, FAB cells, adenine phosphoribosyltransferase-deficient (APRT)-mouse erythroblastemia (MEL) cells (originally obtained from A. Deisseroth, M.D. Anderson Cancer Center, Houston, TX), and the retroviral packaging cell lines PE501, PA317, and PG13 were cultured in Dulbecco’s modified Eagle medium (DMEM) with 10% heat-inactivated (30 minutes at 56°C) fetal bovine serum (FBS). K-562 cells and WEHI cells were grown in RPMI or Iscove’s modified Dulbecco’s medium (IMDM) with 10% heat-inactivated FBS, respectively. Purified cytokines used in hematopoietic cell cultures were of human origin and were obtained from Amgen (Thousand Oaks, CA). WEHI-conditioned medium was prepared as described.

All animal and human cells were isolated under approved institutional guidelines and human samples were obtained with informed consent. Mouse bone marrow was isolated from the femurs of 15-week-old male C57BL/6j mice (Jackson Labs, Bar Harbor, ME) as described and fractionated on a Nycodren 1.080 (Nycodren Pharma, Oslo, Norway) gradient, and the mononuclear cells were cultured in IMDM with 25% FBS and 15% WEHI-conditioned medium until used in transduction experiments 1 day later. Baboon bone marrow buffy-coat cells were isolated as described and enriched for CD34⁺ cells by using the Ceprate LC system (CellPro, Bothel, WA) according to the manufacturer’s instructions. Human bone marrow was obtained from normal donors and enriched for CD34⁺ cells using the Ceprate LC system. Baboon and human CD34⁺ cells were used immediately before use. Human stromal cells were isolated from unseparated normal bone marrow samples by culturing adherent cells in IMDM that contained 20% heat-inactivated FBS without added cytokines for 2 weeks until no suspension cells remained, then culturing for 4 more days in IMDM with 10% FBS before aliquots were frozen for use in experiments.

Vector production. The plasmids pLAPSN and pFGPMAP were used to generate the MLV-derived vector LAPSN and the HFV-derived vector FGPMAP (Fig 1). LAPSN vector stocks were
FOAMY VIRUS VECTOR TRANSDUCTION

Fig 1. HFV and MLV vector constructs. Structures of the wild-type HFV provirus pHRSV13, HFV vector construct pFGPMAP, and MLV vector construct pLAPSN are shown. The positions of the viral gag, pol, env, and bel genes are indicated, as are the HFV, MLV, and gibbon ape leukemia virus (MSV) LTRs. Also shown are the MLV LTR promoter (M), alkaline phosphatase gene (AP), SV40 early promoter (S), and neomycin phosphotransferase gene (N) used as reporter genes. Arrows indicate the location of transcription start sites.

produced in PA317 amphotropic and PG13 gibbon ape leukemia virus (GALV) packaging cell lines as described.28 FGMAP vector stocks were produced by transfecting BHK21 cells with pFGPMAP and pHRSV13 plasmids as described.23 The titers of the filtered FGMAP vector stocks used in these experiments were 3.1 to 3.6 \( \times 10^6 \) AP transducing units per mL, with a sixfold higher level of contaminating helper virus, as assayed on FAB \( \beta \)-galactosidase HFV indicator cells.27

Transduction protocols. All transduction experiments used histochemical staining for human placental AP expression, which produces a purple color in AP-positive cells. Infections with MLV vectors and MLV vector packaging cell lines were performed in the presence of 4 \( \mu \)g/mL Polybrene (Sigma, St Louis, MO). Human fibroblasts were stained for AP expression28 2 days after infecting with LAPSN and FGMAP vector stocks as described previously.20 MEL and K-562 cells were transduced by seeding cultures at \( 5 \times 10^5 \) cells and \( 10^6 \) cells per mL, respectively, in 12-well plates (Corning, Corning, NY) on day 1, changing the medium and infecting with cell-free vector stocks on day 2, and staining for AP expression28 on day 3. Buffer changes during the staining procedure were performed by pelleting cells at 2,500 rpm in a microfuge, and AP-positive cells were counted after plating stained cells in 96-well plates. Under the conditions used, only about 0.02% or 0.2% of MEL or K-562 cells were transduced, respectively, by HFV vectors (Table 1), so assuming equivalent infection rates, helper virus should have coinfected only a small percentage of these cells (0.12% or 1.2%, respectively). In some experiments, cytospin preparations were prepared in a Shandon Cytospin centrifuge (Shandon, Astmoor Runcorn, UK), air-dried, then stained for AP expression.26 Where indicated, AP-stained slides and dishes were counterstained with nuclear fast red (1 mg/mL in 5% aluminum sulfate).

