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Retroviral Vectors Pseudotyped with Lymphocytic Choriomeningitis Virus

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Pseudotyping can improve retroviral vector stability and transduction efficiency. Here, we describe a novel pseudotype of murine leukemia virus packaged with lymphocytic choriomeningitis virus (LCMV). This pseudotype was stable during ultracentrifugation and infected several cell lines from different species. Moreover, LCMV glycoproteins were not cell toxic.

Low titers limit in vivo applications of conventional amphotropic retroviral vectors. Virus particles cannot be concentrated to overcome this problem because of the instability of the retroviral envelope (22). In addition, transduction of some cell types, such as hematopoietic progenitors, has been inefficient (1, 11, 24, 28). Both vector stability and host range have been improved by packaging murine leukemia virus (MLV) vectors with the G protein of vesicular stomatitis virus (VSV) to generate hybrid virions called MLV(VSV) pseudotypes (13, 28, 29). However, a major drawback of these packaging systems is that the VSV G protein is cell toxic (7, 9, 17, 31).

Here we describe a retroviral vector pseudotyped with the glycoproteins of an arenavirus, the lymphocytic choriomeningitis virus (LCMV). LCMV glycoproteins are synthesized as a 74-kDa precursor protein, GP-C, and then cleaved into a 35-kDa transmembrane protein, GP-2, and an external 44-kDa protein, GP-1. In contrast to VSV G, LCMV glycoproteins are not cell toxic (25).

Initially we tested whether LCMV could rescue a retroviral vector. The *env*-negative packaging cell line TELCeB was infected with the LCMV WE strain. TELCeB cells are derived from the human fibroblast cell line Te671 and contain *gag* and *pol* genes as well as a retroviral *lacZ* vector (10). These cells lack viral envelope proteins and do not produce infectious retrovirus unless an appropriate glycoprotein is provided. After infection with LCMV, levels of production of wild-type LCMV and rescued retrovirus were measured daily and expressed as PFU and LacZ-transferring units (LTU), respectively. The titration assays have been described previously (16, 18). Additionally, production of LCMV glycoproteins in infected TELCeB cells was monitored by flow cytometric analysis with a mouse monoclonal antibody against LCMV GP-1 (6). The results of a representative experiment are shown in Fig. 1. Retroviral vector was produced for 6 days, with the maximum level of production of 5×10^4 LTU per ml, together with the highest expression of LCMV glycoprotein, occurring on day 3. The highest titer for wild-type LCMV was 3×10^8 per ml on day 2. No cytopathic effect was observed during replication of LCMV in the packaging cell line, although high levels of LCMV glycoproteins were expressed. Supernatants were then incubated with a neutralizing anti-LCMV gp44 monoclonal antibody (in mouse ascites fluid, 1:100) for 1 h (6). This led to a more than 3-log-unit reduction in vector titer. As a control, the amphotropic pseudotype of the same retroviral vector was used and was not neutralized by the anti-LCMV antibody (data not shown). These data show that the retroviral vector indeed carried on its surface LCMV glycoproteins that mediate cell entry in the absence of retroviral envelope proteins.

We then analyzed whether the LCMV helper function required retroviral *gag* and *pol*. 293 cells and 293gp2 cells, the latter containing MLV *gag* and *pol*, were transfected with an MLV-based retroviral vector containing the *neo* gene (MP1N) and were selected with G418 (12, 28). The resulting cell lines, 293MP1N and 293gp2MP1N, were then infected with LCMV. 293gpMP1N produced infectious retroviral progeny (3×10^3 G418-resistant transfer units [GTU] per ml; for the method of titration, see reference 28), but no vector could be detected in the supernatant of LCMV-infected 293MP1N cells, which did not contain retroviral *gag* or *pol* (detection limit, 1 GTU per ml). As a control, the cells were also infected with a replication-competent amphotropic helper (23). An infectious vector that transferred *neo* resistance was recovered from both cells lines at titers of 1×10^4 and 6×10^4 per ml for 293MP1N and 293gp2MP1N, respectively. Thus, as expected for a classical retroviral pseudotype, retroviral genomic RNA was not packaged by LCMV into infectious virions in the absence of *gag* and *pol* gene products.

To verify that MLV(LCMV) pseudotypes indeed mediated stable transduction with integration of the transgene into the target cell genome, DNA of 12 G418-resistant clones was subjected to Southern blot analysis after restriction with *Hin*dIII, a single cutter, and a *neo* probe (26). In 10 clones, one copy of the integrated retroviral vector genome per cell was detected, and in the remaining 2 clones, two copies were detected (data not shown). Transduction with the MLV(LCMV) pseudotype thus led to stable integration of the transgene, which also supports the conclusion that the MLV(LCMV) pseudotype indeed contained a functional retroviral core.

However, the hybrid vector particles were produced by LCMV infection of *env*-negative packaging cell lines, in which all LCMV proteins were expressed. Therefore, we could not completely exclude the possibility that, in addition to the glycoproteins, further LCMV proteins are required for efficient

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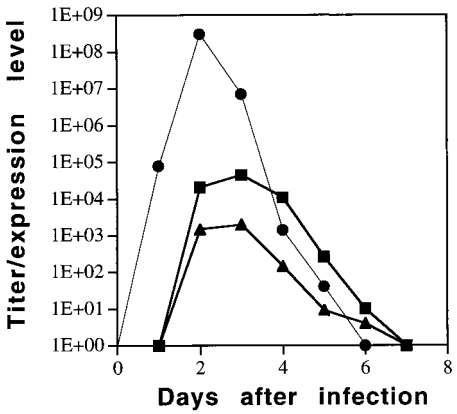

