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Targeted Infection of Human Cells via Major Histocompatibility Complex Class I Molecules by Moloney Murine Leukemia Virus-Derived Viruses Displaying Single-Chain Antibody Fragment-Envelope Fusion Proteins

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As an approach to cell targeting by retroviruses, the lack of which constitutes one major limitation of retroviral vector technology, we engineered the Moloney murine leukemia virus ecotropic envelope glycoprotein. When inserted between amino acids 6 and 7 of the latter, a single-chain antibody fragment (ScFv) specific for human major histocompatibility complex class I molecules was shown to be able to redefine the tropism of ecotropic Moloney murine leukemia virus-derived retroviral particles by allowing infection of major histocompatibility complex class I-positive human cells. At variance with other recently described experimental systems, the type of modification adopted here allowed targeted infection in the absence of coexpressed wild-type *env*-encoded protein molecules. Interestingly, the chimeric ScFv-*env* protein also retained the ability to recognize the ecotropic receptor and allowed infection of murine cells, albeit at a reduced efficiency.

Most of the mammalian retrovirus vectors commonly used for gene transfer are derived from the Moloney murine leukemia virus (MoMuLV) and are usually of either the ecotropic (which infects murine cells only) or the amphotropic (which infects both murine and nonmurine cells) type. The host range is determined primarily by the interaction between viral envelope glycoproteins (*env*) (that differ between amphotropic and ecotropic viruses) and specific proteins on the host cell surface that act as retroviral receptors (for a review, see reference 34). Both ecotropic (RecI) (1, 32) and amphotropic (RamI) (18, 21, 31) receptors have been identified and cloned. Although neither ecotropic nor amphotropic viruses display actual tissue specificity, it is worth noting that the different cell types in a given species are differentially sensitive to infection, some of them even being resistant or nearly so. Finding new receptors for recombinant retroviruses and developing means for redefining the binding specificity of viral particles would have numerous applications in both laboratories and clinics. On the one hand, this may allow better infection of cells of therapeutic interest that are poorly amenable to retroviral infection, such as hepatocytes or early hematopoietic progenitors. On the other hand, this would also allow targeting of specific cell types in vitro in complex cell populations and in vivo in animals. In the in vivo situation, the availability of targeting methods should, for example, permit the development of new disease and gene therapy models.

The envelope glycoprotein is made up of two subunits which are generated by proteolysis of a common precursor protein encoded by the *env* retroviral gene (see reference 33). In MoMuLV, the heterodimer is constituted by a hydrophilic, glycosylated, extracellular protein (SU or gp70), the N moiety of which is responsible for binding to the viral receptor (3, 4, 14), and a hydrophobic transmembrane protein [TM or P15(E)], which is responsible for the tethering of *env* to the viral particle. By using bispecific antibody complexes that recognize gp70 on one side and specific cell surface molecules on the other, we originally demonstrated the possibility of redefining the tropism of ecotropic MoMuLV-derived retroviruses by allowing infection of human cells via major histocompatibility complex (MHC) class I and II molecules (25), as well as via the epidermal growth factor and insulin receptors (11). Neda et al. have also shown that chemical coupling of lactose to viral particles permits infection of human hepatocytes via the galactose receptor (22). More recently, successful targeted infection by various avian (7, 8, 29) and murine (10, 13, 17, 28) retroviruses displaying genetically engineered SU has also been reported (see the Discussion for details).

Single-chain variable fragments (ScFv) are artificial, monovalent, linear molecules composed of antibody heavy and light chain variable domains covalently linked by a short, flexible peptide spacer that usually does not impede functional association of the antibody heavy and light chain variable domains (5, 23, 35) but are devoid of constant regions that are responsible for antibody effector functions. An ScFv corresponding to antibody B9.12.1, which is specific for a monomorphic determinant of human MHC class I molecules (24), which was used in our original targeting experiments (11, 25) has recently been cloned and characterized in detail (20). We report here that when inserted at the N terminus of ecotropic MoMuLV gp70, it allows MHC class I antigen-mediated retroviral infection of human cells. Interestingly, ScFv-*env* molecules also retain the ability to drive infection of mouse cells, albeit with reduced efficiency compared with parental *env*. Our experiments thus

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lend additional support to the notion that MoMuLV gp70 can be engineered by using nonviral genetic information to modify the specificity of infection of MoMuLV-derived recombinant retroviruses.

