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Papillomavirus Capsid Binding and Uptake by Cells from Different Tissues and Species

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The inability of papillomaviruses (PV) to replicate in tissue culture cells has hampered the study of the PV life cycle. We investigated virus-cell interactions by the following two methods: (i) using purified bovine PV virions or human PV type 11 (HPV type 11) virus-like particles (VLP) to test the binding to eukaryotic cells and (ii) using different VLP-reporter plasmid complexes of HPV6b, HPV11 L1 or HPV11 L1/L2, and HPV16 L1 or HPV16 L1/L2 to study uptake of particles into different cell lines. Our studies showed that PV capsids bind to a broad range of cells in culture in a dose-dependent manner. Binding of PV capsids to cells can be blocked by pretreating the cells with the protease trypsin. Penetration of PV into cells was monitored by using complexes in which the purified PV capsids were physically linked to DNA containing the gene for β-galactosidase driven by the human cytomegalovirus promoter. Expression of β-galactosidase occurred in <1% of the cells, and the efficiency of PV receptor-mediated gene delivery was greatly enhanced (up to 10 to 20% positive cells) by the use of a replication-defective adenovirus which promotes endosomal lysis. The data generated by this approach further confirmed the results obtained from the binding assays, showing that PV enter a wide range of cells and that these cells have all functions required for the uptake of PV. Binding and uptake of PV particles can be blocked by PV-specific antisera, and different PV particles compete for particle uptake. Our results suggest that the PV receptor is a conserved cell surface molecule(s) used by different PV and that the tropism of infection by different PV is controlled by events downstream of the initial binding and uptake.

Papillomaviruses (PVs) are epitheliotropic double-stranded DNA viruses which induce benign proliferation of the epithelium and, in some instances, of fibroblasts. Infection of human cervical epithelial cells with certain human PVs (HPV), e.g., HPV types 16 or 18 (HPV16 or HPV18), is associated with the development of lesions which can progress to cervical cancer (39). The PV genome apparently utilizes the host cell machinery to replicate episomally in dividing stem cells (11), while infectious virions are assembled in differentiated epithelial cells (9). However, studying the PV life cycle has proved difficult, because the PV vegetative life cycle is linked to terminal differentiation of keratinocytes, and it has not yet proved possible to replicate the entire life cycle in cell culture (31).

To initiate infection, PVs are presumed, like other viruses, to attach to cells through interaction between the viral capsid and cell membrane components, i.e., the PV receptor. Since the PV capsids are composed of multiple identical units (the structural proteins L1 and L2), the virion very likely possesses the capacity to bind to its receptor through multivalent interactions. This could provide them with high-affinity binding properties either via few high-affinity interactions or multiple low-affinity interactions, as has been shown for adenovirus type 2 (36).

Very little is known about the nature of the PV receptor and mechanisms involved in virus entry into host cells. Bovine PV type 1 (BPV1) virions can enter a number of different types of cells, including epithelial cells and fibroblasts (for review, see reference 20). The range of cells which can be transformed in vitro by BPV1 is broader in terms of both species and tissue specificity than the range of cells that are permissive in vivo for vegetative replication, suggesting that the observed restriction of PV vegetative replication to epithelial cells is determined by cellular regulation of the viral transcription program by cellular factors, rather than by restriction of the PV receptor to a limited host cell range.

The PV L1 protein has the capacity to self-assemble into virus-like particles (VLPs) within intact cells in culture (15, 17, 27, 37, 38), and these can be purified for in vitro studies. Taking advantage of the availability of large quantities of PV capsids, we examined the details of the reaction of PV binding onto the surfaces of different cell lines by using purified PV capsids. The binding of viral particles to cells does not necessarily mean that the particles can be taken up by the cells, since many viruses need to interact with more than one cellular molecule for high-affinity binding and penetration (12, 28, 30, 36). Therefore, we tested if the PVs that are bound to a receptor can penetrate the cells by receptor-mediated endocytosis using a VLP-reporter plasmid complex in which the PV capsid was physically linked to a plasmid containing the *Escherichia coli lacZ* gene. Delivery of the reporter gene into the cells by the PV capsids could then be examined by in situ β-galactosidase (β-Gal) staining. Our results show that PVs can bind to and be internalized by a large range of cell lines in

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vitro and that different PVs seem to use the same receptor to enter their host cells.

MATERIALS AND METHODS

Cells and cell culture. The cell lines that were tested were obtained from the American Type Culture Collection and were grown in Dulbecco's modified Eagle's medium (BRL) with 10% fetal calf serum (FCS) (Sigma) at a density of 10^5 cells per ml at 37°C and with 5% CO_2. *Spodoptera frugiperda* Sf9 cells were cultivated using TNM-FH insect medium (Sigma) with 10% FCS at 27°C. K20 cells were derived from the human lymphoma B-cell line BJAB (16).

