A Novel Lentivirus Vector Derived from Apathogenic Simian Immunodeficiency Virus


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The improvement of gene transfer efficiency in growth-arrested cells using human immunodeficiency virus type 1 (HIV-1)-derived vectors led to the development of vectors derived from other members of the lentivirus family. Here we report the generation of a lentiviral vector derived from the apathogenic molecular virus clone SIVagm3mc of the simian immunodeficiency virus from African green monkeys (Cercocebus pygerythrus). Upon pseudotyping with the G-protein of vesicular stomatitis virus (VSV-G), the SIVagm-derived vector was shown to transduce proliferating and growth-arrested mammalian cell lines, including human cells. After in vivo inoculation into the striatum of the adult rat brain, the vector was shown to transduce terminally differentiated neurons and oligodendrocytes as well as quiescent and reactive astrocytes. Moreover, SIVagm transfer vector mRNA was efficiently packaged by HIV-1 vector particles. Homologous [SIV(SIV)] vectors generated by using the SIVagm-derived envelope glycoproteins allowed selective gene transfer into human CD4+/CCR5 cells. Thus, the SIVagm3mc-derived vector is a useful alternative to HIV-1-derived lentiviral vectors in somatic gene therapy.

Key Words: lentiviral vector; pseudotype vectors; growth-arrested cells.

Introduction. In clinical gene therapy trials, vectors derived from onco-retroviruses similar to the amphotropic murine leukemia virus (MLV-A) have often been used. However, MLV-derived vectors require active proliferation of the target cells for efficient transduction. Growth-arrested or slowly dividing cells are only poorly transduced. In contrast, vectors derived from lentiviruses are capable of transducing nonproliferating cells (1) and may thus be a useful alternative. Lentiviral vectors are generated by triple transfection into mammalian cells of plasmids harbouring the packaging (psi)-signal positive transfer vector gene as well as the psi-negative lentiviral gag/pol gene and a suitable psi-negative envelope gene construct. For efficient transduction of human cells, the G-protein of vesicular stomatitis virus (VSV-G) or the MLV-derived envelope glycoproteins (Env) can be used to generate pseudotype vector particles with a broad cell tropism (2). Most often, human immunodeficiency virus type 1 (HIV-1) has been used to derive lentiviral vectors. As an alternative to the use of HIV-1-derived vectors, other members of the lentivirus family have been used to derive vectors, e.g., HIV-2 (3), simian immunodeficiency virus (SIVmac) from rhesus macaques (Macaca mulatta; Ref. 4, 5), or other nonprimate lentiviruses (6). We report here the generation of a lentivirus vector derived from the molecular clone SIVagm3mc of simian immunodeficiency virus (SIVagm) from African green monkeys (AGMs; Cercocebus pygerythrus). Following natural infection as well as experimental inoculation, SIVagm has been shown to be apathogenic in AGMs and in pig-tailed macaques (Macaca nemestrina; Refs. 7, 8, 9-13). In addition, SIVagm belongs to a phylogenetically distinct group of lentiviruses, which is very different from the HIV-2/SIVsm/SIVmac or the HIV-1/SIVcpz group of lentiviruses. Therefore, we used the apathogenic molecular virus clone SIVagm3mc to derive a new lentiviral vector system.

Results and Discussion. SIVagm vectors can be pseudotyped with VSV-G and packaged into HIV-1 vector particles.

First we tested the ability of the SIVagm-derived packaging construct S11b and the transfer vector S19-lacZ (Fig. 1) to generate infectious vector particles upon cotransfection with the VSV-G envelope construct MD-G (14, 15) into 293T cells. As positive controls, HIV-1- and MLV-derived vectors pseudotyped with VSV-G were produced accordingly. 293T cells transfected with the packaging and transfer vector construct only, but not with an envelope-encoding plasmid, served as negative con-
controls. Furthermore, the capability of SIVagm- and HIV-1-derived packaging constructs to cross-package the respective heterologous vector genome RNA was tested by transfection of the SIVagm-derived transfer vector construct with the HIV-1-derived packaging construct, and vice versa. Vectors were generated as described and titrated in human 293 kidney cells and D17 canine osteosarcoma cells (Table 1). As shown, infectious SIVagm-derived vectors pseudotyped with VSV-G were efficiently produced upon triple cotransfection of respective plasmids and vector titers of $4 \times 10^4$ IU/ml or more were measured in either one of the target cells. Transfer of the SIVagm-derived packaging construct S11b into target cells exposed to SIVagm-derived vector particles was not observed, as tested by immunostaining of the respective targeting cells using an anti-serum of a SIVagm-infected pig-tailed macaque able to detect SIVagm-Gag (Nem 170). This indicated that the deletion in the psi-site was sufficient to avoid copackaging into vector particles (data not shown). Absence of replication-

**FIG. 1.** Components of the SIVagm3mc-derived packaging system and envelope constructs. The genome structure of SIVagm3mc (GenBank Accession No. M30931) is given for comparison.

