Gene Transfer Using Recombinant Rabbit Hemorrhagic Disease Virus Capsids with Genetically Modified DNA Encapsulation Capacity by Addition of Packaging Sequences from the L1 or L2 Protein of Human Papillomavirus Type 16

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The aim of this study was to produce gene transfer vectors consisting of plasmid DNA packaged into virus-like particles (VLPs) with different cell tropisms. For this purpose, we have fused the N-terminally truncated VP60 capsid protein of the rabbit hemorrhagic disease virus (RHDV) with sequences which are expected to be sufficient to confer DNA packaging and gene transfer properties to the chimeric VLPs. Each of the two putative DNA-binding sequences of major L1 and minor L2 capsid proteins of human papillomavirus type 16 (HPV-16) were fused at the N terminus of the truncated VP60 protein. The two recombinant chimeric proteins expressed in insect cells self-assembled into VLPs similar in size and appearance to authentic RHDV virions. The chimeric proteins had acquired the ability to bind DNA. The two chimeric VLPs were therefore able to package plasmid DNA. However, only the chimeric VLPs containing the DNA packaging signal of the L1 protein were able efficiently to transfer genes into Cos-7 cells at a rate similar to that observed with papillomavirus L1 VLPs. It was possible to transfet only a very limited number of RK13 rabbit cells with the chimeric RHDV capsids containing the L2-binding sequence. The chimeric RHDV capsids containing the L1-binding sequence transfer genes into rabbit and hare cells at a higher rate than do HPV-16 L1 VLPs. However, no gene transfer was observed in human cell lines. The findings of this study demonstrate that the insertion of a DNA packaging sequence into a VLP which is not able to encapsidate DNA transforms this capsid into an artificial virus that could be used as a gene transfer vector. This possibility opens the way to designing new vectors with different cell tropisms by inserting such DNA packaging sequences into the major capsid proteins of other viruses.

One limitation of most viral vectors for gene therapy is the cell tropism of such systems, and it has therefore been reported that viral vectors, such as adenovirus vectors, are poorly transduced into target cells (19, 32). For some applications, the vector should ideally have the capacity to transfect a wide range of cells, and for other applications, it must be restricted to one target cell. To overcome these difficulties, modification of the virus genome by the introduction of novel tropism determinants has been investigated, with or without the deletion of endogenous tropism factors (2, 4). In addition to these viral vectors, the use of artificial virus vectors consisting of DNA packaged in vitro into recombinant virus-like particles (VLPs) was recently described as an alternative method for gene transfer (9, 25). By their nature, such vectors suffer from the same cell tropism limits as recombinant viruses. Modification of the cell tropism determinants of the VLPs is one solution. Another solution is the production of a range of VLP vectors with different cell tropisms. However, we have observed in preliminary studies that not all recombinant VLPs have the capacity to package and transfer DNA plasmids, such as the VP60 capsid of rabbit hemorrhagic disease virus (RHDV) (unpublished data).

RHDV is a member of the Caliciviridae family (14). Its genome is a 7.5-kb positive single-stranded RNA with a viral protein linked to its 5’ terminus (VPg) and a short poly(A) tail linked to its 3’ terminus (31). The nonenveloped icosahedral capsid demonstrated a symmetry of T=3 and is 35 nm in diameter (24). The particle is composed of 90 dimers of the VP60 capsid protein. RHDV cannot be propagated in cell culture and is responsible for a lethal acute viral disease in rabbits characterized by necrotic hepatitis and disseminated intravascular coagulation. Its major capsid protein, VP60, is able to self-assemble into VLPs when expressed in insect cells (11, 17, 22). The VP60 protein is not a DNA-binding protein, and it has been recently shown that the N-terminal 42 amino acids can be deleted without affecting its ability to form VLPs (S. Laurent et al., unpublished data).