MATERIALS AND METHODS

Cell lines. The TelCeb6 cell line was derived from the MHC class I-positive TE671 human rhabdomyosarcoma cell line by transfection of both an MoMuLV-derived *gag-pol* expression vector and an nlsLacZ reporter gene-carrying retroviral vector (9). Because of the absence of any *env* expression vector, they produce noninfectious MoMuLV viral core particles. TE671 cells and NIH 3T3 fibroblasts are available from the American Type Culture Collection. All cell lines were grown at 37°C in the presence of 5% CO_2 in Dulbecco's modified Eagle medium containing penicillin and streptomycin (100 U/ml and 100 μg/ml, respectively) and 10% decomplemented fetal calf serum.

Construction of chimeric *env* genes. Anti-human MHC I and anti-chicken egg lysozyme ScFvs have been described by Marin et al. (20) and Ward et al. (33), respectively. They were originally cloned in bacterial expression plasmid pHEN1 (15), from which they can be removed by using restriction enzymes *Sfi*I and *Not*I. An *Sfi*I-*Not*I cloning linker was initially inserted between amino acids 6 and 7 of ecotropic MoMuLV gp70 in the pNot/Sfi-*env* vector (26). The modified *env* gene was subsequently cloned in FBEMOSALF (10), which also carries a phleomycin selection marker. ScFvs were cloned as *Sfi*I-*Not*I restriction fragments at the unique *Sfi*I and *Not*I restriction sites of pFBEMOSALF (see Fig. 1) by using standard cloning procedures (27).

Cell transfection and virus production. TelCeb6 cells were transfected with wild-type and modified *env* expression plasmids by using the calcium phosphate precipitation procedure (2). Both whole populations of transfected cells and individual clones were amplified after selection in the presence of 100 μg of phleomycin (Cayla) per ml (12). For protein analysis, binding assays, and infection experiments, virus-containing cell culture supernatants were harvested after overnight culture of cells freshly grown to confluence. All virus-containing culture supernatants were filtered through 0.45-μm-pore-size cellulose nitrate membranes (Millipore).

Immunoblot assay of *gag* and *env* proteins. For monitoring of ScFv-*env* protein production by virus-producing cells, cell extracts were prepared as follows. Virus-producing cells (5×10^6) were resuspended in 200 μl of a buffer containing 20 mM Tris-HCl (pH 7.5), 1% Triton X-100, 0.05% sodium dodecyl sulfate (SDS), 5 mg of sodium deoxycholate per ml, 150 mM NaCl, and 1 mM phenylmethylsulfonyl fluoride, and lysis was allowed to proceed for 10 min at 4°C. Nuclei were eliminated by centrifugation (10,000 × *g* at 4°C for 10 min), and the protein concentration was determined (27). Cell extracts were then mixed with Laemmli electrophoresis loading buffer (125 mM Tris-borate [pH 6.8], 10 mM β-mercaptoethanol, 2% SDS, 10% glycerol) to give a final protein concentration of 1 mg/ml. To test the incorporation of modified *env* into viral particles, 10 ml of cell culture supernatant was ultracentrifuged for 1 h at 100,000 × *g* in a Beckman SW40 rotor maintained at 4°C. To assay *env*-encoded proteins in culture supernatants, cells were grown for 24 h in the absence of serum. A 1-ml volume of culture supernatant was then mixed with 1 ml of cold acetone, and precipitation was allowed to proceed for 30 min at −20°C. In the case of precipitation at acidic pH, 1 volume of 10% trichloroacetic acid was added to culture supernatants and incubated for 30 min at 0°C. Precipitates were pelleted by centrifugation (18,000 × *g* for 20 min at 4°C), dried under a vacuum, washed three times with acetone in the case of acidic precipitation, and resuspended in 100 μl of Laemmli loading buffer. For electrophoresis analysis, viral samples (20 μl), culture supernatant proteins (50 μl), and cell extracts (20 μg) were boiled for 5 min and subsequently electrophoresed through SDS–7.5% polyacrylamide gels as described by Laemmli (19). Proteins were then electrotransferred onto BA85 nitrocellulose membranes (Schleicher & Schuell). Immunodetection experiments were conducted exactly as described by Marin et al. (20), by using as first antibodies either a 1/1,000 dilution of a goat antiserum raised against Rauscher leukemia virus SU protein (04-0109; Quality Biotech Inc.) for detection of *env* proteins or anti-*gag* rat monoclonal antibody R187b (6). Final detection was obtained by using either a horseradish peroxidase-conjugated rabbit anti-goat serum (Amersham) or rabbit anti-rat immunoglobulin antibodies (Amersham) as secondary antibodies and the electrochemiluminescence kit from Amersham. Scanning analysis of autoluminograms was performed in the linear range of detection by using different exposure times with the Sun ImageAnalyser from Millipore.