<table>
<thead>
<tr>
<th>Vector particle</th>
<th>Transfer vector</th>
<th>293 (human kidney)</th>
<th>D17 (canine osteosarcoma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[SIVagm(VSV-G)]</td>
<td>SIVlacZ (pS19-lacZ)</td>
<td>$9.2 \times 10^4; 7.9 \times 10^4$</td>
<td>$5.0 \times 10^4; 3.7 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>HIVlacZ (pHR'CMV-lacZ)</td>
<td>$8.0 \times 10^4; 5.0 \times 10^4$</td>
<td>$5.7 \times 10^4; 4.0 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>MLVlacZ (pHIT110)</td>
<td>$&lt;10; &lt;10$</td>
<td>$&lt;10; &lt;10$</td>
</tr>
<tr>
<td>[HIV(VSV-G)]</td>
<td>SIVlacZ</td>
<td>$1.5 \times 10^4; 2.4 \times 10^4$</td>
<td>$3.1 \times 10^4; 1.6 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>HIVlacZ</td>
<td>$4.4 \times 10^5; 1.4 \times 10^5$</td>
<td>$9.8 \times 10^5; 2.1 \times 10^5$</td>
</tr>
<tr>
<td></td>
<td>MLVlacZ</td>
<td>$&lt;10; &lt;10$</td>
<td>$&lt;10; &lt;10$</td>
</tr>
<tr>
<td>[MLV(VSV-G)]</td>
<td>SIVlacZ</td>
<td>$1.1 \times 10^5; 9.1 \times 10^5$</td>
<td>$3.8 \times 10^5; 1.6 \times 10^5$</td>
</tr>
</tbody>
</table>

* Results from two independently performed experiments.
competent viruses in vector preparations was further confirmed by the lack of reverse transcriptase (RT) activity in C8166 T cells, as determined with an assay specific for lentiviral RTs (Cavidi Tech, Uppsala, Sweden) for 4 weeks of cultivation after transduction with both SIVagm- and HIV-1-based pseudotype vectors (data not shown). Replication of SIVagm was used as a positive control with an m.o.i. of 0.001 was readily detected. HIV-1-derived vectors reached vector titers about five times higher than those of SIVagm-based vectors. Interestingly, cross-packaging of SIVagm- and HIV-1-derived transfer vectors by the respective heterologous core particles was observed (Table 1). Whereas the packaging of HIV-1-transfer vectors into SIVagm core particles seemed to be inefficient, resulting in low vector titers, encapsidation of SIVagm-derived vector genomes by HIV-1 core particles led to high-titer vector preparations, indicating efficient cross-packaging. This capability of vector components derived from SIVagm and HIV-1 to allow formation of RNA pseudotypes is consistent with the observed cross-packaging between SIVmac and HIV-1 (4). The use of HIV-1-derived packaging constructs and transfer vectors derived from apathogenic SIVagm3mc may prove suitable for the generation of lentiviral vectors with potentially increased safety in humans, since the sequence homology between these two vector components is further minimised and makes recombination and generation of replication competent lentiviruses less likely. In addition, it is feasible to assume that cross-packaging of SIVagm-derived vector genomes will lead to a further enhanced inhibitory effect on HIV replication due to a TAR and RRE decoy effect when used to transduce HIV-1-infected cells (16). Therefore, this novel vector system may especially contribute to future gene therapy strategies against HIV-1 infection and AIDS.