Construction of recombinant plasmids. Plasmid pVL11L1 (HPV11 L1) was constructed by PCR amplification of the L1 gene from a *Bam*HI-digested and religated HPV11 genomic clone using the following primers: 5′-AAA GAA TTC GAC GCC GTA AAC GTA TTC CC-3′ and 5′-AAA AGA TCT ACA ACA ACA TAT AAA T-3′. The amplified fragment was then ligated into the *Eco*RI and *Bgl*II sites of the transfer vector pVL1393 (Invitrogen). The plasmid pVL11L2 (HPV11 L2) was cloned using the primers 5′-AAA GAA TTC TTT ATA CAG TGT GTG GTG TA-3′ and 5′-AAA GGA TCC ACA GGG TTG GGA GGA GGC AC-3′ and was ligated into *Eco*RI and *Bgl*II of pVL1393. The HPV6b L1 open reading frame was cloned using primers 5′-CG CCC GGG TTA CCT TTT AGT TTG GCG CGC TTA CGT TTA GG-3′ and 5′-GC GGA TCC AGA TGT GGC GGC CTA GCG ACA GCA CAG TAT ATG-3′.

Production of baculovirus recombinants. *S. frugiperda* Sf9 cells were grown as suspension or monolayer cultures in TNM-FH insect medium (Sigma) with 10% FCS–2 mM glutamine at 27°C. For HPV6b and HPV11 recombinant baculovirus construction, 5 μg of plasmid was used to transfect Sf9 cells together with 5 μg of linearized Baculo-Gold DNA (Pharmingen, San Diego, Calif.). Recombinant viruses were purified by standard methods as suggested by the manufacturer. The HPV16 L1- and L1/L2-expressing baculovirus recombinants were gifts from R. Kirnbauer (18). For expression of PV capsid proteins, 10^6 Sf9 cells were infected with baculovirus recombinants at a multiplicity of infection of 5 to 10. After incubation, medium was removed, and the cells were washed with phosphate-buffered saline (PBS). The cells were then lysed in sodium dodecyl sulfate (SDS) sample buffer for SDS-polyacrylamide gel electrophoresis and immune blotting assays.

PV capsid purification. All VLPs were generated from baculovirus recombinants. For VLP purification, 500 ml of Sf9 cells (1×10^6 to 3×10^6 cells per ml) was infected with L1 (L1/L2) recombinant baculovirus. After incubation for 3 days, the cells were harvested and lysed in 10 ml of extraction buffer (1 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.], 150 mM NaCl, 50 mM $CaCl_2$, 10 mM $MgCl_2$) containing 2 mM phenylmethylsulfonyl fluoride. The lysate was then cleared by centrifugation at 10,000 × *g* for 10 min and enriched for particles by centrifugation through a 40% sucrose cushion (5 mM HEPES [pH 7.5], 10 mM $CaCl_2$, 10 mM $MgCl_2$) at 100,000 × *g* for 2 h. The pellet was resuspended in extraction buffer containing 31% CsCl and further purified by equilibrium centrifugation. VLPs were collected and examined by electron microscopy (Fig. 1). For the purification of BPV complete particles, cattle warts were minced and then extracted essentially in the way described for the PV VLPs.

Electron microscopy. Fractions of CsCl density gradients containing PV L1 proteins, which were demonstrated by immunoblots with appropriate antibodies, were dialyzed against 5 mM HEPES buffer (pH 7.4). Virus particles were stained with 2% aqueous uranyl acetate and examined in a Zeiss EM 10 electron microscope.

Binding assays. (i) Indirect binding assay. BPV particles (about 1.5×10^4 to 1.25×10^5 particles in 100 μl) of CsCl-purified BPV virions were mixed with suspensions of cells (2×10^5 to 1×10^6 cells per well) for 45 min at 37°C in a 96-well microtiter plate previously blocked with gelatin, in a total volume of 200 μl. Cells grown as monolayer were lifted from the culture flasks by EDTA treatment. Particles absorbed to cells were removed by low-speed centrifugation. Cell free-supernatant (100 μl) was collected and used in an antigen capture enzyme-linked immunosorbent assay (ELISA) to quantify the remaining BPV particles. For the ELISA, 96-well microtiter plates were coated with a 1:500 dilution of anti-BPV1 antibody AU2 (5) overnight in 0.1 M sodium carbonate buffer (pH 9.6). The plates were washed and blocked with 5% bovine serum albumin (BSA) in PBS. The plates were then incubated with different dilutions of BPV particles. After extensive washing, the plates were then incubated with a rabbit anti-BPV1 antiserum and then with anti-rabbit peroxidase-conjugated immunoglobulin G (Southern Biotechnology) at a 1:5,000 dilution. The plates were stained by adding 100 μl of ABTS [2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid)] staining solution (1 mg/ml in a 100 mM sodium acetate-phosphate buffer [pH 4.2] containing 0.015% H_2O_2). After color development, the A_{405} was measured in an automated ELISA reader. To examine whether trypsin pretreatment of cells could alter PV particle binding, cells were exposed to 0.1% trypsin for 5 min, and trypsin activity was neutralized by the addition of medium containing 10% FCS and then by repetitive washing of the cells with PBS. Binding results are expressed as a percentage of virions bound to each cell type. To establish BPV binding to each cell type, suspensions of BPV particles to which the cell type being tested or an equal volume of saline had been added were assayed for BPV protein after the cell with adsorbed virus had been removed by centrifugation. The background of the antigen capture ELISA was measured on antibody-coated wells without the addition of any BPV antigen and was subsequently subtracted from values of all other wells. The percentage of BPV binding to cells was then calculated as 100× (OD with saline − OD with cells)/OD with saline added, where OD is optical density.