Binding assays. Binding assays were carried out as described elsewhere (10). Briefly, 10^6 cells, previously washed in phosphate-buffered saline (PBS) and detached by 10 min of incubation in PBS containing 1 mM EDTA, were incubated with virus-containing cell culture supernatants from various sources for 30 min at 4°C in PBA (PBS containing 2% fetal calf serum and 0.1% sodium azide). Cells were then washed with PBA and incubated in PBA containing a 1/200 dilution of anti-Rauscher leukemia virus *gp70* serum for another 30 min at 4°C. Cells were washed twice with PBA and incubated with rabbit anti-goat immunoglobulin fluorescein isothiocyanate-conjugated antibodies (Dako). Five minutes before the two final washes in PBA, cells were stained with 20 μg of propidium iodide per ml. Fluorescence of living cells was analyzed with a FACScan apparatus from Becton Dickinson. To monitor the presence of MHC class I antigens, cells were incubated first in the presence of 0.2 μg of purified B9.12.1 per ml for 30 min at 4°C and then with anti-mouse immunoglobulin fluorescein isothiocyanate-conjugated antibodies, and final analysis was performed as described above.

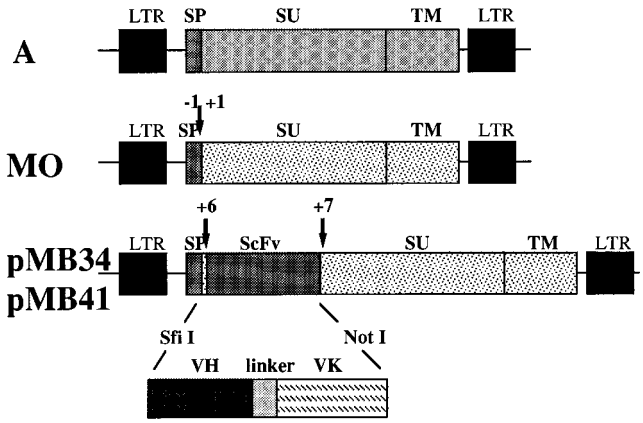

FIG. 1. Envelope glycoprotein expression vectors. Ecotropic and amphotropic envelope glycoproteins were expressed from MO and A expression vectors as described by Cosset et al. (10). Anti-MHC class I antigen (20) and anti-chicken egg lysozyme (33) ScFvs were cloned between amino acids 6 and 7 of ecotropic MoMuLV *env* in the *Sfi*I-*Not*I linker of pFBEMOSALF (see Materials and Methods and reference 10) to give pMB34 and pMB41, respectively. Variable regions VH and VK are separated by a $(\text{Ser Gly}_4)_3$ linker. LTR, long terminal repeat; SP, signal peptide; SU, surface protein; TM, transmembrane protein; VH, immunoglobulin heavy chain variable region; VK, immunoglobulin K light chain variable region.

Assay of viruses. Virus stocks were assayed on either murine NIH 3T3 fibroblasts or TE671 cells. NIH 3T3 cells (2×10^4 per well of 12-well culture plates [Nunc]) were plated in the presence of Dulbecco's modified Eagle medium containing 10% fetal calf serum. At 24 h later, the medium was replaced with 0.5 ml of fresh culture medium containing serially diluted virus-containing culture supernatants. Infection was allowed to proceed for 3 to 5 h at 37°C in the presence of 4 μg of Polybrene per ml before the culture medium was renewed. TE671 cells (2×10^5 per well of six-well culture plates [Nunc]) were plated in the presence of 2 ml of culture medium. At 24 h later, the medium was replaced with 1 ml of fresh virus-containing culture supernatant containing 4 μg of Polybrene per ml. Infection was allowed to proceed for 24 h before the culture medium was changed. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) staining of infected cells was performed 72 h after infection as described in the *Current Protocols in Molecular Biology* cookbook (2). For inhibition of MHC class I molecule-mediated infection, 0.2 μg of anti-human MHC class I antibody B9.12.1 per ml was added to the virus-containing culture supernatant.