In the present study we built synthetic gene vector systems using recombinant pseudoviruses composed of the major capsid protein of RHDV, self-assembled into VLPs in which each of the two putative DNA-binding domains of human papillomavirus type 16 (HPV-16) L1 and HPV-16 L2 proteins have been incorporated (L1BS and L2BS, respectively). The DNA-binding activity of these two domains is sequence independent. Papillomavirus contains two proteins, L1 and L2, which encapsidate a closed, circular, double-stranded DNA of about 8 kbp. The viral capsid of 50 to 55 nm contains 72 pentamers of L1 centered on the vertices of a T=7 icosahedral lattice (1, 28). L2 is present at about 1/30 the abundance of L1 (10). The
major capsid protein L1 of HPV can self-assemble into VLPs (10, 20, 21, 26), and capsids consisting of L1 or L1 plus L2 proteins have the ability to transfer genes to a wide range of cell types (25). The two DNA-binding sequences used for the construction of these chimeric VLPs are derived from those identified on the two structural proteins of HPV-16. The first DNA-binding sequence used has been identified at the N-terminal end of the minor capsid protein, L2 (35). The second DNA-binding domain used was identified in a preliminary study at the C-terminal of the major capsid protein (L1) of HPV-16 (27). Moreover, it has been shown that a binding to bovine papillomavirus type 1 (BPV-1) L1 plus L2 VLPs is enhanced by the presence of a 120-bp DNA sequence located in the BPV-1 E1 gene (33).

The two chimeric capsids obtained, VP60Δ-L1BS and VP60A-L2BS, were investigated for DNA binding, DNA packaging, and their ability to deliver foreign DNA into a variety of cells with the subsequent expression of the encoded gene.

**MATERIALS AND METHODS**

**Generation of RDHV–HPV-16 L1 or L2 recombinant baculovirus.** The VP60 fragment (VP60a) was first amplified from a plasmid carrying the VP60 gene (14) by PCR, the upper primer containing the 5′ end of the L1 (CCGAAAAGGTCAGTGTCAGACGAGAAGTCATCTAGCAGAT) or L2 (GCCAACCGACAAAGGTCTGACGAGAATCTCTACAACGCAGATC) DNA-binding domain and with a lower primer containing a HindIII restriction site (CTCGAG ATTG). A chimeric plasmid containing the C-terminal end of the L1 (CCGAAAAGGTCAGTGTCAGACGAGAAGTCATCTAGCAGAT) or L2 (GCCAACCGACAAAGGTCTGACGAGAATCTCTACAACGCAGATC) DNA-binding domain and with a lower primer containing a HindIII restriction site (CTCGAG ATTG) or L2 (GCCAACCGACAAAGGTCTGACGAGAATCTCTACAACGCAGATC) DNA-binding domain and contain a BamHI restriction site. Amplification was performed with 0.7 μM concentrations of each primer and 1.25 IU of Taq polymerase (Life Technologies, Eggenstein, France). Following amplification, the PCR products were cloned into the pCRII Topo vector (Topo TA cloning, Invitrogen, San Diego, Calif.) and then subcloned into the pFast Bac vector (Baculo Technologies) after digestion with BamHI and HindIII restriction enzymes. Recombinant baculoviruses encoding VP60A-L1BS and VP60A-L2BS were generated using the Bac-to-Bac baculovirus expression system according to the manufacturer's instructions (Life Technologies).

**Generation of HPV-16 L1 and HPV-16 L1+L2 recombinant baculoviruses.** The HPV-16 L1 gene was first amplified from an HPV-16 DNA-positive biopsy (26) using primers containing BglII sites (upper primer, CCAGCATGTTCTCTTGCGGTATGGAGG, and lower primer, CCAGCATTTTACAGCTTCTGGTTTGTGCTTTT).