Transductions in the presence of human stromal cultures were performed by plating \( 5 \times 10^5 \) viable human CD34+ cells per well in 200 \( \mu \)L of IMDM containing 20% FBS, 10 ng/mL interleukin (IL)-3, 10 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF), 50 ng/mL IL-6, 100 ng/mL stem-cell factor (SCF), and 5 U/mL erythropoietin (EPO) in 12-well plates previously seeded with human stromal cells. A 120 \( \mu \)L quantity of FGMAP cell-free filtered vector stock was added to each well 1 day later. Six days after infection, the wells were stained for AP expression.26

The procedure for transducing hematopoietic progenitors is outlined in Table 2. On day 1, BHK21 cells were plated at \( 3 \times 10^6 \) cells per 10-cm dish. On day 2, the cells were transfected with 20 \( \mu \)g each of pFGPMAP and pHRSV13 or 20 \( \mu \)g of pFGPMAP alone by calcium phosphate-DNA coprecipitation.27 On day 3, the transduced cells were treated with trypsin and plated at 2.5 to \( 5 \times 10^6 \) cells per 35-mm dish. On day 7, these cells were irradiated with 1,700 rad from a \( ^3 \)Cs Gammacell 40 Irradiator source (Atomic Energy of Canada, Ottawa), the medium was changed, and hematopoietic cells were added as described later. Mouse experiments used \( 3 \times 10^5 \) bone marrow cells per dish in 2.5 mL of IMDM that contained 20% FBS and 15% WEHI-conditioned medium. Baboon experiments used \( 3 \times 10^5 \) CD34+ -enriched cells per dish in 5 mL IMDM that contained 20% FBS, 100 ng/mL IL-3, 100 ng/mL GM-CSF, 100 ng/mL IL-6, 100 ng/mL SCF, and 4 U/mL EPO. Human experiments used \( 1.6 \times 10^7 \) CD34+ -enriched cells per dish in 2.5 mL IMDM that contained 20% FBS, 10 ng/mL IL-3, 10 ng/mL GM-CSF, 50 ng/mL IL-6, 100 ng/mL SCF, and 5 U/mL EPO. On day 9, after 2 days of cocultivation, nonadherent cells were removed from the dishes and dilutions were plated in 24-mm Transwells (polycarbonate membrane, 0.4-mm pore size; Costar, Cambridge, MA) in 0.27% soft agar in the same medium used during infection. In some experiments, suspension cells were also kept in liquid culture under the same conditions, and cytospin preparations made from them were used for AP staining. Colonies were allowed to grow for 7 (mouse) or 10 (baboon and human) days before staining. Transduction with MLV vector packaging lines producing LAPSN(PE501) and LAPSN(PG13) was performed by an identical irradiation and cocultivation procedure 2 days after plating packaging cells at \( 2 \times 10^5 \) cells per dish.