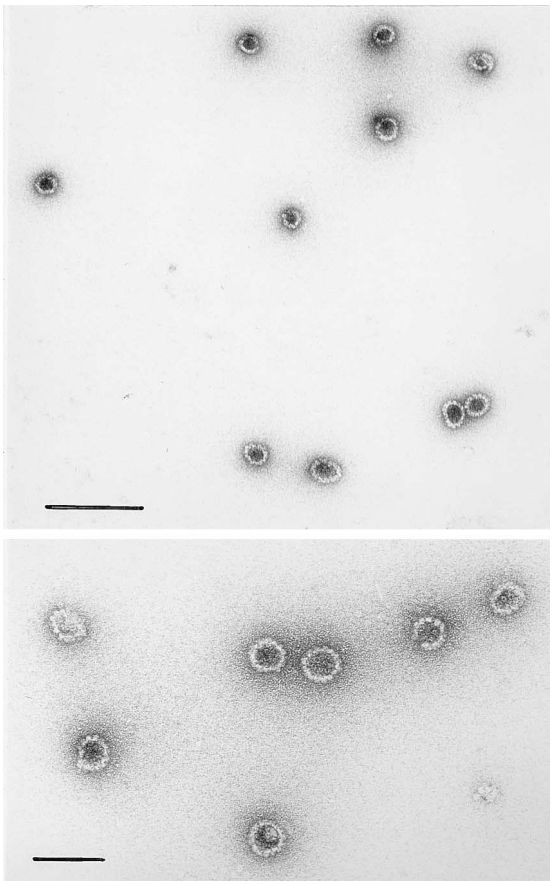

FIG. 1. Electron micrograph of HPV11 L1 VLP purified from recombinant baculovirus-infected cells. The bars in the upper and lower parts indicate lengths of 200 and 100 nm, respectively.

(ii) Direct binding assay. Cells grown in 96-well plates were fixed with ice-cold methanol-acetone (1:1). The plates were then washed six times with PBS–0.05% Tween 20 and incubated with HPV11 VLPs (about 0.1 μg/ml in 3% BSA–PBS–0.05% Tween 20) for 1 h at 37°C. The cells were then washed four times with PBS–0.05% Tween 20 and subsequently incubated with a monoclonal antibody (MAb), A3 (1:5,000), against HPV11 L1 (2). After washing, a goat horseradish peroxidase-conjugated anti-mouse immunoglobulin G (Southern Biotechnology) was added (1:3,000), and the amount of immunoglobulin G retained in wells was measured as described above.

Preparation of VLP-reporter plasmid complexes. The reporter plasmid (pNeoβ) was constructed as follows. The β-galactosidase (β-Gal) gene was isolated by *Not*I digestion from plasmid pSVβ (Clonetech) and was cloned into the *Not*I site downstream from the cytomegalovirus promoter of pCNDA3 (Invitrogen), which in addition contains a G418 resistance gene. The resulting plasmid (pNeoβ [8.9 kb]) was purified twice by CsCl gradient centrifugation.

Purified VLPs were coupled to polylysine according to previously described methods (8, 9). Briefly, 100 μg of PV capsids purified by CsCl gradient centrifugation was dialyzed against HBS buffer (150 mM NaCl, 20 mM HEPES [pH 7.3]) to remove CsCl. The PV capsids were then mixed with polylysine (molecular mass, 23.7 kDa; Sigma) with a final concentration of 150 μM in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) at 2 mM in 4 ml of HBS buffer. After incubation at 4°C overnight, the capsids were purified by CsCl gradient centrifugation to remove unreacted chemicals. The modified VLPs were then dialyzed against HBS before the addition of 10 μg of plasmid DNA to approximately 25 μg of VLPs.

VLP-reporter plasmid complexes were made in 500 μl of HBS. Ten micrograms of pNeoβ plasmid in 250 μl of HBS was mixed with 250 μl of modified PV capsids (25 μg). After incubation at room temperature for 30 min, the complex was added to cells for uptake analysis.