**Production and purification of chimeric VLPs.** St21 cells, maintained in Grace's insect medium supplemented with 10% fetal calf serum (FCS), were infected with the different recombinant baculoviruses at a multiplicity of infection in the presence of 5- bromo-4-choloro-3-indolylphosphate (BCIP) and nitroblue tetrazolium (NBT; Sigma Aldrich). After 3 days of infection, the BCIP and NBT solutions were washed three times with PBS. Then 100 μl of PBS and 100 μl of 1× lysis reagent were added. Cell lysate was harvested after 30 min of incubation at room temperature, and luciferase was measured on a VICTOR II (Wallac) plate reader at 460 nm. The luciferase (luc) activity was expressed as counts per second per milligram of cell protein (micro-BCA assay; Pierce).

Cell binding was evaluated by the capacity of the VLPs to inhibit gene transfer
The electron micrographs were taken at a magnification of 30,000 or 50,000.

Analysis of transfection efficiency was performed by comparing the means of blue cells observed or the means of luciferase counts per second/per milligram by the F test using EPI-Info 6.04c(US) software. Blue cells observed or the means of luciferase counts per second/per milligram by PBS was considered 100 and 0% of inhibition, respectively.


d-D-L1BS pseudovirions to incubation of cells with the plasmid alone or with VP60

Results were expressed as the percent inhibition of the gene transfer activity. Luciferase activity corresponding different VLPs before being added to the cells. Results were expressed as the percent inhibition of the gene transfer activity. Luciferase activity corresponding to incubation of cells with the plasmid alone or with VP60-L1BS pseudovirions were mixed with a 20-fold excess of D-L1BS proteins was generated using the Bac-to-Bac baculovirus expression system. The two chimeric proteins VP60-L1BS strongly bound DNA.

In order to test VLP subcellular localization, the insect cells were fixed, sectioned, and embedded in Epon resin. Ultrathin sections were then made and stained with uranyl acetate and lead citrate. Analysis of the presence of VLPs by electron microscopy showed that the two VP60 chimeric proteins self-assembled into VLPs (Fig. 4). As expected, the VP60Δ VLPs were found in the cytoplasm of infected cells (Fig. 4a and b). However, VP60Δ-L1BS and VP60Δ-L2BS recombinant VLPs were detected in the nucleus (Fig. 4c and d). In addition, the amount of VLPs was titrated by enzyme-linked immunosorbent assay using anti-VP60 monoclonal antibody, in both the nuclear and cytoplasmic fractions of Sf21-infected cells. For VP60Δ, VP60Δ-L1BS, and VP60Δ-L2BS, the cytoplasm fractions contained 80, 25, and 24% of the total VLP reactivity, respectively. Accordingly, the nuclear fractions contained 20, 75, and 76% of the VLPs, respectively.

The different VLPs were purified by CsCl gradient ultracentrifugation and observed by electron microscopy after negative staining. These VLPs were similar in size and appearance to authentic RHDV virions (Fig. 5). Some tubular structures and aggregates could also be observed (data not shown). In addition, Fig. 5f shows VLPs obtained by expression of a recombinant baculovirus coding for both HPV-16 L1 and L2 proteins. These VLPs were more regular in size and shape than HPV-16 L1 VLPs (Fig. 5c).