FIG. 1. Rescue of the retroviral vector MFGlnsLacZ by LCMV. The retroviral *env*-negative packaging cell line TELCeB was infected with LCMV at a multiplicity of 0.01. Between days 1 and 7 after infection, supernatants were replaced daily and the titers of wild-type LCMV and of the LacZ vector were determined by a plaque assay on L-929 cells and by measurement of *lacZ* gene transfer to Sc-1 cells, respectively. Additionally, a portion of the LCMV-infected TECLeB cells was stained daily with a monoclonal antibody to the LCMV glycoprotein GP-1 and analyzed by flow cytometry (FACScalibur; Becton Dickinson, Heidelberg, Germany). The mean fluorescence is shown. ●, titer of wild-type LCMV in PFU per ml; ■, LTU per ml; ▲, mean fluorescence of LCMV glycoprotein GP expression.

vector production. This issue is important for the generation of helper-free packaging systems and is currently addressed by expressing isolated LCMV genes in *env*-negative packaging cells.

Amphotropic retroviruses lose infectivity upon ultracentrifugation, most likely because of the lability of the retroviral envelope glycoproteins (22). We tested whether the MLV (LCMV) pseudotypes are more stable. TELCeB cells were infected with LCMV or with amphotropic helper virus. Equal amounts of both virus progeny were pelleted and purified by ultracentrifugation through a 0 to 40% Urografin gradient as described previously (19). Titers of a representative gradient (in LTU per ml) are shown in Fig. 2. LCMV pseudotypes were recovered without loss of infectivity, in contrast to a >3-log-unit loss of infectious amphotropic virus. The reverse transcriptase activities in the virion peaks showed that the amounts of virus particles harvested from the gradient were similar for both pseudotypes (data not shown).

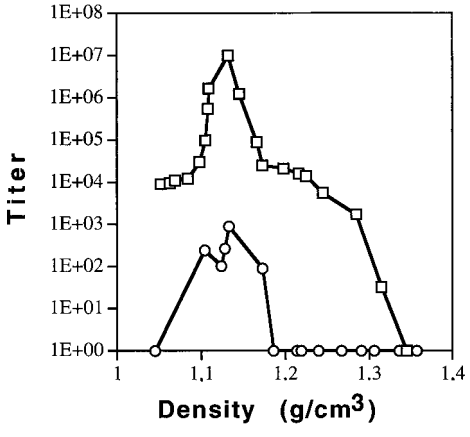

FIG. 2. MLV(LCMV) pseudotypes retain infectivity upon ultracentrifugation. TELCeB cells were infected with LCMV or amphotropic helper virus. Supernatants were harvested and frozen. The MLV(LCMV) and amphotropic pseudotype titers were determined. Equal quantities of the infectious vector were pelleted by ultracentrifugation and then subjected to purification on a 0 to 40% Urografin gradient. Vector titers and densities in each fraction were determined. ○, amphotropic pseudotype; □, MLV(LCMV) pseudotype.

TABLE 1. Host range of MLV(LCMV) pseudotypes

Cell line (no. of expts)	Cell type	Mean vector titer relative to titer on SC-1 cells (range)
293 (5)	Epithelial, human	5.1 (1.2–16.7)
Te671 (5)	Fibroblast, Human	16.5 (3.5–25)
K562 (5)	Myeloid progenitor, human	0.26 (0.06–0.5)
TF-1 (4)	Myeloid progenitor, human	0.14 (0.03–0.27)
HUH-7 (5)	Hepatoma, human	0.08 (0.02–0.19)
nce-G112 (3)	Glioma, human	3.44 (0.1–10)
CHO (4)	Epithelial, hamster	0.7 (0.02–2.5)
Cf2Th (4)	Thymus stroma, dog	15.3 (6–20)
Sc-1[a]	Fibroblast, mouse	1

[a] The titers on Sc-1 cells of the different virus stocks used here (non concentrated supernatants) ranged from 0.2×10^4 to 5×10^4 LTU per ml.

LCMV pseudotypes were also stable when they were stored at 4°C. Within the observation period of 3 days, the loss of titer was less than twofold. One cycle of freezing (−80°C) and thawing led to a twofold reduction in pseudotype titer.

The tropism of MLV(LCMV) pseudotypes was analyzed. Vector titers on different cell lines relative to the titer on the mouse fibroblast line Sc-1 are shown in Table 1. The human hematopoietic progenitor cell lines K562 and TF-1, the human hepatoma cell line HUH-7, and the human glioma cell line nce-G112 could be efficiently infected (14, 30). All these lines are derived from cell types that are relevant targets in gene therapy. Moreover, canine thymus cells (Cf2Th) and hamster cells, the latter of which are normally resistant to transduction with MLV-derived vectors, were also highly susceptible to the MLV(LCMV) pseudotypes.

Wild-type LCMV has been shown to infect several cell types from different tissues and species (2, 3, 5, 15, 20, 27). Alpha-dystroglycan, which is widely expressed in different tissues, was recently found to be a receptor for LCMV (4, 8). It was, therefore, not surprising that in our analysis MLV(LCMV) pseudotypes also infected several different cell lines, including hamster cells, which are resistant to amphotropic retroviral vectors (21). An interesting property of LCMV is that single amino acid changes in the glycoprotein can alter cell tropism, indicating that the LCMV glycoproteins may use different closely related receptors (or one receptor with different post-translational modifications) on different cell types (20, 27). This flexibility of the glycoprotein may be of considerable advantage for the design of vectors with preferential tropism for specific tissues and cell types.

In conclusion, although several questions remain open, especially regarding the feasibility of a helper-free packaging system, MLV(LCMV) pseudotypes are a promising alternative to current retroviral pseudotypes and may allow stable production of broad-host-range retroviral vectors which can be concentrated by ultracentrifugation.

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