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Toward Highly Efficient Cell-Type-Specific Gene Transfer with Retroviral Vectors Displaying Single-Chain Antibodies

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Recently, we constructed retroviral vector particles derived from spleen necrosis virus (SNV) that display a single-chain antibody (scA) on the viral surface. By transient transfection protocols, we showed that such particles are competent for infection and cell type specific. Efficient infection was dependent on the presence of wild-type envelope, although wild-type SNV was not infectious on target cells (T.-H. T. Chu and R. Dornburg, J. Virol. 69:2659–2663, 1995; T.-H. T. Chu, I. Martinez, W. C. Sheay, and R. Dornburg, Gene Ther. 1:292–299, 1994). In this study, stable packaging lines were constructed and detailed biological and biochemical studies were performed. Chimeric scA-envelope fusion proteins were expressed as efficiently as wild-type envelope and were stable over a period of at least 6 h. Only a fully functional wild-type envelope could act as a helper for efficient virus penetration. The ratio of wild-type envelope protein to chimeric envelope protein appears to determine the efficiency of infection. Virus titers of targeting vectors obtained from stable packaging lines were as high as 10^4 CFU/ml. A 25-fold concentration of vector virus stocks resulted in a 200-fold increase in virus titers (up to 10^6 CFU/ml). These data indicate that an inhibitor of infection was (at least partially) removed by the concentration protocol. Our data show that this technology has several variables for further improvements and, therefore, has the potential to become a powerful tool for cell-type-specific in vivo human gene therapy.

Human gene therapy has the potential to become a major new clinical practice to cure not only inborn errors of metabolism but also a large variety of acquired diseases, like cancer, diabetes, and AIDS (5, 8, 20, 26). However, the lack of a cell-type-specific gene delivery system forces most current gene transfer protocols to be performed in tissue culture. Such ex vivo protocols are too expensive and technically problematic to be used on a widespread basis.

Several attempts have been made to construct cell-type-specific gene delivery tools. For example, retroviral particles were constructed that display the CD4 molecule to target human immunodeficiency virus-infected human cells (25). In another approach, two different antibodies, one directed against a cell surface protein and the other directed against the viral envelope (Env) protein, were linked by streptavidin at their carboxy termini to direct a retroviral vector to a specific target cell (9, 21). More recently, a peptide hormone was fused to the Env protein to specifically infect cells that express the corresponding hormone receptor (12). However, no or only very low levels of infectivity were observed.

During the past 6 years, we have been developing retroviral vector particles, derived from spleen necrosis virus (SNV) (7), that display the antigen binding site of an antibody on the viral surface. This has been achieved by fusing a single-chain antibody (scA) to the Env protein. First, by using a dinitrophenol hapten model system, we showed that such particles were competent for infection (4). Similar results have been obtained by Russell et al. with a different hapten model system and murine leukemia virus (MLV)-derived retroviral vector particles (22). To further investigate the versatility of this system for gene transfer into human cells, we next used an scA (termed B6.2) directed against a surface antigen expressed on human colon cells and various carcinomas. By transient transfection assays, we showed that SNV vector particles that displayed the B6.2 scA on the viral surface were competent for infection as well. The efficiency of infection was strongly enhanced by the presence of the SNV wild-type envelope, which does not confer infectivity on human cells. The infectivity was antigen and cell type specific (2–4). Most recently, similar findings have been obtained with an scA directed against the low-density lipoprotein receptor (24). In this paper, we describe the construction of stable packaging lines producing such targeting vectors. Detailed biochemical and biological analyses were performed. Our data show that such engineered particles have the potential to become a powerful tool for cell-type-specific in vivo gene delivery.

Establishment of stable cell lines. To test the biological and biochemical properties of retroviral targeting vectors, stable cell lines were established from single selected cell colonies transfected (13) with the construct of interest. All stable cell lines were derived from DSgp13-cxl cells, which express SNV Gag-Pol proteins and the retroviral vector pCXL (Fig. 1). The retroviral vector pCXL transduces the bacterial β-galactosidase gene (19). Transfection of an envelope expression plasmid into this cell line creates a complete retroviral packaging system.