### RESULTS

The short DNA-binding sequences of HPV-16 L1 or L2 were fused by using PCR at the N-terminal extremity of a truncated RHDV-VP60 protein sequence and then cloned into a pFast-BacI vector. Recombinant baculovirus encoding the VP60Δ-L1BS or the VP60Δ-L2BS proteins was generated using the Bac-to-Bac baculovirus expression system. The two chimeric VLPs consisting of the truncated VP60 capsid protein of RHDV with the addition of one of the two DNA-binding domains of the capsid proteins of HPV-16 (Fig. 1) were expressed in Sf21 insect cells by using the recombinant baculovirus. Three days postinfection, corresponding to the maximal level of expression, the cytoplasmic and nuclear fractions of infected cells were analyzed by SDS-PAGE and immunoblotting using an anti-VP60 monoclonal antibody. A band of around 60 kDa, corresponding to the expected size of the VP60Δ protein, was found, as expected, in the cytoplasmic fraction of infected cells. A band of similar size was identified for both VP60Δ-L1BS and VP60Δ-L2BS, but it was in the nuclear fraction of infected cells (Fig. 2). The DNA-binding capacity of the different proteins was investigated by Southwestern blotting using a digoxigenin-labeled DNA plasmid.
Packaging and gene transfer capacity of the RHDV–HPV-16 chimeric VLPs. To explore the potential of the VLPs for the packaging of exogenous DNA we investigated whether the VLPs were dissociable and whether reassociation could be initiated to reconstitute intact VLPs. The principle of dissociation is the removal of Ca\(^{2+}\) ions and reduction of disulfide bonds. As shown previously for HPV-16 L1 VLPs (25), it was possible to disassemble RHDV capsids as the two chimeric RHDV-HPV VLPs. Figure 6 shows that under the conditions used, VLPs (Fig. 6a) were completely disassembled into structures resembling capsomers (Fig. 6b). Intact VLPs could then be reconstituted during a multistep reaction by increasing the concentration of calcium and decreasing the concentration of the reducing agent. To investigate whether foreign DNA could be packaged into VLPs, plasmid DNA was added to capsomers obtained after dissociation of the VLPs, and the preparation was then diluted in a buffer containing 5 mM CaCl\(_2\) and 1% DMSO in order to refold the VLPs (Fig. 6c). About 30 to 50% of the capsomers seemed to reassemble into VLPs.

To demonstrate that plasmid DNA was packaged into VLPs, reconstituted VLPs were treated with DNase. After disassembly of the VLPs and reassembly in the presence of DNA, the mixture was treated with Benzonase. The detection of DNA after treatment is indicative of its uptake by VLPs. As shown in Fig. 7, VP60\(\Delta\)-L1BS and VP60\(\Delta\)-L2BS VLPs, but not VP60\(\Delta\) VLPs, are able to protect pCMV-\(\beta\) plasmid DNA from digestion. DNA protection was estimated to be 12% for DNA introduced during the reassociation of the VP60\(\Delta\)-L1BS VLPs (Table 1), compared to 18% observed when DNA was packaged in HPV-16 L1 VLPs. A lower level of protection from Benzonase degradation (9.5%) was observed with the VP60\(\Delta\)-L2BS VLPs.

Transfer efficiency of the pCMV-\(\beta\) plasmid packaged into the different VLPs was initially investigated in Cos-7 cells. As shown in Fig. 8, the VP60\(\Delta\)-L1BS VLPs and HPV-16 L1 VLPs can efficiently transduce packaged plasmid DNA into cells, resulting in the expression of a functional \(\beta\)-galactosidase as demonstrated by the presence of blue cells after addition of 5-bromo-4-chloro-3-indolyl-\(\beta\)-D-galactopyranoside (X-Gal). The results indicate that VP60\(\Delta\) and VP60\(\Delta\)-L2BS VLPs could not transfer genes into Cos-7 cells. As we had previously demonstrated that many cell lines could be transfected with
HPV-16 VLPs, we investigated 10 cell lines for their capacity to be transfected by the pCMV-β plasmid packaged in HPV-16 L1, VP60Δ-L1BS, and VP60Δ-L2BS VLPs. The results (Table 2) indicate that all cell lines investigated could be transfected by the HPV-16 L1 VLP vector. VP60Δ-L1BS VLPs were not able to transfect human cell lines or L929 rodent cells. However, it must be noted that rabbit RK13 and hare R17 cell lines are efficiently transfected with VP60Δ-L1BS VLPs. The level of transfection in these two cell lines was higher than that observed with HPV-16 L1 VLPs, as demonstrated by the observation of a higher number of blue cells (Table 2). Transfection with the VP60Δ-L2BS vector gave no transfer in the different cell lines tested, with the exception of a very limited level of gene transfer into the rabbit RK13 cell line.