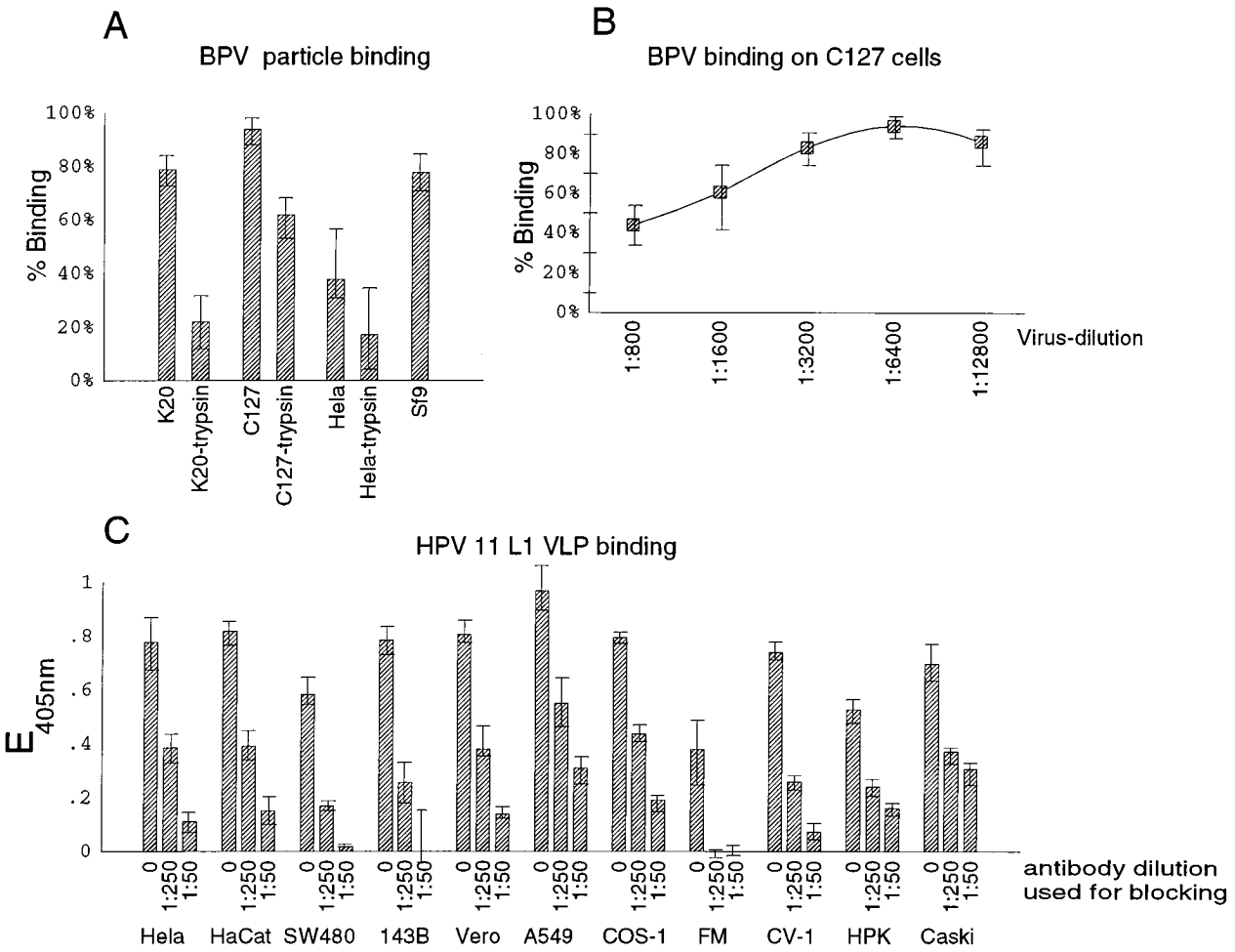

FIG. 2. Binding of PV capsids to different cell lines. (A) Adsorption of BPV virions to various cell lines. Results are expressed as percentages of virions bound to each cell type. To establish BPV binding, suspensions of BPV particles to which the cells being tested or an equal volume of saline had been added were assayed for BPV protein after the cells with absorbed virus had been removed by centrifugation. Percentages of BPV binding to cells were then calculated as described in Materials and Methods. Results for K20 cells (human lymphoma B-cell line), C127 cells (mouse mammary tumor cells), and HeLa cells (human cervical carcinoma cells), either untreated or exposed to trypsin prior to assaying, are given. (B) Dose-dependent binding of BPV particles to C127 cells. Decreasing amounts of BPV particles were incubated with a constant number of cells in the assay described for panel A. At a low concentration of BPV (1:6,400 dilution) containing approximately 1.25×10^5 particles, more than 90% of the BPV antigen was immobilized by 8×10^4 C127 cells. (C) Binding of HPV11 L1-formed VLPs to cell monolayers assessed by ELISA. Cells were fixed in 96-well plates, and VLPs were added. Results show binding of the VLPs to a range of cell lines assessed by ELISA for L1 protein. Results are expressed as the L1 protein ELISA (E) optical density readings obtained with cells exposed to VLP minus the ELISA optical densities of the same cells that were not exposed. For each cell type (see also Table 1), inhibition of binding by prior exposure of the particles to an HPV6b L1-specific polyclonal rabbit antiserum is also shown.

Purification of adenovirus particles. The replication-defective adenovirus type 5 (strain *dl*312) was grown in 293 cells (6, 7). The cells that were infected with *dl*312 virus were frozen and thawed three times, and the crude virus extracts were purified by spinning in a Beckman SW28 rotor at 26,000 rpm for 2 h and then by CsCl gradient centrifugation. The titer of purified *dl*312 viruses was determined as previously described (6).