RESULTS

Expression of ScFv-*env* chimeric proteins in transfected TelCeb6 cells. It was previously shown that insertion of an ScFv between amino acids 6 and 7 of ecotropic MoMuLV gp70 allows display of functional chimeric *env*-encoded proteins on the surface of viral particles (26). A recently characterized ScFv (pMB4.3; 20) corresponding to anti-human MHC class I antigen mouse monoclonal antibody B9.12.1 (24), which was previously used for targeting of human cells by ecotropic viruses by using the bispecific-adapter approach (11, 25), was thus cloned at this position to give eucaryotic expression vector pMB34 (Fig. 1; see Materials and Methods). As a negative control for targeted-infection experiments, we also made a comparable construct (pMB41) by using an ScFv specific for chicken egg lysozyme (33). pMB34, pMB41, and wild-type ecotropic (MO) and amphotropic (A) *env* expression vectors (10) were then independently used to transfect TelCeb6 cells (9); this produced *env*-deficient MoMuLV particles carrying the genetic information for the reporter *Escherichia coli* β-galactosidase. For further analysis, we selected one individual clone, 34.23, and a population of transfected cells, 34.1 (mixture of

more than 100 clones), for pMB34; one individual clone (41.2) for pMB41; and populations of transfected cells for MO (popMO) and A (popA). To allow direct comparison of infection efficiencies, it was verified prior to any investigation that transfection with and/or expression of the different *env* genes did not dramatically affect the production of viral particles, as estimated by measuring the amounts of protein $p30^{gag}$ in culture supernatants (Fig. 2D).

As a first step in the functional characterization of expression vectors pMB34 and pMB41, their ability to direct the production of chimeric *env*-encoded protein was compared with that of MO and A. Cytosolic extracts from transfected and nontransfected TelCeb6 cell lysates were fractionated by electrophoresis, and the presence of *env*-encoded glycoproteins was tested in an immunoblotting assay using an anti-Rauscher leukemia virus SU serum which cross-reacts with both ecotropic (70-kDa) and amphotropic (80-kDa) MoMuLV SU proteins. Consistent with ScFv size (approximately 30 kDa; see below), immunoreactive proteins of approximately 100 kDa were detected (Fig. 2A). Presumably because of differences in transfection efficiency, the abundances of the different *env*-encoded proteins were different (Fig. 2A and Table 1).

Incorporation of ScFv-*env* proteins into viral particles. As a second step in our investigation, we tested for the presence of chimeric *env* in viral particles. Viral particles were prepared by ultracentrifugation from culture supernatants of clones 34.1, 34.23, 41.2, popA, and popMO, and the presence of *env*-encoded proteins was monitored by using the immunoblotting assay described above (Fig. 2B). In three independent experiments, chimeric envelope glycoproteins were found to be associated with virions, albeit to a lesser degree than were wild-type *env*-encoded proteins. Although data may slightly vary from one set of experiments to another, those presented in Fig. 2 are typical: (i) eightfold less *env*-encoded protein was found associated with clone 34.1 and 34.23 viruses than with popMO viruses, and (ii) twofold less *env*-encoded protein was found associated with clone 41.2 viruses than with popMO viruses. The results obtained with the anti-SU antibody, recognizing ampho- and ecotropic envelopes to comparable extents but not exactly to the same extent, also indicate that roughly comparable amounts of *env*-encoded protein are associated with popA and popMO viruses (Table 1; also, see below).

The lower abundance of virus-associated ScFv-*env* may be interpreted in several ways. First, incorporation into virions may be less efficient than that of wild-type *env*. Second, since wild-type SU is known to be loosely attached to the transmembrane protein, release from viral particles may be enhanced. Three, because of their structural alteration, chimeric *env*-encoded proteins might become sensitive to a protease(s) in the culture medium or that produced by TelCeb6 cells. To address this issue, we reasoned that with comparable incorporation into viral particles and favored release, abundances of the different *env*-encoded proteins in culture supernatants (which contain both free and virus-associated *env*-encoded proteins) would directly reflect abundances within virus-producing cells. Total proteins from clones 34.1, 34.23, and 41.2 and from popMO and popA culture supernatants were quantitatively precipitated either at acidic pH (data not shown) or in the presence of acetone, and *env*-encoded proteins were assayed by immunoblotting. Relative abundances (Table 1) of *env*-encoded proteins in culture supernatant (Fig. 2C) were close to those of virus-associated *env*-encoded proteins (Fig. 2B) and departed significantly from the abundances of *env*-encoded proteins in virus-producing cells (Fig. 2A). This indicates either that incorporation into virions is reduced or that modified *env*-encoded proteins got more sensitive to degradation. Further experiments are, however, required for discrimination between these two possibilities.