It has been shown that the presence of L2 in HPV VLPs dramatically increased their gene transfer efficiency (29) and

FIG. 5. Identification of chimeric RHDV and HPV-16 VLPs by transmission electron microscopy. (a) VP60; (b) VP60Δ; (c) VP60Δ-L1BS; (d) VP60Δ-L2BS; (e) HPV-16 L1; (f) HPV-16 L1+L2. Bar, 100 nm.
that packaging with BPV-1 L1+L2 VLPs is increased in the presence of a papillomavirus DNA sequence (33), suggesting that specific DNA binding to L2 is necessary to obtain efficient packaging. We therefore investigated gene transfer into RK13 cells using VLPs consisting of HPV-16 L1, HPV-16 L1+L2, VP60Δ-L1BS, or VP60Δ-L2BS proteins in which the pCMV-β plasmid or the same plasmid containing the BPV EPS was packaged (Table 3). No transfection of RK13 cells was observed with VP60Δ-L2BS VLP vectors, whatever the plasmid used. However, when L1 VLPs, L1+L2 VLPs, or VP60Δ-L1BS VLPs were used, a two- to threefold increase in the level of gene transfer was observed when the packaged plasmid contained the BPV EPS in comparison to the level obtained with a similar plasmid without this DNA sequence (P = 0.0005, 0.0015, and 0.04, respectively).

In addition, the number of transfected cells increased from 380 with L1 VLPs to 1,130 with L1+L2 VLPs (P = 0.0001) when the pCMVβ plasmid was used and from 915 to 2,875 (P < 0.01) when the pCMVβ/BPV-1-EPS plasmid was used. These results were confirmed using the pCMV-Luc plasmid. In experiments using RK13 cells, luciferase activity of (1.2 ± 0.3) × 10^4 and (1.5 ± 0.1) × 10^6 cps/mg was observed with VP60Δ-L2BS pseudovirions and plasmid DNA alone, respectively. With L1 VLPs, L1+L2 VLPs, and VP60Δ-L1BS VLPs, the results were (7 ± 1.3) × 10^6, (2 ± 0.8) × 10^7, and (1.2 ± 0.2) × 10^7 cps/mg, respectively.

In order to investigate the capacity of VP60Δ-L2BS VLPs to bind to RK13 cells, VP60Δ-L1BS pseudovirions were incubated with a 20-fold excess of L1, VP60Δ-L1BS, and VP60Δ-L2BS VLPs and then added to the cells (Table 4). Levels of inhibition of the luciferase gene transfer were 95 and 98% when VP60Δ-L1BS pseudovirions were mixed with VP60Δ-L1BS and L1 VLPs, respectively. However, only 2% inhibition was detected when VP60Δ-L2BS VLPs were used as competitors.

**DISCUSSION**

We have shown in this study that the insertion of one of the DNA-binding sequences present in the two structural proteins of HPV-16 at the N-terminal extremity of the RHDV VP60 gene is able to transform this protein into a DNA-binding protein. Moreover, the chimeric VP60 proteins conserved their ability to form VLPs that are similar in size and morphology to authentic virions.

<table>
<thead>
<tr>
<th>VLP</th>
<th>Nuclear localization</th>
<th>DNA binding</th>
<th>DNA protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHDV VP60</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>HPV-16 L1</td>
<td>+</td>
<td>+</td>
<td>18</td>
</tr>
<tr>
<td>VP60Δ-L1BS</td>
<td>+</td>
<td>+</td>
<td>12</td>
</tr>
<tr>
<td>VP60Δ-L2BS</td>
<td>+</td>
<td>+</td>
<td>9.5</td>
</tr>
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</table>
plasm of infected cells, as observed for the full-length VP60 protein (18). The chimeric VP60Δ-L1BS and VP60Δ-L2BS VLPs still formed VLPs but were found in the nuclei of infected cells. This confirms the observation of Zhou et al. (34) that the 11 C-terminal amino acids of the HPV-16 L1 protein

**FIG. 8.** Demonstration of gene expression of β-galactosidase in Cos-7 (a and b), R17 (c), HuH-7 (d), and CaCo2 (e and f) cells after gene transfer using VP60Δ-L1BS (a, c, d, and e) or HPV-16 L1 (b and f) capsids. Transfected cells expressing β-galactosidase were stained with X-Gal.