Analysis of the uptake of VLP-reporter plasmid complexes in tissue culture cells. The cells were plated at 10^5 per well into 24-well tissue culture plates and were grown in 0.25 ml of Dulbecco's modified Eagle's medium medium with 10% FCS. Before the addition of the VLP-reporter plasmid complexes, the cells were washed twice with fresh medium without FCS, and the VLP-reporter plasmid complex was mixed with medium containing 10% FCS and added to the cells for 4 h at 37°C. At the end of this period, the medium was removed and replaced by prewarmed fresh medium with 10% FCS, and the cells continued to be incubated at 37°C for 36 h. The cells were then washed with PBS and stained for β-Gal expression.

β-Gal staining. The cells that were infected by VLP-reporter plasmid complexes containing the β-Gal gene were fixed with 2% formaldehyde in PBS for 5 min. The cells were incubated with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) staining buffer [2 mM $MgCl_2$, 3 mM $K_3Fe(CN)_6$, 3 mM $K_4Fe(CN6)$, 1 mg of X-Gal (Sigma) per ml in PBS] at room temperature for up to 1 h. Then, the cells were washed with 5% dimethyl sulfoxide in PBS and visualized using a light microscope. The number of blue cells per 1,000 cells was determined for each experiment.

RESULTS

Binding of PV particles to cells of different lines. We used two assay systems to measure binding of PV particles to cultured cell lines. First, cells were suspended in a preparation of BPV particles that were purified from cattle warts. The abilities of different cell types to adsorb BPV particles were assayed by measuring the BPV particles remaining in suspension by an antigen-capture ELISA after the cells had been removed by low-speed centrifugation. All cell lines tested (K20, C127, Sf9, and HeLa) adsorbed PV particles (Fig. 2A). The best adsorption was seen with C127 cells; more than 90% of immunoreactive BPV antigen was removed from the supernatant by these cells. The fraction of BPV particles bound by each cell type was inversely proportional to the concentration of parti-

FIG. 3. Analysis of β-Gal expression of HeLa cells after incubation with an HPV6b VLP-reporter plasmid complex at 0.5 μg per well. (A) Cells exposed to a mixture of unmodified VLPs and the reporter plasmid; (B) cells exposed to VLP-reporter plasmid complexes; (C) cells infected with *dl*312 adenovirus and exposed to a mixture of unmodified VLPs and reporter plasmid; (D) cells infected with adenovirus *dl*312 (500 particles per cell) and exposed to VLP-reporter plasmid complexes.

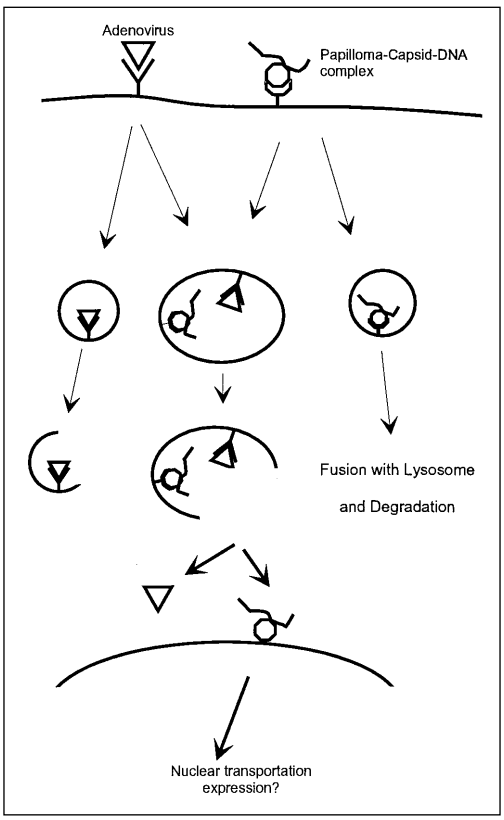

FIG. 4. Hypothesis of the uptake mechanism of VLP-reporter plasmid complexes by receptor-mediated endocytosis according to Cristiano et al. (6, 7). Coincubation of the adenovirus and VLP-reporter plasmid complex results in the binding and uptake of each component through its own receptor. The cointernalization of these components into the same endosome results in the release of the complex into the cytoplasm, allowing expression.

cles and reached almost 100% at high dilutions (about 1.25×10^5 particles per 8×10^4 [Fig. 2B]). Binding of BPV particles to cells could in each case be reduced by pretreatment of the cells with trypsin (Fig. 2A). Since even insect Sf9 cells were able to bind more than 75% of the BPV preparation, other assays were developed to confirm that the observed binding was specific. To further investigate the interaction between PV particles and cells, a direct ELISA was developed in which monolayers of fixed adherent cells were exposed to PV VLPs prepared from Sf9 cells infected with HPV11 L1 recombinant baculovirus (Fig. 1). Binding of VLPs was assessed with a MAb specific for HPV11 L1 capsids (2). Each of 11 tested cell lines was able to bind VLPs, and in each case this binding could in part be prevented by prior exposure of the particles to a rabbit polyclonal antiserum raised against HPV6b L1 which was cross-reactive with HPV11 L1 (Fig. 2C).