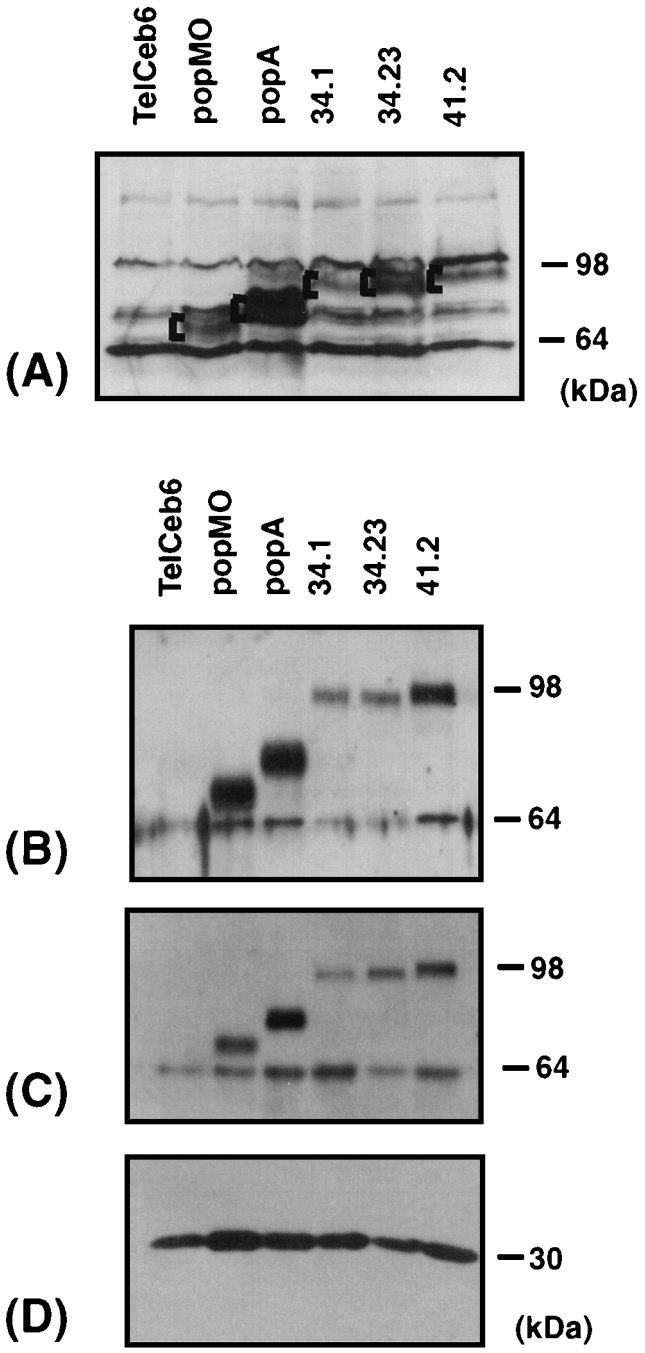

FIG. 2. Abundances of $p30^{gag}$ and *env* proteins. Procedures used for immunoblotting analysis and preparation of viral particles, total proteins from culture supernatants, and cell extracts are described in Materials and Methods. (A) Envelope glycoproteins in cellular extracts. Brackets indicate positions of wild-type and modified *env* proteins. (B) Virion-associated *env* glycoproteins. (C) *env* proteins in cell culture supernatants. (D) Virion-associated *gag* proteins.

Careful analysis of the immunoblots in Fig. 2 suggests that the two ScFvs used in our experiments may differently affect the behavior of ScFv-*env* proteins. First, although the molecular mass of anti-lysozyme ScFv (27 kDa) is lower than that of the anti-MHC class I antigen ScFv (30 kDa) (20), pMB41-

TABLE 1. Relative abundances of wild-type and modified *env* proteins in popMO and popA and clones 34.1, 34.23, and 41.2

Samples[a]	Relative abundance of *env* proteins in:					
	TelCeb6	popMO	popA[b]	34.1	34.23	41.2
Cell extracts	0	1	>10	0.6	2.1	0.8
Viral particles	0	1	1.3	0.14	0.12	0.57
Culture supernatants	0	1	1.8	0.18	0.24	0.45

[a] Data were deduced by scanning of the autoradiographs in Fig. 2. Relative abundances are expressed with respect to *env* abundance in popMO. $p30^{gag}$ was used as a reference for normalization of *env* abundances in the different protein samples.