<table>
<thead>
<tr>
<th>Vector</th>
<th>Envelope</th>
<th>Titer* on Fibroblasts†</th>
<th>Titer Relative to K-562‡</th>
<th>% AP' Cells (MOI 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>( 8 \times 10^5 )</td>
<td>0.05</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>LAPS N(PA317)</td>
<td>Amphotropic</td>
<td>( 4 \times 10^4 )</td>
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<td>0.13</td>
</tr>
<tr>
<td>LAPS N(PG13)</td>
<td>GALV</td>
<td>( 3 \times 10^6 )</td>
<td>&lt;0.0003</td>
<td>0.23</td>
</tr>
<tr>
<td>FGMAP (HSRV13)</td>
<td>HFV</td>
<td>( 3 \times 10^6 )</td>
<td>0.003</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Normal human fibroblasts, MEL cells, and K-562 cells were infected with MLV and HFV AP vectors of the indicated envelope pseudotype and stained for AP expression (AP') 2 days later.

* Titer were measured in triplicate and each value varied by \( <30\% \) from the mean.
† Fibroblast titers are AP' focus-forming units per mL of vector stock.
‡ MEL or K-562 titers were calculated as (fraction of AP' cells) \( \times \) (total no. of cells exposed to vector)/(mL of vector stock).
§ MEL or K-562 titer divided by fibroblast titer.
### Table 2. Foamy Virus Vector Transduction of Hematopoietic Progenitors

| Day 1. | Seed BHK21 cells for transfection |
| Day 2. | Cotransfect with vector (pFGPMAF) and helper (pHSRV13) constructs |
| Day 3. | Split transfected cells into wells |
| Day 7. | Irradiate producer cells with 1,700 rad |
| Day 9. | Collect suspension cells |
| Day 18. | Fix soft agar wells |
| Day 19. | Count plain and purple colonies |

Mouse marrow experiments were fixed and stained on day 15 instead of 18.

Soft agar cultures were stained for AP expression by gently replacing the medium in the well surrounding the Transwell insert as follows: three phosphate-buffered saline (PBS) washes (15 minutes each), fixation in 0.25% glutaraldehyde in PBS for 20 minutes, four PBS washes (30 minutes each), incubation in PBS for 60 to 90 minutes at 68°C; overnight incubation in 100 mmol/L Tris pH 8.5, 100 mmol/L NaCl, 50 mmol/L MgCl₂, 0.1 mg/mL 5-bromo-4-chloro-3-indolyl phosphate, and 1 mg/mL nitro blue tetrazolium, followed by two washes (30 minutes each) in 100 mmol/L Tris pH 8.5, 100 mmol/L NaCl, and 50 mmol/L MgCl₂. After staining, colonies (>50 cells) were counted under a dissecting microscope and scored for AP expression.

### RESULTS

**Transduction of hematopoietic cell lines.** The foamy virus vector FGPMAF was used to transduce mouse (MEL) and human (K-562) hematopoietic cell lines. This vector was derived from the HFV infectious provirus pHSRV13, and contains the human placental AP gene under the control of an internal MLV long terminal repeat (LTR) promoter in place of a portion of the HFV env gene (Fig 1). The env deletion used destroys the internal promoter for the bel1 gene, which encodes a transactivator of the HFV LTR. This construct produces no detectable transactivation of the LTR in transduced cells, and therefore should not express the viral gag and pol genes in the absence of helper virus. Southern analysis of cell clones transduced by HFV vectors has shown that all transfected clones contain integrated vector proviruses, without episomal forms or detectable helper virus, which indicates that transduction by HFV vectors occurs through typical retroviral integration mechanisms. We compared transduction by the foamy virus vector FGPMAF to transduction by the analogous MLV vector LAPSN, which also contains the AP gene under the control of the MLV LTR promoter. LAPSN stocks were generated in PA317 or PG13 packaging cell lines that express the amphotropic or gibbon ape leukemia virus env genes, respectively.

Titters obtained after transduction of MEL and K-562 cells were compared with those obtained on normal human fibroblasts, which are permissive for all three vector types (Table 1). The GALV-pseudotyped LAPSN(PG13) vector transduced K-562 cells at approximately one fourth the frequency of primary fibroblasts, and the amphotropic LAPSN(AP317) vector transduced both MEL and K-562 cells at 20- to 40-fold lower frequencies. Relative to primary fibroblasts, the HFV FGPMAF vector transduced K-562 cells as efficiently as LAPSN(AP317), and MEL cells at a 10-fold lower rate. Although this demonstrates that hematopoietic cells can be transduced by HFV vectors, transduction was inefficient as compared with several adherent cell types from different species we assayed previously.