Internalization of PV particles into cultured cells. Many viruses are able to bind nonspecifically to cells, which usually does not result in virus uptake and infection of a cell (32). To measure uptake and internalization of PV particles (BPV or baculovirus-derived HPV6b, HPV11, and HPV16 VLPs composed of L1 alone or of the L1 and L2 proteins), VLPs were physically linked to a β-Gal reporter plasmid whose expression was used as a measure of particle uptake (4). When VLP-reporter plasmid complexes were applied to HeLa cells, β-Gal expression was detected after 48 h. However, enzyme activity was detected only in less than 1% of all exposed cells (Fig. 3B). No cells expressed β-Gal when a mixture of unlinked PV and reporter plasmid DNA was used (Fig. 3A). To investigate whether failure of release of the intact reporter construct from lysosomes was the reason why only a small proportion of cells exhibited β-Gal activity, we infected cells with a replication-defective adenovirus which facilitates lysis of lysosome membranes (24) (Fig. 4) at the time of addition of VLP-reporter plasmid complexes. Cells infected with adenovirus and simultaneously exposed to VLP-reporter plasmid constructs expressed β-Gal 10 to 20 times more frequently than cells not infected with adenovirus, resulting in about 10 to 20% positive cells (Fig. 3D). VLP-reporter plasmid constructs composed of HPV16 L1 and L2 proteins delivered the DNA into cells as efficiently as the VLP-reporter plasmid complex made of L1 alone, suggesting that the L2 protein is not necessary for PV binding and penetration. No positive cells were found when a mixture of unmodified PV capsids and reporter plasmid construct was added to cells along with *dl*312 adenovirus (Fig. 3C).

Inhibition of VLP-reporter plasmid complex uptake by PV-specific antisera. PV capsid-reporter plasmid complexes were mixed with a rabbit antiserum raised against HPV6b L1 and were then added to confluent monolayers of HeLa cells together with adenovirus strain *dl*312. After 48 h of incubation, the cells were stained for β-Gal activity. Reduction of β-Gal-positive cells was observed in a PV genotype-specific fashion (Fig. 5A). The antiserum was able to reduce uptake and expression of β-Gal linked to HPV6b L1 VLPs or HPV11 L1 VLPs but did not block uptake of HPV16 L1 VLPs or BPV particles. A MAb (H3) that was reported to be neutralizing for HPV11 (2) also blocked the uptake of HPV6b L1 and HPV11 L1 VLP-reporter gene complexes (Fig. 5A), while a nonneutralizing MAb directed against the HPV11 L1 protein (A3) failed to do so (data not shown). Uptake of PV-reporter plasmid complexes was not affected by exposure to a preimmune rabbit serum. These results suggest that binding and uptake of the VLP-reporter plasmid complexes are mediated through a specific interaction between the virus and a cellular receptor(s).

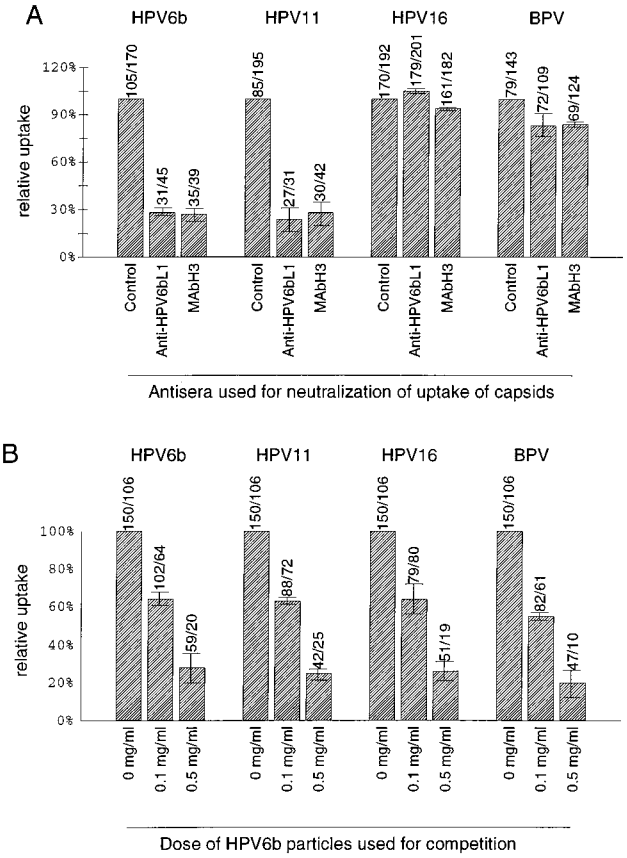

FIG. 5. (A) Inhibition of the uptake of PV-reporter plasmid complexes by L1-specific antisera. Results for cells exposed to VLP-reporter plasmid complexes preincubated with antisera are as indicated. Cells were stained for β-Gal activity after 48 h, and the percentages of blue cells (of 1,000 cells counted) were determined. The control (cells exposed to complexes treated with preimmune serum) was set at 100%. (B) Inhibition of the uptake of different PV-reporter plasmid complexes by unmodified HPV6b VLPs. Particle-reporter plasmid complexes of HPV6b, HPV11, HPV16, and BPV were mixed with unmodified HPV6b VLPs (0, 0.1, and 0.5 mg/ml), and the mixtures were added to HeLa cells along with adenovirus *dl*312 at 500 particles per cell. The controls in both panels A and B were set at 100%, and the mean values of reduction for different antibody or particle concentrations are indicated. In both panels A and B, the actual numbers of blue cells per 1,000 cells, which were counted in two independent experiments, are indicated at the top of each column. The reduction of the number of blue cells was calculated individually for both experiments. The variations for the relative inhibition are shown by error bars.