First, a stable packaging cell line was made that expresses a chimeric scA-Env fusion protein by introducing the plasmid pTC25 into DSgp13-cxl cells. The resulting cell line was termed gp13-cxl-TC25 (Fig. 1). In pTC25, the scA gene is fused to the complete transmembrane (TM) coding region of the SNV *env* gene. Since the conserved SU/TM cleavage motif has not been retained in this construct, this chimeric scA-*env* gene product

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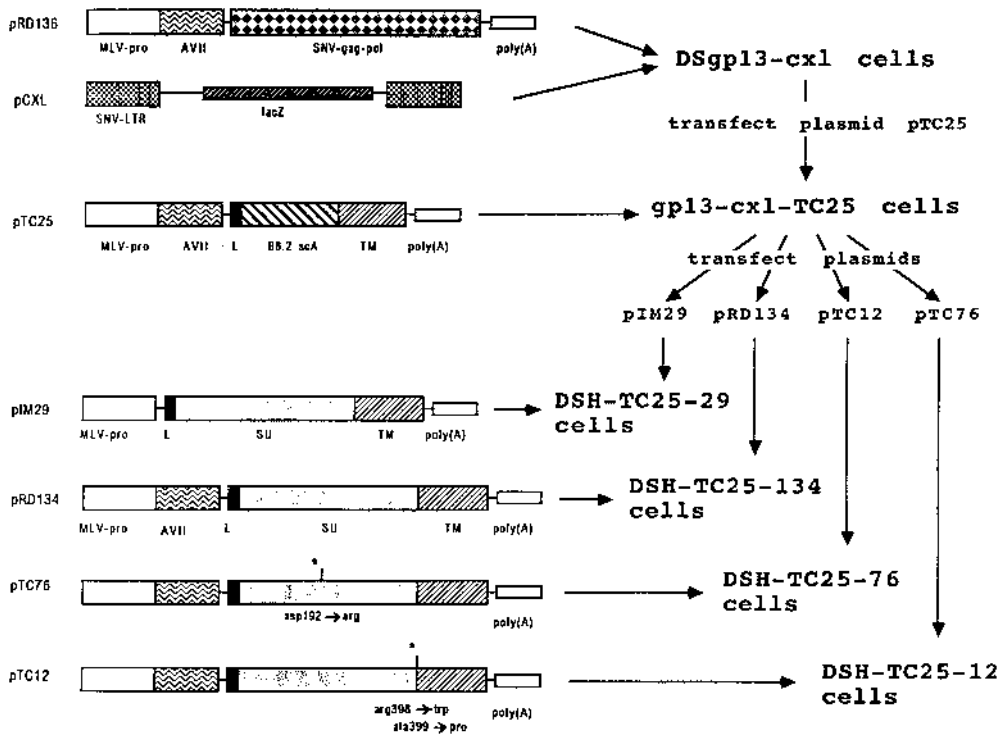

FIG. 1. Pedigree of retroviral packaging lines for production of vectors that display the B6.2 scA and plasmids used for their construction. In pRD136 (15–17), SNV Gag-Pol proteins are expressed from the MLV U3 promoter (MLV-pro) followed by the adenovirus tripartite leader sequence (AVtl). Plasmid pCXL contains a standard SNV-derived retroviral vector with SNV long terminal repeats (LTRs), which transduces the bacterial *lacZ* gene (19). In plasmid pTC25, the scA gene B6.2 was fused to TM of the SNV envelope (2, 3). Plasmids pRD134 and pIM29 contain the complete wild-type envelope gene of SNV and have been described recently (15, 16). Plasmids pTC12 and pTC76 contain mutant SNV envelope genes with point mutations at amino acid position 398-399 (SU/TM cleavage site) and at position 192 (Asp to Arg in the middle of SU), respectively. These point mutations abolish membrane fusion and receptor binding, respectively (15, 18). DSgp13-cxl cells have been derived from a single selected dog D17 cell clone and contain plasmids pRD136 and pCXL (2). DSgp13-cxl cells were transfected (13) with plasmid pTC25, which contains the B6.2 scA gene fused to TM of the SNV envelope gene. A cell line was established from a single cell colony and termed gp13-cxl-TC25. gp13-cxl-TC25 cells were transfected with plasmids pIM29, pRD134, pTC76, and pTC12 to give DSH-TC25-29, DSH-TC25-134, DSH-TC25-76, and DSH-Tc25-12 cells, respectively. These cell lines express both the chimeric and the wild-type SNV envelope genes. L, hydrophobic leader sequence of the SNV *env* gene. Plasmid sequences (derived from pUC19) that abut the above-mentioned vectors are not shown.

was not expected to be proteolytically cleaved. Therefore, it could be distinguished from wild-type envelope in polyacrylamide gels after immunoprecipitation with an anti-TM antibody.