**Transduction of bone marrow cells occurs by direct contact with vector-producing cells.** We initially attempted to transduce hematopoietic progenitors with filtered medium collected after transfecting BHK21 cells with vector and helper constructs. Human CD34⁺-enriched bone marrow cells were infected with cell-free FGPMAF vector supernatants at a multiplicity of infection (MOI) of 0.2 transducing units (as measured on human fibroblasts) per CD34⁺ cell. These infections were performed in the presence of human bone marrow stromal cells, in an attempt to improve gene transfer rates as was previously observed with MLV vectors. Histochemical staining of cyotospin preparations of suspension cells from these cultures demonstrated AP-positive cells at 8 and 11 days after infection; however, the percentage of transduced cells in these preparations remained low (<1%). A 1% transduction rate was also observed in hematopoietic progenitors infected with cell-free vector supernatants as assayed by AP staining of colonies grown in soft agar (see Materials and Methods).

When liquid cultures that contained stromal support layers and human CD34⁺ cells were stained without disturbing cellular attachments 6 days after exposure to FGPMAF vector stock, we could identify both transduced stromal and hematopoietic cells in situ (Fig 2A). These cultures clearly showed that transduced hematopoietic cells were almost exclusively found in contact with transduced stromal cells, while isolated hematopoietic cells and those in contact with untransduced stromal cells were consistently AP-negative. Some of the AP-positive stromal cells also exhibited syncytia formation characteristic of HFV infection. We interpreted these results as indicating that efficient transduction of CD34⁺ cells by HFV vectors requires direct contact with vector-producing cells. In these experiments, vector stocks contained contaminating helper virus, which enabled coinfected stromal cells to continuously produce additional FGPMAF vector particles while in contact with CD34⁺ cells.

**Transduction of hematopoietic progenitors by HFV vectors.** Based on these findings, we developed a cocultivation method for transduction of hematopoietic progenitors by HFV vectors that is outlined in Table 2. Because HFV infection causes significant cytopathic effects and ultimately kills the BHK21 cells used to produce vector stocks, we adopted a transient transfection system to generate vector-producing cells. At the time of peak vector production, but before maximum cytopathic effect had occurred, transfected producer cells were irradiated at the minimum dose that completely abolished subsequent cell division, and cocultivated with hematopoietic progenitors. After 2 days of cocultivation, the loosely attached hematopoietic cells were recovered, plated
Fig 2. Photomicrographs of human CD34+ cells and colonies derived from them after transduction by the HFV vector FGPMAP. Specimens were stained for AP expression, which produces a purple color. (A) CD34+ cells cultured in the presence of human marrow stromal cells and stained 6 days after infection. Large, flat cells are stromal cells. Black arrows indicate untransduced CD34+ cells. White arrows indicate transduced CD34+ cells. (B) Cytospin preparation of CD34+ cells maintained in liquid culture for 5 days after a 2-day cocultivation with irradiated FGPMAP-producing BHK21 cells. (A) and (B) were counterstained with nuclear fast red. (C-G) Examples of AP-negative (C, F) and AP-positive (C-E, G) colonies (C, D) and bursts (E-G) derived from hematopoietic progenitors grown in soft agar for 10 days after a 2-day cocultivation with irradiated FGPMAP-producing BHK21 cells. The burst in (G) shows sectored of transduced cells.

in soft agar, and allowed to develop into colonies. By using Transwell plates (Costar), which have a porous bottom membrane that allows diffusion of solutions into the culture well, we were able to process these cultures for histochemical staining without disturbing the soft agar gel. Transduction efficiencies were directly calculated from each soft agar culture as the percentage of AP-positive colonies. Both CFU and burst-forming unit (BFU) were detected in these cultures. However, BFUs could not be definitively identified as erythroid, since the cells in bursts did not always develop a red color, and AP-positive cells were too dark to detect any hemoglobin that might have been present. Examples of human hematopoietic colonies transduced by the FGPMAP vector are shown in Fig 2C through G.