[b] Amphotropic and ecotropic proteins were recognized with comparable, but not exactly identical, efficiencies by anti-SU serum. Values for popA thus cannot be compared directly to those for popMO and clones 34.1, 34.23, and 41.2.

derived ScFv-*env* reproducibly showed slightly decreased electrophoretic mobility (Fig. 2A to C). Whether the shift reflects a direct effect on the modified *env* structure or an undirect one on glycosylation is, however, not known. Second, the abundances of the different ScFv-*env* proteins in virions do not reflect the abundances in producing cells. For example, although produced at a 3-fold lower level in 41-2 cells, ScFv-*env* chimeric proteins were 4.5-fold more abundant in clone 34.23 viruses. It must be emphasized that this situation also concerns wild-type envelopes, since amphotropic *env* was found much less efficiently associated with virions than was ecotropic *env*. The decrease in the efficiency of incorporation was still greater when clone 34.1 and 41.2 viruses and cell lines were compared. Why the anti-MHC class I ScFv-*env* chimeric protein is proportionally less efficiently incorporated in clone 34.23 than in clone 34.1 viruses was not determined. However, the equivalent amount of the chimera reproducibly found associated with clone 34.23 and 34.1 viruses suggests that control of its incorporation into viral particles operates at the level of producing cells (see Discussion).

Binding of ScFv-*env* to human MHC class I molecules. It was important to determine next whether ScFv-*env* is able to recognize cell membrane-associated MHC class I molecules. MHC class I-positive TE671 cells were thus incubated with culture supernatants from clone 34.23 and popMO and popA (which contain both free and virus-bound *env*-encoded proteins), and the binding of SU was tested by using an immunocytometric assay as described in reference 10 (Fig. 3). It is noteworthy that binding of MoMuLV amphotropic *env* to the RamI receptor is much more efficient at 37°C than at 4°C (3). However, binding experiments were conducted at a low temperature to inhibit internalization into cells, which may possibly proceed differently for RamI and MHC class I antigens at a higher temperature. Results were as follows. (i) Because of the absence of the ecotropic receptor on TE671, no binding was observed in the case of ecotropic *env*, (ii) because of the presence of RamI, significant binding of amphotropic *env* was detected, and (iii) binding of pMB34-derived *env* was comparable to that of A, which is consistent with the idea that this modified version of *env* is capable of binding to MHC class I molecules.

Infection via RecI and MHC class I antigens. Next, we determined whether ScFv-*env*-displaying viral particles were still capable of ecotropic infection. Indicator NIH 3T3 mouse fibroblasts harboring the RecI receptor were infected by using serial dilutions of clones 34.1, 34.23, and 41.2 and popMO and popA culture supernatants in a classical virus assay using *E. coli* β-galactosidase activity as a reporter for identification of infected cells. We were able to detect infection in all situations,