Earlier, we found that the efficiency of infection was enhanced when wild-type envelope was coexpressed on retroviral particles containing the B6.2 scA-Env fusion protein (2). To test this further, gp13-cxl-TC25 cells were used for the construction of cell lines that express chimeric plus wild-type or mutant SNV envelope genes (Fig. 1). Thus, all cell lines derived from such clones were expected to express equal levels of Gag-Pol and the retroviral vector pCXL. In fact, although the complete loss of reverse transcriptase activity was recurrently observed in some single isolated DSgp13-cxl cell clones, stable cell lines derived from DSgp13-cxl cells used in this study expressed about equal levels of reverse transcriptase (data not shown).

Four different cell lines were established from gp13-cxl-TC25 cells that produce retroviral vector particles displaying both chimeric and wild-type Env proteins: two cell lines that express fully functional SNV wild-type Env proteins were made and were termed DSH-TC25-134 and DSH-TC25-29 (Fig. 1). These cell lines contain the envelope expression plasmids pRD134 and pIM29, respectively. Plasmids pIM29 and pRD134 are identical except that pIM29 does not contain the adenovirus tripartite leader sequence, which enhances envelope expression in D17 cells about 10-fold (16, 23). Thus, DSH-TC25-134 and DSH-TC25-29 cells differ only in the level of expression of wild-type envelope (Fig. 1 and 2). The rationale for using these two *env* expression vectors was to get a first insight into whether the wild-type/chimeric envelope expression ratio has an effect on protein processing and/or on the efficiency of infection.

To shed light on the role of wild-type envelope in infection of composite targeting vectors, two more cell lines were established from gp13-cxl-TC25 cells. These cell lines express mutant SNV envelopes and were termed DSH-TC25-76 and DSH-TC25-12 (Fig. 1). Cell line DSH-TC25-12 contains plasmid pTC12, which has two point mutations within the SU/TM cleavage site. These point mutations abolish cleavage of the envelope precursor and, hence, the membrane fusion function (15). However, they do not abolish transport of the protein to the cell surface (14a). Cell line DSH-TC25-76 contains plasmid pTC76, which carries a point mutation in the middle of SU. This mutation does not abolish protein processing or transport to the cell surface (18). However, it reduces the efficiency of wild-type SNV infection by about 4,000-fold. Our data indicate that the latter mutation interferes with receptor binding (15, 18).

The rationale for using these mutant envelope proteins was to determine whether the wild-type envelope helps virus penetration only by supplying a functional membrane fusion do-