Human CD34+ progenitors were isolated and transduced by FGPMAP using the protocol shown in Table 2. The total number and percentage of AP-positive colonies were determined after cocultivation with producer cells transfected
with the pFGPMAP vector and pHSRV13 helper constructs (Fig 3). For comparison, CD34+ cells were also cocultivated with irradiated packaging cells producing the GALV-pseudotyped MLV vector LAPSN(PG13). Cocultivation with BHK21 cells producing HFV vectors had only a minor inhibitory effect on the subsequent growth of hematopoietic colonies, and no apparent effect on the number of BFU observed. This effect could have been due to differences in cytokine expression between the BHK21 cells used to produce HFV vectors and MLV vector packaging cells, and/or growth inhibition caused by contaminating wild-type HFV. An average of 27% of CFUs and 40% of BFUs were transduced by the FGPMAP vector, which was higher than the transduction rate observed with the LAPSN(PG13) vector under similar conditions. Cytospin preparations of suspension cells collected and maintained in liquid culture for 5 days after cocultivation with FGPMAP-producing cells showed a transduction rate of 15% to 20% (see Fig 2B), similar to that obtained by colony assay. Cocultivation with control cells transfected with the FGPMAP construct alone and no helper virus construct did not produce AP-positive colonies, which confirms that gene transfer requires vector encapsidation by viral proteins, and rules out AP protein transfer as an explanation for AP positivity.

Similar experiments were performed with mouse and baboon hematopoietic progenitors. In both cases, exposure to BHK21 cells transfected with the HFV helper construct led to a slight decrease in the total number of CFUs obtained. Lower colony numbers were also obtained after cocultivation with cells transfected with pFGPMAP alone, which suggests that BHK21 cells provide less effective support for hematopoietic progenitors than MLV packaging lines such as PE501 and PG13. Forty-three percent of murine bone marrow CFUs (Fig 4A), and 11% of baboon CD34+ CFUs were transduced by cocultivation with FGPMAP-producing cells (Fig 4B). These transduction rates compared favorably with those obtained with mouse bone marrow cells cocultivated with cells producing the ecotropic MLV vector LAPSN(PE501) and baboon CD34+ cells cocultivated with cells producing LAPSN(PG13). The lower transduction rates observed with baboon cells could have several explanations, including the use of human instead of baboon cytokines, or the presence of endogenous retroviruses in primary baboon cell cultures. Foamy viruses in particular can often be isolated from baboon hematopoietic cells, and we have commonly observed cytopathic effects typical of foamy viruses in baboon bone marrow cultures (unpublished results, April 1995).

**DISCUSSION**

We have found that vectors based on foamy viruses are able to transduce hematopoietic cell lines and normal hematopoietic progenitors from mice, baboons, and humans. Earlier studies suggested that wild-type HFV could infect hematopoietic cell lines, but did not study primary hematopoietic progenitors. These findings extend our previous report describing the transduction of a wide variety of adherent cells from several species by HFV vectors, adding marrow stromal cells and primary hematopoietic progenitors to the list of cell types that can be transduced. To date, we have analyzed more than 25 different cell types from species ranging from chickens to humans, and have failed to find one that was not transduced by HFV vectors. Although a foamy virus receptor has not yet been identified, these results suggest that it is ubiquitously expressed in vertebrate cells.

The in situ staining protocol used to detect AP expression in colonies grown in soft agar is well suited for transduction studies of hematopoietic progenitors. After heat-inactivation there is no background AP expression, and both transduced and untransduced colonies can be scored in the same dish. This allows a more accurate measurement of the percentage of transduced cells than the commonly used neomycin phosphotransferase reporter gene, which requires comparisons of separate culture dishes and subjective estimates of colony size after growth in the presence of toxic concentrations of the neomycin analog G418. In addition, sectored colonies can be identified that contain both AP-positive and AP-negative cells, which allows one to monitor the segregation of cells that contain the vector provirus during colony development.