**TABLE 2.** Efficacy of gene transfer in 10 cell lines using chimeric HPV-16–RHDV VLPs

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Gene transfer (no. of blue cells/well) obtained with:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>VP60Δ-L1BS</td>
</tr>
<tr>
<td>RK13</td>
<td>416</td>
</tr>
<tr>
<td>R17</td>
<td>757</td>
</tr>
<tr>
<td>L929</td>
<td>0</td>
</tr>
<tr>
<td>CHO</td>
<td>0</td>
</tr>
<tr>
<td>Cos-7</td>
<td>3,200</td>
</tr>
<tr>
<td>HeLa</td>
<td>0</td>
</tr>
<tr>
<td>HepG2</td>
<td>0</td>
</tr>
<tr>
<td>CaCo2</td>
<td>2</td>
</tr>
<tr>
<td>HuH-7</td>
<td>0</td>
</tr>
<tr>
<td>MRC5</td>
<td>0</td>
</tr>
</tbody>
</table>

**TABLE 3.** β-Galactosidase gene transfer into RK13 cells using plasmids with or without the BPV-1 EPS

<table>
<thead>
<tr>
<th>VLP</th>
<th>Gene transfer (no. of blue cells/well) obtained with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pCMV-β</td>
</tr>
<tr>
<td>L1</td>
<td>380 ± 42</td>
</tr>
<tr>
<td>L1+L2</td>
<td>1,130 ± 155</td>
</tr>
<tr>
<td>VP60Δ-L1BS</td>
<td>680 ± 183</td>
</tr>
<tr>
<td>VP60Δ-L2BS</td>
<td>0</td>
</tr>
</tbody>
</table>
TABLE 4. Inhibition of luciferase gene transfer in RK13 cells mediated by VP60δ-L1BS pseudovirions

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Luciferase activity (cts/mg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>1.0 x 10^7</td>
<td>0</td>
</tr>
<tr>
<td>VP60δ-L1BS</td>
<td>5.0 x 10^7</td>
<td>95</td>
</tr>
<tr>
<td>L1</td>
<td>1.7 x 10^7</td>
<td>98</td>
</tr>
<tr>
<td>VP60δ-L2BS</td>
<td>0.98 x 10^7</td>
<td>2</td>
</tr>
</tbody>
</table>

continued a strong nuclear localization signal. As VP60δ-L2BS VLPs also localized in the nuclei of infected cells, the 12 N-terminal amino acids of the HPV-16 L2 protein also contained a nuclear localization signal, in agreement with results observed for HPV-6 L2 (23).