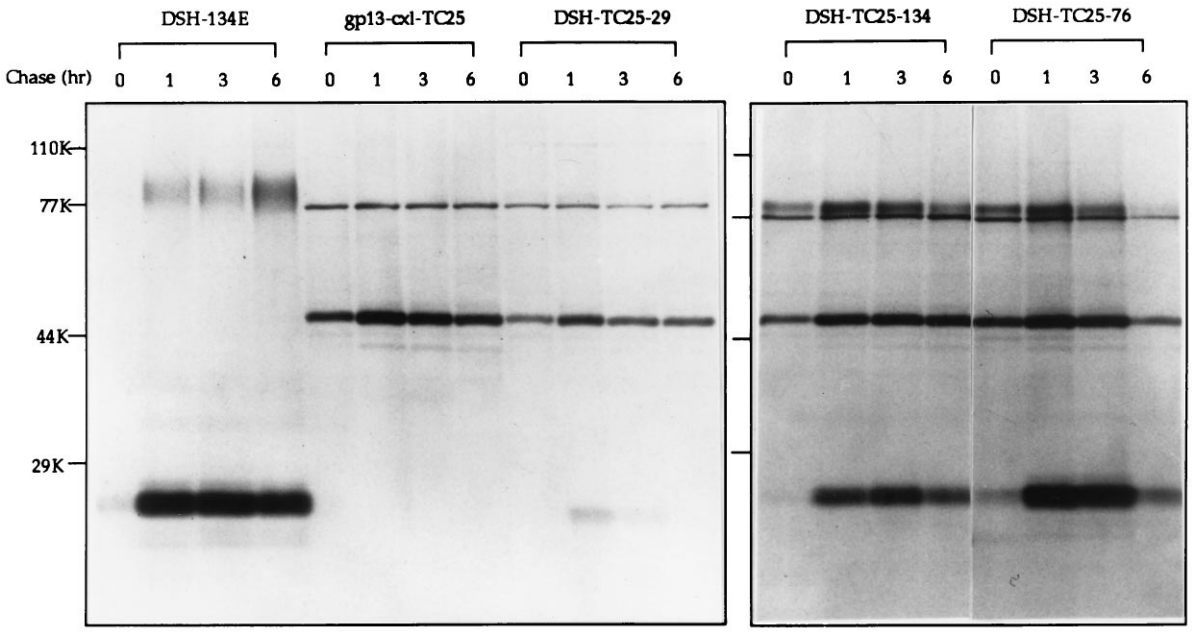

FIG. 2. Pulse-chase experiment to study expression and stability of envelope proteins. Confluent cell monolayers were labelled with 40 μCi of [^{35}S]methionine and [^{35}S]cysteine (Tran^{35}S-label; ICN Biomedicals, Costa Mesa, Calif.) per ml for 40 min; this was followed by incubation with Dulbecco's minimal essential medium supplemented with 7% fetal bovine serum for 0, 1, 3, or 6 h. Cell lysates were prepared as described previously (10). Incorporated [^{35}S]methionine and [^{35}S]cysteine were determined by scintillation counting. An excess (0.1 μl of ascites fluid) of an anti-TM antibody (6) (kindly provided by L. Lee, Regional Poultry Research Laboratory, East Lansing, Mich.) was preabsorbed with protein A-Sepharose beads (Pharmacia-LKB, Piscataway, N.J.). Lysate (3×10^7 CPM) from each sample was then incubated with the immunocomplex, precipitated, and subjected to sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis as described previously (10). The gels were fixed and exposed to Kodak X-ray film as described previously (10).

main or whether some interaction of SU with a cellular receptor is also involved. In the first case, a mutation in SU would not abolish the helper function of wild-type Env, whereas a mutation in TM would. In the second case, more complex interactions may occur; SNV can infect a large variety of species and cell lines, including dog D17 cells. Thus, it appears that SNV is docking to a housekeeping receptor, like some other retroviruses. The corresponding receptor may also be present on human cells. However, it may carry mutations which reduce the affinity of the SNV envelope below a threshold level necessary to mediate infection, similar to what is observed with ecotropic MLV (14). Thus, it may be possible that the targeting scA-envelope restores this affinity by anchoring the particle to the cell surface. Once bound to the cell surface, the complete SNV envelope may be able to interact with its cellular receptor to initiate membrane fusion. In this case, a mutation in a receptor-interacting domain of SU would also diminish the helper function of wild-type Env.

For control experiments, a standard retroviral packaging cell line has also been made from DSgp13-cxl cells by transfection of plasmid pRD134. This cell line was termed DSH134E, and it expresses only the wild-type SNV envelope.

Expression and stability of chimeric and wild-type envelope proteins. Intracellular expression and stability of the chimeric and wild-type envelopes were examined by pulse-chase analysis and immunoprecipitation. ^{35}S-labeled Env proteins were precipitated by anti-TM monoclonal antibodies (kindly provided by L. Lee, Regional Poultry Research Laboratory, East Lansing, Mich.) followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and radioautography (Fig. 2). The monoclonal antibody is very specific for the SNV envelope TM peptide; no bands were detected when immunoprecipitations were performed with control D17 cells or DSgp13-cxl cells (15, 18) (data not shown).

The anti-TM monoclonal antibody precipitated a 24-kDa protein in cells expressing the wild-type SNV envelope protein (Fig. 2, DSH-134E). The size of this band corresponds to the predicted size of the TM peptide. Coinciding with earlier observations (18), only small amounts of the SU peptide were coprecipitated by this antibody. TM was hardly detected immediately after labelling and could be immunoprecipitated after 1 h (Fig. 2, DSH-134E). Since the anti-TM antibody does not bind to noncleaved precursor envelope proteins (data not shown), it can be concluded that SU/TM cleavage was not complete after 1 h. The wild-type Env appeared to be stable over a period of at least 6 h.