**SIVagm3 Vectors Efficiently Transduce Growth-Arrested Cells.** To test the capability of the SIVagm3mc-derived vectors to transduce growth-arrested cells, transduction experiments were performed using proliferating and growth-arrested human target cells in parallel. Vector stocks were therefore generated as described and titrated in 293 cells. To achieve comparable transduction efficiencies, vector stocks were adjusted to 1 × 105 IU/ml and again titrated in human 293 kidney cells using serial dilutions. Growth arrest of target cells was achieved by seeding cells 24 h prior to infection in standard culture media containing 20 μg/ml of the DNA polymerase inhibitor aphidicolin. Growth arrest in G1/S of more than 97% of the cells was confirmed by FACS analysis after propidium iodide staining as described elsewhere (data not shown; Ref. 17). Two days posttransduction, LacZ-positive cells were visualised by X-Gal staining (Table 2). As expected, MLV-derived vectors were not capable of transducing aphidicolin-treated cells. In contrast, SIVagm- and HIV-1-derived vector particles mediated gene transfer into growth-arrested cells with comparable efficiencies. Obtained transduction efficiencies in growth-arrested cells ranged between 20 and 40% for the SIVagm-derived vectors and 40 and 80% for the HIV-1-based vector particles, compared to the respective titers in proliferating target cells. These results clearly demonstrate the ability of vector particles derived from apathogenic SIVagm3mc to enable the transduction of growth-arrested human cells. This property is believed to be crucial for successful gene delivery in vivo in future gene therapy applications (15).

**SIVagm Vectors are Capable of Transduction of Neuronal and Glial Cells In Vivo.** Adult female Fischer 344 rats (n = 5, injection sites n = 10) were anaesthetised (44 mg/kg ketamine, 0.75 mg/kg acepromazine, 4mg/kg xylazine, in 0.9% NaCl ip) and 3 μl of an [SIVagm(VSV-G)] vector preparation generated by cotransfection of 293T cells with pS11b, pS19-lacZ, and pMD-G followed by concentration via ultracentrifugation to 3 × 108 IU/ml was injected into the striatum (AP + 0.2, ML ± 3.5, DV − 4.5) bilaterally using a 5-μl Hamilton syringe (15, 18, 19). After 4 weeks, animals were sacrificed and analysed for β-Gal-positive cells in the striatum (Fig. 2). All adult rat brain injection sites showed expression of the transgene. No inflammatory reaction was noticed. Immunofluorescent staining with a rabbit polyclonal antibody against β-Gal revealed transduction of different cell types of the central nervous system. Counterstaining for cell-specific markers, such as NeuN for terminally differentiated neurons, GFAP for astrocytes, and RFP for oligodendrocytes, showed that the vectors transduced neu-
rons (Figs. 2A1–A3), astrocytes (Figs. 2B1–B3), and oligodendrocytes (Fig. 2C1–C3). The capability of SIVagm(VSV-G) vectors to transduce a variety of cells in the adult rat brain was thus demonstrated and is in accordance with previous findings using other lentiviral vectors (18, 20, 21).

SIVagm Vectors Are Capable of Selectively Transducing CD4⁺ Cells Upon Pseudotyping with Variants of the Homologous and Heterologous Lentiviral Envelope Glycoproteins. HIV-1-derived vectors have been designed to transfer therapeutic genes selectively into human cell types permissive for HIV-1 infection (16). Such HIV-1 vector particles use their natural envelope glycoproteins for entry and thus display the CD4-dependent cell tropism known from wild-type HIV-1. Although the resulting vector titers are relatively low compared to those of lentiviral vectors pseudotyped with the VSV-G envelope, these vectors may prove useful for the establishment of an in vivo gene therapy against HIV-1 infection and AIDS, as selective delivery of therapeutic genes into the host