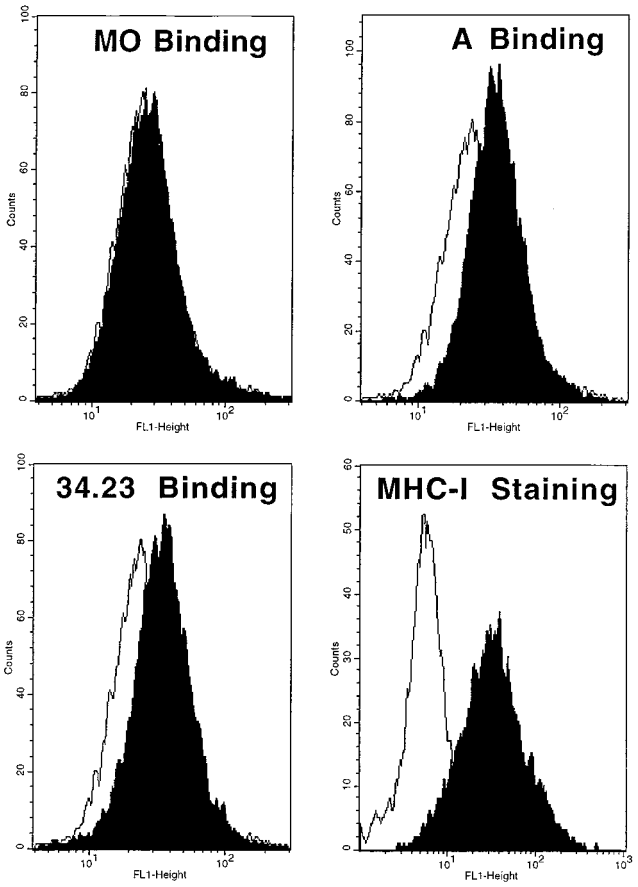

FIG. 3. Binding of wild-type and ScFv-*env* proteins to TE671 human cells. Binding assays were carried out as described by Cosset et al. (10) (also, see text). TE671 cells were incubated at 4°C with either culture supernatants from clone popMO, popA, or 34.23 or purified antibody B9.12.1 before fluorescence-activated cell sorter analysis (see Materials and Methods).

indicating that ScFv-*env* proteins may be functionally exposed at the surface of virions. However, titers were approximately 10^4 lower in clones 34.1, 34.23, and 41.2 than in popMO and popA (Table 2). Whether this discrepancy reflects differences in interactions of viral particles with the ecotropic receptor or abortive infection at a postbinding step was not determined.

For testing of the redefined specificity of infection, TE671

TABLE 2. Infection of mouse NIH 3T3 fibroblasts and human TE671 cells[a]

Cell population or clone	Virus titers in:	
	NIH 3T3 cells	TE671 cells
popMO	10^7, 10^7, 10^7	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0
popA	10^7, 10^7, 10^7	10^7, 10^7, 10^7
34.1	10^3, 10^3, 10^3	3, 5, 5, 9, 10, 10, 50, 58, 112
34.23	2×10^3, 2×10^3, 2×10^3	3, 8, 8, 12, 12, 14, 16, 16, 16, 20, 20, 42
41.2	10^3, 10^3, 10^3	0, 0, 0, 0, 0

[a] Infection assays were conducted as described in Materials and Methods. The data indicate the number of PFU obtained with 1 ml of culture supernatant from the different cell lines. Each value corresponds to an individual assay.

TABLE 3. Inhibition of targeted infection by antibody B.9.12.1[a]

Condition	Virus titers in TE671 cells	
	popA[b]	34.23[c]
Without B9.12.1	10^7, 10^7, 10^7	12, 14, 20, 25
With B9.12.1[d]	10^7, 10^7, 10^7	2, 2, 5, 5

[a] Infection experiments were carried out with TE671 cells by using 1 ml of culture supernatant from popA and clone 34.23.

[b] Three independent experiments were conducted with popA culture supernatants.

[c] Four independent experiments were conducted with clone 34.23 culture supernatant. Values obtained in the presence or absence of the antibody are presented in the order in which the experiments were done.

[d] Inhibition experiments were conducted in the presence of 0.2 μg of purified antibody B9.12.1 per ml, which was added to virions before infection.

cells were incubated in the presence of serial dilutions of clone 34.1, 34.23, 41.2, popMO, and popA culture supernatants. As expected, efficient infection was obtained with amphotropic popA viruses whereas no infected cell was observed in the 21 and 5 independent experiments conducted with popMO and clone 41.2, respectively (Table 2). In contrast, TE671 cells were infected reproducibly in the 9 and 12 independent experiments conducted with clone 34.1 and 34.23 culture supernatants, respectively, although titers were low, ranging from 3 to 112 PFU/ml. To demonstrate that targeted infection actually occurred through interaction with MHC class I antigens, four infection experiments were conducted in the presence of 0.2 μg of monoclonal antibody B9.12.1 per ml. Although B9.12.1 did not inhibit infection by amphotropic viruses, it quantitatively inhibited infection by clone 34.1-produced viruses (Table 3).