The anti-TM antibody precipitated a 49-kDa protein from cells expressing the scA-TM fusion protein immediately after labelling (Fig. 2, gp13-cxl-TC25). The molecular weight of this peptide precisely reflects the predicted size of the chimeric envelope. Another protein, with a molecular mass of about 77 kDa, was always coprecipitated from cells that express gpTC25. This protein was not detected in immunoprecipitates from envelope-negative D17 control cells (data not shown) or cells expressing the wild-type envelope alone. Since the molecular weight of this protein is too low for it to be interpreted as a dimeric scA-Env fusion protein, it appears that this protein is of cellular origin. It was probably associated with and coprecipitated with the chimeric envelope protein. The chimeric envelope was also stable over a period of 6 h.

When the wild-type Env was coexpressed with the chimeric protein, both proteins were detected. The ratio of chimeric Env to wild-type Env was approximately 1:1 in cells expressing wild-type Env and the chimeric construct from identical gene expression vectors (Fig. 2, DSH-TC25-134). Neither protein appeared to have a negative effect on the stability of the other peptide. Similar results were obtained with the receptor-binding mutant gpTC76 coexpressed with the chimeric envelope

TABLE 1. Biological activity of retroviral particles[a]

Cell line	Viral titer (CFU/ml) in[b]:					
	D17 cells		HeLa cells		DLD-1 cells	
	B.C.	Conc.	B.C.	Conc.	B.C.	Conc.
DSgp13-cxl	0.5	2×10^1	0.5	1×10^2	0.5	6×10^1
DSH134-E	1.5×10^6	4×10^8	3×10^1	2.1×10^2	1.5×10^1	8×10^1
gp13-cxl-TC25	0.5×10^1	1.8×10^2	3×10^1	8×10^2	0.5×10^1	3.7×10^2
DSH-TC25-29	3.6×10^5	2.1×10^8	7×10^3	8×10^5	4.5×10^2	1.7×10^4
DSH-TC25-134	1.7×10^5	ND[c]	7×10^2	ND	ND	ND
DSH-TC25-12	<1	<1	<1	<1	<1	<1
DSH-TC25-76[d]	4.7×10^3	1×10^1	8×10^1	<1	2×10^1	<1
DSH-TC25-76[e]	4.7×10^3	1.3×10^4	8×10^1	5×10^3	2×10^1	2.3×10^3

[a] Virus particles were harvested from various packaging cell lines expressing chimeric and/or wild-type Env proteins (see also Fig. 1) and subjected to infection of D17, HeLa, or DLD-1 cells in serial dilutions to determine virus titers. Infectivity studies were performed as described recently (2). To determine the number of cells expressing the bacterial *lacZ* gene, the cells were stained with 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside as described previously (19). D17 cells (a dog osteosarcoma cell line obtained from the American Type Culture Collection) were grown in Dulbecco's minimal essential medium (DMEM) containing 6% calf serum. DLD-1 cells (human colon carcinoma) and HeLa cells (human cervical carcinoma) were grown in DMEM containing 10% fetal bovine serum.

[b] B.C., infectivity of virus particles harvested from confluent cell cultures before concentration; Conc., infectivity of virus particles after a 25-fold concentration by ultrafiltration. Concentration was performed as follows. A 15-ml volume of supernatant was added to a Centriprep-100 tube containing a cellulose membrane with a 100-kDa molecular mass cutoff (Amicon, Beverly, Mass.). The Centriprep-100 was centrifuged in a tabletop centrifuge (Sorvall RC5B) with swing-out buckets at 3,000 rpm for 30 min at 4°C. The filtrate was discarded and spun for another 10 min. The same procedure was repeated until a final volume of approximate 0.6 ml was obtained (usually three spins were performed). The concentrated supernatant was either subjected directly to infection or stored at −20°C for further analysis.

[c] ND, not done.

[d] The concentration conditions were identical to those used for the other samples indicated above.

[e] Gentle concentration conditions were used (see the text).

(Fig. 2, DSH-TC25-76), although this mutant Env appeared to be slightly less stable (see also below). Coinciding with earlier observations (16, 23), considerably less wild-type Env was detected in cells expressing the wild-type Env without the adenovirus tripartite leader sequence (Fig. 2, DSH-TC25-29).

The chimeric envelope was also detected with anti-TM antibodies in cells coexpressing the SU/TM cleavage mutant gpTC12 envelope protein (data not shown). Thus, the presence of this mutant envelope did not have a significant negative effect on the expression of the chimeric scA-Env protein. Although the SU/TM cleavage mutant protein could be detected on the cell surface with a polyclonal anti-SU antiserum by fluorescence-activated cell sorter analysis, it could not be precipitated with anti-TM antibodies (data not shown). These data suggest that the anti-TM antibody recognizes the TM epitope only if it is properly folded.