We observed that VP60δ-L1BS and VP60δ-L2BS VLPs could be disassembled and reassembled under the same conditions as those used previously for HPV-16 L1 VLPs and to package a 7.2-kbp DNA plasmid. However, the DNA protection from enzymatic degradation was lower with VP60δ-L2BS VLPs than with VP60δ-L1BS VLPs. The results obtained are in agreement with the fact that truncation of the DNA-binding nuclear localization sequence of the HPV-16 L1 protein results in the loss of its DNA packaging capacities in VLP disassembly-reassembly experiments (27). A similar result was also observed for polyomavirus VLPs (7). These findings suggest that the addition of a short amino acid sequence containing a DNA-binding sequence is sufficient to confer on a VLP the capacity to encapsidate DNA. However, only the VP60δ-L1BS VLPs were able to efficiently transfer the gene into Cos-7 cells. It has recently been shown that DNA encapsidation by BPV-1 VLPs is enhanced by a specific DNA sequence of the papillomavirus genome (33). This 120-bp sequence, named EPS, was also recognized by HPV-6b VLPs despite the phylogenetic difference between these two papillomavirus types. Zhao et al. (33) have therefore suggested that other papillomaviruses may use the same packaging sequence. In order to test whether the low level of gene transfer observed with VP60δ-L2BS VLPs was due to the absence of a papillomavirus DNA sequence recognized by L2BS, we investigated gene transfer using plasmids with and without the EPS packaged into VLPs containing L1 or L2 binding sequences. Our results indicate that gene transfer was enhanced in the presence of the BPV EPS with capsids containing the L1BS protein. If no gene transfer was observed with VP60δ-L2BS VLPs whatever the plasmid used, an increase in DNA packaging was observed with the plasmid containing the BPV EPS (data not shown). This confirmed that the EPS is not papillomavirus type specific as suggested by Zhao et al. (33) and that the BPV EPS is recognized by both L1BS and L2BS. In addition, the results suggested that the low level of gene transfer observed with VP60δ-L2BS capsids is not a consequence of specificity of the DNA binding. The difference between the gene transfer capacities of VP60δ-L1BS and VP60δ-L2BS VLPs could be related to the presence of a heparin-binding sequence in the L1BS inserted at the N terminus of the RHDV VP60 protein (8). Its presence in VP60δ-L1BS might allow the interaction between VLPs and cells, thus favoring attachment to a cell receptor and internalization of the capsid. Inhibition experiments are in agreement with such hypotheses, since an absence of gene transfer inhibition was observed between VP60δ-L1BS pseudovirions and VP60δ-L2BS VLPs.

To investigate the cell tropism of the VP60δ-L1BS VLPs, we compared gene transfer in 10 cell lines to the transfer observed with HPV-16 VLPs. All cell lines investigated could be transfected with HPV-16 L1 VLPs. In addition to previously published findings (25), we transfected rabbit and hare kidney cells, HepG2 and HuH-7 human liver cells, and L929 mouse cells, confirming the presence of HPV-16 receptors on numerous cells. In contrast, no gene transfer was observed in human cell lines with VP60δ-L1BS VLPs. However, the results were better than those observed with HPV-16 VLPs in rabbit and hare kidney cells. This suggests that RHDV and HPV-16 cell receptors are different. Alpha-6 integrin has been identified as a cell receptor candidate for HPV-16 (5, 13), but no cell receptor has so far been identified for RHDV. Glycosaminoglycans (GAGs) have also been proposed as cell receptors for HPV-16, and virus sequences interacting with these cell components have been identified at the C terminus of the L1 protein (8). As this HPV-16 viral sequence was introduced into the VP60-L1BS, this could explain why gene transfer was much more efficient with VP60δ-L1BS VLPs than with VP60δ-L2BS VLPs. However, if GAGs act as the only cell receptor for HPV-16 VLPs, similar cell tropism would be observed with VP60δ-L1BS and L1VLPs. Our results thus support the hypothesis that proposes the presence of two cell receptors in HPV-16 VLPs, explaining a difference in cell tropism for HPV-16 VLPs and chimeric L1-RHDV capsids. This could also suggest that virus binding to cells is mediated by interaction between the virus and the cell GAGs followed by internalization of the capsid by a cell receptor, which is the alpha-6 integrin in the case of HPV-16 and is an unknown receptor for RHDV.

In conclusion, we demonstrated the possibility of producing recombinant chimeric VLPs by the addition of a DNA-binding sequence to the capsid protein of RHDV, a protein unable to bind DNA and transfer plasmid DNA by itself. These VLPs have a restricted cell tropism compared to that observed previously for HPV-16 L1 (25) or polyomavirus VP1 VLPs (6). This suggests the possibility of developing new vectors by producing chimeric VLPs based on different capsids with different DNA packaging capacities and different cell tropisms.

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when expressed in the baculovirus system. Virus Res. 58:149–160.