DISCUSSION

Thanks to genetic engineering of the *env*-encoded glycoprotein, successful redefinition of retroviral infection specificity has recently been obtained. (i) Replacement of an internal region of the envelope glycoprotein of an avian retrovirus of subgroup A with a 16-amino-acid-long RGD-containing peptide known to attach to several cellular integrin receptors has permitted infection of otherwise resistant rat glioblastoma cells (29). (ii) Substitution of human erythropoietin for the N moiety of ecotropic MoMuLV gp70 allowed infection of human cells expressing the human erythropoietin receptor (17), although the interpretation of this work has been contested (16). (iii) A series of ScFv-*env* fusion proteins involving the envelope glycoprotein of the spleen necrosis virus has been constructed for targeting of human and chicken cells (7, 8). (iv) Addition of part of amphotropic MoMuLV *env* at the N terminus of ecotropic gp70 allowed infection of human cells via the RamI amphotropic receptor (10). (v) Substitution of heregulin, which is a ligand for human epidermal growth factor receptors 2 and 4, for the N moiety of MoMuLV ecotropic *env* allowed targeted infection of human breast cancer cells overexpressing human epidermal growth factor receptors 2 and/or 4 (HER2 and/or HER4) (13). (vi) Somia et al. have shown that when inserted in ecotropic MoMuLV gp70, an ScFv directed against the human low-density lipoprotein receptor (LDL-R) allows specific infection of specifically engineered human and quail cells (28). (vii) By using a similar approach in this study, we targeted infection of human cells through recognition of MHC class I antigens.

The rationales used by us and Somia et al. (28) for construction of ScFv-MoMuLV *env* molecules are similar. However, MHC class I antigen-mediated infection was much poorer than that mediated by LDL-R (10^2- to 10^3-fold difference), although its efficiency was comparable to those of most of the other above-mentioned targeting approaches. This difference cannot be accounted for by a different ScFv-*env* chimera design since the ScFv insertion site was the same in both situations. Two nonexclusive possibilities may, however, be considered for explaining this difference. (i) The nature of the targeted receptor may be determining. Indeed, we have previously reported that different molecules at the surface of the same cell may mediate MoMuLV infection with very different efficacies likely because of differences in intracellular fate after internalization (10, 11, 25). Along this line, it must be emphasized that in our experiments MHC class I antigens were endogenous molecules whereas in the experiments of Somia et al. targeted infection was achieved via ectopically overexpressed LDL-R, whose behavior may differ from that of physiologically expressed LDL-R at the surface of hepatocytes. (ii) It must also be considered that the differences in target cell context may be crucial since it has already been demonstrated that the same molecule at the surface of different cell types may be differentially efficient in allowing targeted infection (25).

We feel that four other points are worthy of mention concerning the experiments we conducted. First, insertion of an ScFv at position +6 reduced the incorporation of *env*-encoded proteins into virions. Although the mechanism of this inhibition is not known, it is reasonable to assume that limiting amounts of envelope glycoproteins might partially account for lowered infection yields. Second, it is of note that modification at position +6 did not totally inhibit the ecotropic infection potential in our experiments (see references 9 and 30), whereas insertion of an anti-LDL-R ScFv seems to abolish it (28). Whether this differential effect is linked to the particular structures of the different miniantibodies used in the different laboratories requires further investigation. Third, successful targeted infection with the MHC class I antigen was independent of the presence of coexpressed wild-type ecotropic *env*, whereas coexpression was required with LDL-R (28). Although the point has not been studied extensively, preliminary experiments suggest that coexpression of wild-type *env* does not stimulate MHC class I antigen-mediated infection significantly. Again, differences in structure between anti-LDL-R- and anti-MHC class I ScFv-bearing molecules might explain the observed differential effect and requires further investigation. It is of note that with the ScFv-spleen necrosis virus *env* (7, 8), heregulin-*env* (13), and erythropoietin-MoMuLV *env* (17) chimeras, successful targeting also required coexpression of wild-type *env*. Whether in these situations wild-type *env* exerts its effect in virus-producing cells, for efficient chimera maturation, assembly, or incorporation into virions, or in infected cells, to avoid trafficking of viruses to the lysosomes, is not known and has to be investigated. Fourth, anti-MHC class I ScFv-*env* molecules were less efficiently incorporated into virions than were anti-lysosyme ScFv-*env* molecules. Although we cannot rule out a structural effect for explaining this difference, one possibility is that binding to MHC class I molecules expressed by TelCeb6 cells entails retention of PM34-derived *env*-encoded proteins in the endoplasmic reticulum and/or at the cell surface.

In conclusion, together with reports by others, our work supports the notion that retroviral envelope glycoproteins can be engineered for the purpose of cell targeting. However, efficacies of targeted infection are still very low and improvements are necessary before actual in vivo gene therapy strategies can be considered. Toward this aim, extensive study of

wild-type *env* and analysis of the influence of different types of genetic modifications are required.

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