Cell surface expression of gpTC25 and wild-type envelopes has been demonstrated earlier (3, 16). Attempts were also made to compare the levels of wild-type and chimeric envelopes in virus particles. However, the sensitivity of the assays was not sufficient to detect any of these proteins (including wild-type Env), even in preparations of concentrated virus stocks (data not shown).

Infection studies of cell-type-specific SNV vector particles. Virus titers on D17, DLD-1, and HeLa cells were determined in serial dilutions with supernatant media harvested from confluent cell cultures. The cell line releasing wild-type vector particles (DSH-134E) produced 1.5×10^6 CFU/ml of supernatant medium on D17 cells (Table 1). Minimal infectivity was seen on HeLa and DLD-1 cells. Particles displaying the scA-Env fusion protein alone showed hardly any infectivity on D17 or DLD-1 cells. Consistent with our data from transient transfection experiments (2), a low level of infectivity was consistently observed on HeLa cells. No infectivity was observed on 293 or 143T cells, which do not express the B6.2 antigen (data not shown).

Particles displaying both wild-type and chimeric envelope proteins infected D17 cells about 10 times less efficiently than those containing the wild-type envelope alone. These particles also infected HeLa cells, with an average titer of 7×10^3 CFU/ml. Interestingly, particles from cells expressing low levels of wild-type Env (DSH-TC25-29) were about 10 times more infectious than those from cells expressing high levels of Env (DSH-TC25-134, Table 1 and Fig. 3). Again, no infectivity was observed on human 293 or 143T cells (data not shown).

Particles harvested from cells that express the chimeric envelope with the wild-type envelope containing an impaired membrane fusion function (DSH-TC25-12) could not infect D17, HeLa, or DLD-1 cells at all (Table 1). The low level of infectivity conferred by the chimeric protein alone was completely inhibited by this mutant Env. These data indicate that a membrane fusion function has to be supplied by the wild-type envelope. Mixed particles, containing an SU mutant envelope and the chimeric Env (DSH-TC25-76), were about 200-fold less infectious on D17 cells than wild-type vector particles. The infectivity of such virions on the human cells was reduced about 10- to 100-fold compared with particles coexpressing wild-type envelope. The infectivity on HeLa cells was still slightly higher than that of particles containing the chimeric envelope alone (Table 1). These data indicate that a fully functional wild-type envelope is needed to act as a helper for infectivity.

Infection studies on concentrated viruses. High vector virus titers are the most important feature of targeting vectors for application in human gene therapy. To test if concentrated virus stocks of targeting vectors can be obtained, ultracentrifugation or ultrafiltration was applied.

First, virus particles were pelleted by ultracentrifugation. Tissue culture supernatant media were collected and clarified by low-speed centrifugation, and this was followed by centrifugation in an SW27 rotor (Beckman Instruments, Palo Alto, Calif.) at 25,000 rpm for 90 min at 4°C. The pellets were resuspended in phosphate-buffered saline and either subjected to infection immediately or stored at −20°C for later analysis. As observed with other retroviruses (11), the particles containing the wild-type SNV envelope alone had a decreased infectivity on D17 cells after ultracentrifugation. Surprisingly, all particles, even with wild-type Env alone, became significantly infectious on human cells (data not shown). These data suggest that the virus particles were modified by ultracentrifugation