Foamy virus vectors transduced 11% to 43% of hematopoietic progenitors, which is similar to transduction rates obtained with MLV vectors of various pseudotypes, includ-
The MLV vectors used in progenitor transduction experiments can typically be produced at titers over $10^6$/mL by established packaging cell lines. Our results with foamy virus vectors are especially encouraging considering that the relatively crude vector production protocol used produces cell-free vector supernatants with titers of approximately $10^4$ transducing units per mL when assayed on permissive, adherent cells. This is 10- to 100-fold lower than the titers of LAPSN (PE501) and LAPSN(PG13) vector supernatants generated by the packaging cell lines used in these studies. Efficient transduction of hematopoietic progenitors by HFV vectors required cocultivation with producer cells. As shown in Fig 2, this seemed to reflect a requirement for direct cell contact, rather than a simple prolonged exposure to the medium surrounding producer cells. Budding of vector virions present at the surface of producer cells into hematopoietic progenitors apparently leads to more efficient gene transfer than diffusion of cell-free vector particles. It is possible that the low transduction rates obtained in MEL and K-562 cells exposed to cell-free FGPMAP stocks (Table 1) could also have been improved by cocultivation.

The cotransfection procedure used to generate HFV vector stocks and producer cells also leads to the production of varying amounts of replication-competent helper virus. However, we do not believe the presence of helper virus affected the transduction rates reported here. The experiments with MEL and K-562 cells listed in Table 1 were completed 2 days after infecting with vector stocks, before coinfection with helper virus would have led to significant production of additional vector particles. Similarly, the transduction experiments with hematopoietic progenitors involved a 2-day cocultivation with producer cells, followed by plating in soft agar. Suspension in the agar gel would limit the subsequent spread of viral particles, and prevent the direct contact with producer cells required for efficient transduction. The majority of transduced colonies obtained were completely AP-positive, rather than sectored into AP-positive and AP-negative fractions (data not shown), which suggests that the transduction events occurred before colony growth in soft agar. Although the vector production system used in these experiments is adequate for studying transduction in vitro, an accurate assessment of transduction rates in animal transplantation experiments will require helper-free vector production. The construction of stable HFV vector packaging lines that do not produce replication-competent helper virus is difficult for several reasons, including the toxicity of the HFV envelope protein, the high helper genome copy numbers that may be required to produce adequate vector titers, and the regulated transcription levels of the internal and LTR promoters. In addition, important cis-acting elements such as the HFV packaging signal will have to be mapped before helper constructs can be designed with minimized packaging potential.

Foamy virus vectors offer several potential advantages over MLV vectors, including a larger packaging capacity, resistance to inactivation by human serum, lack of transcription from the LTR in the absence of the viral Rev protein, wide host range, and improved transduction of nondividing cells. These last two properties could prove especially important in transducing human hematopoietic stem cells. Foamy virus vectors seem to survive in nondividing cells and retain the ability to transduce these cells when they are stimulated to divide later on. This may allow for efficient transduction of stem cells that are quiescent at the time of infection, but subsequently divide during reconstitution of...
the hematopoietic system. In addition, if one factor in the poor stem cell transduction rates observed in large animals is low expression of the receptor for the amphotropic MLV vectors used, the apparently ubiquitous expression of the HFV receptor could overcome this limitation. The common isolation of foamy viruses from primate marrow cultures suggests that they are able to infect stem cells and survive in a latent state until the marrow is cultured ex vivo. While our results demonstrate efficient transduction of hematopoietic progenitors, only hematopoietic reconstitution after transplantation can measure stem cell transduction rates. We hope to develop a high-tier, helper-free HFV vector production system for animal transplantation experiments, and subsequent use in human gene therapy.

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