Presence of the PV receptor on different cell lines. Since the PV binding assays suggested that the putative PV receptor is not restricted to epithelial cells, we examined 16 different cell lines for their abilities to take up the VLP-reporter plasmid complexes (Table 1). All cells demonstrated expression of the β-Gal gene after lipofection of this plasmid. The cells were then infected with adenovirus *dl*312, together with PV-reporter plasmid complexes linked to HPV6b L1 VLPs. All cells but one (FM cat skin cells) of those tested were shown to express the *lacZ* gene (Table 1), suggesting that PV particles can be taken up by a mechanism or receptor present in many tissues of a variety of mammalian species.

Different PVs compete for the same entry pathway. To test if different PVs use the same cellular uptake mechanism, we examined unmodified HPV6b capsids for their abilities to interfere with uptake of the reporter plasmid linked to VLPs (HPV6b) and complete viral particles (BPV). An HPV6b L1 VLP-reporter plasmid complex (0.1 μg of protein per well) was mixed with a suspension of PV capsids (HPV11, HPV16, or BPV virions) at concentrations of 100 or 500 μg/ml, and the mixtures were added to HeLa cell monolayers along with adenovirus *dl*312. PV capsids competed with uptake of the HPV6b VLP-reporter plasmid complex in dose-dependent fashion, and VLPs of each genotype were inhibitory with similar efficiency (Fig. 5B). When HPV11 or HPV16 capsids were added 2 h after the HPV6b VLP-reporter plasmid complex, no inhibition of β-Gal activity was observed. These results indicated that different PV capsids use the same saturable pathway to enter their host cells.

DISCUSSION

PVs are nonenveloped 50- to 60-nm icosahedral structures (7) that are composed of highly conserved L1 major capsid and less well-conserved L2 minor capsid proteins. The major capsid protein has a molecular mass of approximately 55,000 Da (13, 14) and represents approximately 80% of the total viral protein. The minor capsid protein has a calculated molecular mass of approximately 50,000 Da but migrates as about 70 to 80 kDa in SDS-polyacrylamide gels (10, 19, 34). Recent high-resolution cryoelectron microscopic analysis of BPV1 and HPV1 virions has determined that the two viruses have very similar structures, with 72 pentameric capsomeres, each of which is presumably composed of five L1 molecules, forming a virion shell with T = 7 symmetry (1).

Virus-cell interaction, which represents the initial step in virus infection, occurs on the plasma membranes of susceptive cells and, in many instances, determines the virus host range (3, 23, 28, 29). PVs have a restricted tissue and host tropism; however, the basis for this restriction is unknown. On the basis of the following observations, it can be assumed that different PV use related (or even identical) cellular receptor molecules which do not seem to be restricted to a certain type of epithelium. (i) The major structural protein L1 (which makes contact with the cellular receptor, as shown in this report) is very well conserved among different PVs (33). (ii) Although most of the HPVs are restricted either to the skin (e.g., HPV1, -4, -60, -63, and -65) or to the mucosa (e.g., HPV6, -11, -16, -18, -31, and -45), there are certain HPV types, such as HPV2, -3, -7, -27, and -57 that infect both the cutaneous and the mucosal epithelia (8). (iii) BPV1 naturally infects bovine skin keratinocytes and fibroblasts; however, under experimental conditions, it can transform cells from different species in culture (20–22). (iv) A recent study using radioactively labeled BPV1 particles isolated from cattle warts as well as BPV and HPV16 VLPs demonstrated binding to cells of 14 lines derived from different tissues (epithelium, connective tissue, and neural crest) and different species (humans, mice, and monkeys) (25, 26). It is, therefore, reasonable to propose that the tropism of the PV infection is (or is only partially) attributable not to specific virus-cell interactions but to other steps of the PV life cycle, such as regulation on the transcriptional level in host cells.