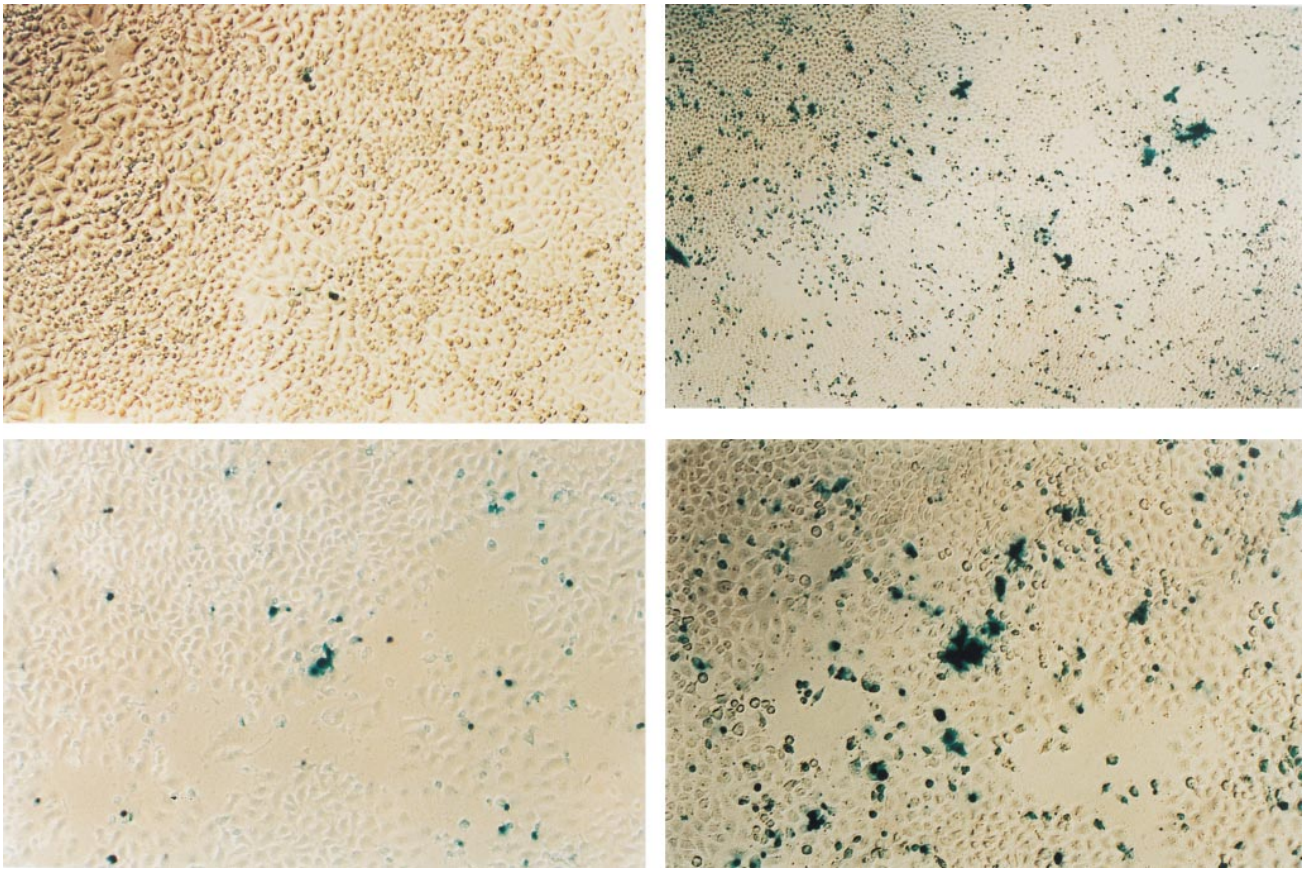

FIG. 3. Infection of HeLa cells with retroviral particles transducing the bacterial *lacZ* gene. Top left: HeLa cells infected with 200 μl of undiluted vector virus containing the SNV wild-type envelope (harvested from DSH helper cells); bottom left: HeLa cells infected with 200 μl of vector virus displaying the B6.2 scA and wild-type envelope expressed from pIM29; top right: HeLa cells infected with 100 μl of vector virus containing the B6.2 scA plus the SNV wild-type Env after a 25-fold concentration by ultrafiltration; bottom right: same as the panel above it but at a higher magnification.

and that the increase in infectivity was most certainly nonspecific (data not shown).

However, the biological properties of the virus particles did not appear to be altered when the particles were concentrated by ultrafiltration, using Amicon Centriprep-100 tubes. Although the virus particles were concentrated only 25-fold in these experiments, the infectivity of wild-type vector particles (RD134) on D17 cells increased about 250-fold (Table 1), producing titers of up to 4×10^8 CFU/ml. A similar increase was seen with all other vector stocks. For example, the virus titer of particles with mixed envelopes increased from 7×10^3 to 8×10^5 CFU/ml on HeLa cells. The infectivity of virus particles with no viral envelope was also increased by ultrafiltration. Particles produced from envelope-negative cells may incorporate various cellular proteins which may mediate low levels of nonspecific infection.