FIG. 2. Transduction of neuronal and glial cells in vivo. High magnifications of striatal injection sites are shown, presenting β-Gal-expressing cells following lentiviral gene transfer. Staining with NeuN for terminally differentiated neurons (A3, Cy3 = red) and β-Gal (A2, FITC = green) showed overlap in striatal medium spiny neurons (A1). GFAP for astrocytes (B3, Cy3 = red) and β-Gal staining (B2, FITC = green) revealed coexpression in astrocytes (B1). RIP for oligodendrocytes (C3) and β-Gal staining (C2, FITC = green) showed oligodendrocytes stably expressing the transgene (C1). The overlay in the first row projected double labeling in a single confocal scanned area (A1, B1, C1).
tropic HIV-1-strain mediating specific infection of CD4
lope construct Tr712 has been derived from a T-cell
ing a similarly modified envelope derived from HIV-1
titers. As SIVagm is only poorly neutralised by sera from
very likely that this will have a negative impact on vector
genes interfere with the viral vector components, it is
epitopes, respectively. Assuming that these inhibitory
(scFv) directed against conserved HIV-1 sequences or
are transferred that inhibit the replication of HIV-1 , such
the very likely further decreased vector titer when genes
HIV-1-specific antibodies in HIV-1-infected patients and
these vectors may be the expected fast neutralisation by
HIV-1 , such inhibitory effects are not expected using
SIVagm-derived vector particles displaying the homolo-
gous envelope glycoproteins. We therefore wanted to
test the possibility of generating SIVagm-derived vectors
displaying their natural CD4-dependent tropism. Thus,
293T cells were cotransfected with the packaging con-
struct S11b, the plasmid pS19-lacZ harbouring the transfer-
vector, and the envelope construct Rep Δ10 env (Fig.
1) encoding a C-terminally truncated variant of the trans-
membrane protein and the full-length surface envelope
glycoprotein of SIVagm3mc (22). The resulting [SIV(SIV)]
 vectors were expected to mediate cell entry into CD4+/CCR5+
and CD4+/CXCR4+ cells. [HIV(HIV)] vectors bear-
ing a similarly modified envelope derived from HIV-1
(designated as Tr712; Ref. 23) served as controls. Enve-
lope construct Tr712 has been derived from a T-cell
 tropic HIV-1-strain mediating specific infection of CD4+/CXCR4+ cells. Furthermore, hybrid vectors [HIV(SIV)] and
[SIV(HIV)] were generated by cotransfection of 293T cells with pCMVΔR8.2, pHRCMV-lacZ, and pRepΔ10env, or with pS11b, pS19-lacZ, and Tr712, respectively. The cell-
free supernatants of the transfected 293T cells were
subsequently used to transduce derivatives of the megaglioma cell line U87 expressing human CD4 and
one of the coreceptors CCR5 or CXCR4 (24), respectively.
X-Gal staining performed 2 days posttransduction re-
vealed selective and efficient transduction of the CD4/
CCR5-expressing target cells by SIVagm-derived vectors and
the [HIV(SIV)] hybrid vectors, as indicated in Table 3.
In contrast, only CD4+/CXCR4+ cells were transduced by
[HIV(HIV)] vectors or [SIV(HIV)] hybrid vectors, both dis-
playing the envelope glycoprotein variants of T-cell tropic
HIV-1. The titers of up to 1.6 × 10^4 IU/ml obtained for the
[SIV(SIV)] or [HIV(HIV)] vectors were comparable to those
mentioned in previous reports (25). The titers of the
respective [SIV(HIV)] and [HIV(SIV)] vectors were about
5 × 10^3 to 4 × 10^3 IU/ml and thus lower but sufficient for
the determination of the respective coreceptor usage.
The 10-fold lower titer of [HIV(SIV)] vectors detected in
CD4+/CXCR4+ U87 cells was higher than expected. In
summary, these observations showed that selective
gene transfer into human CD4+/CCR5+ cells is achieved
using [SIV(SIV)] vectors carrying the envelope glycopro-
teins of SIVagm3mc. To exclude rapid generation of rep-
ication-competent virus during vector preparation, an
assay similar to that described above was performed.
Following transduction of C8166 T cells with each of the
vectors listed in Table 3 and subsequent cultivation of
the respective cells for another 4 weeks, no RT activity
was detected (data not shown). Thus, using such enve-
lope mutants, these vectors derived from apathogenic
SIVagm3mc should be able to circumvent the major
drawbacks of HIV-1-based vectors.

Materials and Methods. Vector components. Expression
constructs used for the generation of vectors are shown in Fig. 1. Details of the construction are available
from the authors upon request. Briefly, the packaging
construct S11b encompasses the functional genes gag/
pol, vif, vpx, tat, and rev. The env gene was truncated to
a final extent of only 1035 nt of originally 2631 nt by
inserting a stop codon at nt position 6809. The putative
packaging signal psi (Ψ) was removed by deleting a
19-nt fragment (nt 334–353). The 5’LTR was replaced by
a CMV promoter, whereas the 3’LTR was substituted by
the polyadenylation signal (pA) of the bovine growth
hormone gene (BGH). The transfer vector S19-lacZ con-
tains the flanking LTRs of SIVagm3mc, the rev-respon-
sive element (RRE), and the splice donor (SD) and ac-
ceptor (SA) of SIVagm3mc, as indicated. The first 1559 nt