Coinciding with our earlier observations, particles displaying the targeting envelopes together with the mutant Env gpTC12 could not infect any of the cells, either before or after concentration. These data suggest that the increase of infectivity was not caused by nonspecific effects. It is noteworthy that poorly reproducible results were obtained with particles containing the chimeric protein plus the SU envelope point mutant, gpTC76. In general, the mutation in SU appeared to result in less stable envelope proteins. The usual ultrafiltration protocol applied to the other vector virus solutions almost completely abolished infectivity. However, a gentle concentration (shorter spinning periods) increased infectivity about 10-fold.

Discussion. Previously, we and others have shown that retroviral vectors that display an scA on the viral surface enable cell-type-specific gene delivery (3, 24). However, several questions still needed to be addressed to prove the potential of this gene delivery system for future application in human gene therapy. In this study, we constructed stable packaging cell lines and investigated the stability of targeting vectors and their efficiency of infection.

Chimeric scA-Env fusion proteins were expressed as efficiently as wild-type envelope. When the wild-type or mutant SNV *env* genes were copresent with the chimeric *env* gene, both proteins were expressed corresponding to the promoter strength. Moreover, both wild-type and chimeric Env proteins were stable, and neither protein had a negative effect on the stability of the other protein.

Earlier we reported that the addition of wild-type envelope to particles containing a targeting envelope bestows infectivity on cells that do not contain a suitable receptor for SNV (3, 4). We hypothesized that (i) the chimeric Env protein can act as a targeting molecule to bind the virus to the cell surface of target cells and (ii) wild-type envelope is needed as a helper, probably for efficient membrane fusion. In this study we investigated this phenomenon in more detail. We found that a mutant wild-type envelope defective in membrane fusion did not confer infectivity. On the contrary, this mutant Env protein abolished infectivity completely. We expected that an envelope protein defective in receptor binding would still be able to act as a viable helper by supplying a fully functional membrane fusion

domain. However, this mutant envelope increased the infectivity of targeting vectors only minimally.

These data show that a fully functional wild-type Env is needed to act as a helper for virus penetration. We hypothesize that the targeting envelope anchors the virus particle on the cell surface. Once bound to the cell surface, the unmodified SNV wild-type envelope can interact with its corresponding cellular receptor on human cells, to which it normally has insufficient affinity to initiate membrane fusion. However, as a result of the stabilized virus binding via the targeting envelope, all necessary conformational changes can take place to trigger membrane fusion.

We further hypothesize that the ratio of wild-type envelope to targeting envelope plays an important role in efficient infection. Thus, two different packaging lines were constructed which express different amounts of wild-type Env. Earlier, we found that the density of wild-type envelope on the cell surface does not influence the level of infectivity of vector virus particles containing wild-type Env alone (16). However, the level of wild-type Env expression did play a role in the efficiency of infectivity in particles with mixed envelopes. Surprisingly, smaller amounts of wild-type Env resulted in more infectious particles (Table 1). These data indicate that the ratio of wild-type Env to scA-Env protein is important for efficient infectivity. For example, the presence of too much wild-type Env may inhibit efficient binding of the scA to its antigen on the cell surface.

Vector virus titers obtained from stable producer lines were as high as 10^4 CFU/ml. The utility of the cell-type-specific gene delivery system described here for human gene therapy depends upon high virus titers. Thus, experiments were also performed to obtain higher virus titers by concentration. Concentration of retrovirus particles has proven to be very problematic in the past, and technologies have been developed to bypass such difficulties, e.g., by pseudotyping MLV with the envelope of vesicular stomatitis virus (1). Ultracentrifugation of particles with mixed envelopes led to nonspecific results. However, higher titers for all vectors were obtained by concentration of the viral particles by ultrafiltration. This procedure did not have any negative effects on the stability of vector particles. Surprisingly, the level of infectivity increased up to 250-fold, although the virus solution had been concentrated only about 25-fold. These data indicate that this procedure not only concentrates the amount of infectious virus in solution but also may remove an inhibitor of viral infection or particle maturation or simply create more individual virus particles available for infection. The infection efficiency of the targeting vectors increased to a titer of close to 10^6 CFU/ml.

In summary, data presented in this paper demonstrate that retroviral vectors displaying the antigen binding site of an antibody have the potential for direct in vivo gene therapy, although higher vector virus titers still need to be obtained. We have shown that this cell-type-specific gene delivery system has several variables that can be further optimized.

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