<table>
<thead>
<tr>
<th>Vector particle</th>
<th>Transfer vector</th>
<th>Envelope construct</th>
<th>CD4+/CCR5+</th>
<th>CD4+/CXCR4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>[SIVagm(SIVagm)]</td>
<td>SIVlacZ</td>
<td>pRepSIVagmΔ10env</td>
<td>7.7 × 10^3; 7.7 × 10^3</td>
<td>&lt;10; &lt;10</td>
</tr>
<tr>
<td>[HIV(HIV)]</td>
<td>HIVlacZ</td>
<td>pHIV-1 Tr712</td>
<td>&lt;10; &lt;10</td>
<td>1.2 × 10^4; 1.6 × 10^4</td>
</tr>
<tr>
<td>[SIVagm(HIV)]</td>
<td>SIVlacZ</td>
<td>pHIV-1 Tr712</td>
<td>&lt;10; &lt;10</td>
<td>4.7 × 10^3; 5.9 × 10^3</td>
</tr>
<tr>
<td>[HIV(SIVagm)]</td>
<td>HIVlacZ</td>
<td>pRepSIVagmΔ10env</td>
<td>4.2 × 10^3; 5.2 × 10^3</td>
<td>5.8 × 10^3; 3.5 × 10^3</td>
</tr>
</tbody>
</table>

* Results from two independently performed experiments.
of the gag gene were included but transcription was abrogated by deleting the start codon. The lacZ reporter gene is driven by a CMV promoter. The envelope construct pMD-G was used to pseudotype all vector particles described here with VSV-G. The envelope constructs pTr712 and pRepSilVagmΔ10env have been previously described (22) and encompass truncated variants of the envelope genes derived from T-cell tropic HIV-1 and SilVagm3mc driven by the human β-actin promoter and the 3’LTR of Rous sarcoma virus (RSV), respectively.

Generation of Vector Particles and Infection of Target Cells. SilVagm3-derived constructs were designed to allow generation of the vectors by cotransfection of three plasmids into 293T cells. The transfer vector harbouring the reporter gene lacZ, the packaging construct encompassing the lentiviral genes (gag/pol, vif, vpx, tat, and rev), and the VSV-G encoding gene were located on three separate plasmids (Fig. 1). Packaging constructs and transfer vectors derived from HIV-1 (pCMVΔR8.2 and pHR’CMV-lacZ; ref. 15) and MLV (pHIT60 and pHIT110; Ref. 26) served as controls. Vector particles were generated by lipofection of 5 × 10^5 293T cells using the reagent Lipofectamin P (Gibco, Eggenstein, Germany) according to the manufacturer’s instructions. Forty-eight hours posttransfection cell culture supernatants were harvested by passing through a 0.45-μm filter and subsequently used in various dilutions to infect 2 × 10^5 target cells in a total volume of 1 ml. After 2 h of coincubation of cells and vector particles, target cells were washed and expanded. All cells were cultured using DMEM (Gibco) supplemented with 10% fetal calf serum (FCS, Seromed, Berlin, Germany). Determination of vector titers was performed by X-Gal assay and counting of β-Gal-positive cells.

Analysis of Rat Brains for Expression of β-Galactosidase. Animals were perfused intracardially with saline, 4% paraformaldehyde, and 0.2% glutaraldehyde. The brains were removed, postfixed, and saturated in 30% sucrose. Brains were frozen sectioned on a sliding microtome into 50-μm slices. Primary antibodies (Ab) used for immunohistochemistry were incubated overnight at 4°C in the following dilution: rabbit AB against β-galactosidase (Cortex, 1:10,000), mouse Ab against NeuN detecting terminally differentiated neurons (Chemi-Con, 1:500), guinea pig Ab (Advanced Immunocchemicals, 1:250), or mouse Ab against GFAP (Chemi-Con, 1:1000) detecting astrocytes and mouse Ab against Rip-detecting oligodendrocytes (Chemi-Con, 1:20,000). Corresponding fluorescent-coupled secondary antibodies (Cy3, FITC, Jackson, 1:250) were used and nuclear staining was achieved by diaminodino-2-phenylindole (Molecular Probes). The sections were analysed by confocal scanning laser microscopy (Leica). The signals were collected, digitally colour enhanced, and superimposed with Photoshop 5.0 (Adobe Photoshop).

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