Details of the early events of PV infection, including the nature and distribution of the PV receptor and the mechanisms involved in the process of viral penetration, remain unclear, since no tissue culture system exists to support virus propagation. To this end, many laboratories (including ours) have developed the method of producing PV VLPs by expression of structural proteins in recombinant vectors (15, 17, 27, 37, 38). Here, we report that synthetic PV capsids consisting of the PV L1 protein as well as complete infectious BPV virions can be applied to the study of the initial interaction of PVs with cells, i.e., attachment and penetration. In the binding assay, we used an antigen capture ELISA to quantify the amount of L1

TABLE 1. Uptake of HPV6b VLP-reporter plasmid complexes by adherent cell lines derived from different species

Cell line	Source	Tissue	Species	ATCC no.[a]	Uptake
143B	Osteosarcoma	Bone	Human	CRL8303	Yes
RK13	Epithelium	Kidney	Rabbit	CCL37	Yes
CV-1	Epithelium	Kidney	Monkey	CCL70	Yes
3T3	Fibroblast	Embryo	Mouse	CL173	Yes
HeLa	Epithelium	Cervix	Human	CCL2	Yes
Hacat	Epithelium	Skin	Human	NA	Yes
PC-3	Epithelium	Prostate	Human	CRL1435	Yes
DBT	Gliocyte	Brain	Human	NA	Yes
MDCK	Epithelium-like	Kidney	Canine	CCL34	Yes
293	Epithelium-like	Kidney	Human	CRL1573	Yes
BHK	Fibroblast	Kidney	Hamster	CCL10	Yes
FR	Epithelium	Skin	Rat	CRL1213	Yes
SW480	Epithelium	Colon	Human	CCL228	Yes
Wes	Fibroblast	Skin	Chimpanzee	CRL1069	Yes
FM	Epithelium	Skin	Cat	CRL6169	No?[b]
A549	Epithelium	Lung	Human	CCL185	Yes

[a] ATCC, American Type Culture Collection. Reference numbers are indicated when available. NA, not available.
[b] Not obtained directly from ATCC.

protein which failed to bind to cells in vitro, and we found that a large range of cells (including SF9 insect cells) was able to interact with PV capsids. Trypsinization of the cells prior to the addition of the particles reduced binding ability, suggesting that the cellular receptor might be of a protein nature.

Since this assay does not differentiate between particles attached to the surface and those which have been taken into the cells, we adapted a system which has been successful in the delivery of many genes via different receptors (4, 6, 7, 35) to study penetration of PV capsids into cells. We used complete BPV particles as well as VLPs of HPV6b, HPV11, and HPV16, which were all physically linked to DNA which codes for the cytomegalovirus promoter-driven *E. coli lacZ* gene coding for β-Gal, whose activity within the cells can be easily measured. Penetration of VLP-reporter plasmid complexes seems to be dependent on specific interactions between the PV capsids and a cellular molecule(s) since unlinked PV capsids consistently failed to deliver the reporter gene into the cell lines. Coinfection of cells with replication-defective adenovirus (*dl*312) enhanced the uptake of the VLP-reporter plasmid complexes by more than 10- to 20-fold, very likely by the adenovirus-induced lysis of lysosome membranes (24), indicating that the uptake of PV capsids occurs through an endocytotic pathway (Fig. 4).

All but one (namely, FM cells derived from cat skin) of 16 different cell lines tested in this study that originate from different tissues and species (Table 1) turned out to be β-Gal positive after coinfection by VLP-reporter plasmid complexes and adenovirus *dl*312. The numbers of blue cells were similar (about 10 to 20% of the cells) in all instances in the positive cell lines. These data suggest that the VLP-reporter plasmid complexes are delivered into the cells by receptor-mediated endocytosis and that tissue difference and evolutionary divergence have no effects on the abundance of the PV receptor molecule(s). It has not yet been analyzed why the FM cells are negative for uptake of the PV complexes. This deficiency might be due to the absence of a functional PV-specific receptor. However, the FM cells exhibited low affinity for HPV11 VLPs in our binding assays and also showed a low level of DNA transfection efficiency in a transfection assay. Further experiments are required to characterize the ability of this cell line to interact with PVs.

Specific antisera were shown to decrease capsid binding and/or uptake, probably by blocking the binding to the PV receptor, suggesting that the inhibition of the uptake of PV capsids is virus type specific. However, even at a relatively high concentration of antiserum, the blocking was not complete. It is possible that during the attachment of poly-L-lysine to the PV capsids, the particles disassembled into capsomeres, each of which retained the ability to deliver DNA into the cells, resulting in a deficiency of neutralizing antibodies. It also cannot be ruled out that partial denaturation of the particles changed the conformational epitopes but still retained the ability of the particles to interact with the cellular receptor. Incomplete blocking of binding of BPV1 has also been reported by others, showing that a neutralizing BPV1 MAb only partially blocked binding even at a high antibody-to-PV ratio (26).

In our competition assay, a 5,000-fold excess of unlinked PV capsids blocked only about two-thirds of PV-DNA capsids from entering into HeLa cells. HPV6b, HPV16, and HPV11 capsids or BPV particles could block HPV6b capsid binding and/or uptake with similar efficiencies. This result suggests that all PVs share the same receptor molecule(s) to enter their host cells. However, at the present time we are unable to distinguish whether the competition of VLPs from different genotypes was at the stage of binding or of uptake of the particles. Since we failed to completely block the entry of HPV6b capsids into cells, the PV receptor very likely is an abundant molecule that is conserved in many different cells. The method described here for uptake of PV capsids will be useful in determining the neutralizing activities of MAbs raised against PV particles and in detecting such antibodies in human sera or mucosal secretions. Furthermore, this method can be applied to studying the entry pathway of PVs into cells.

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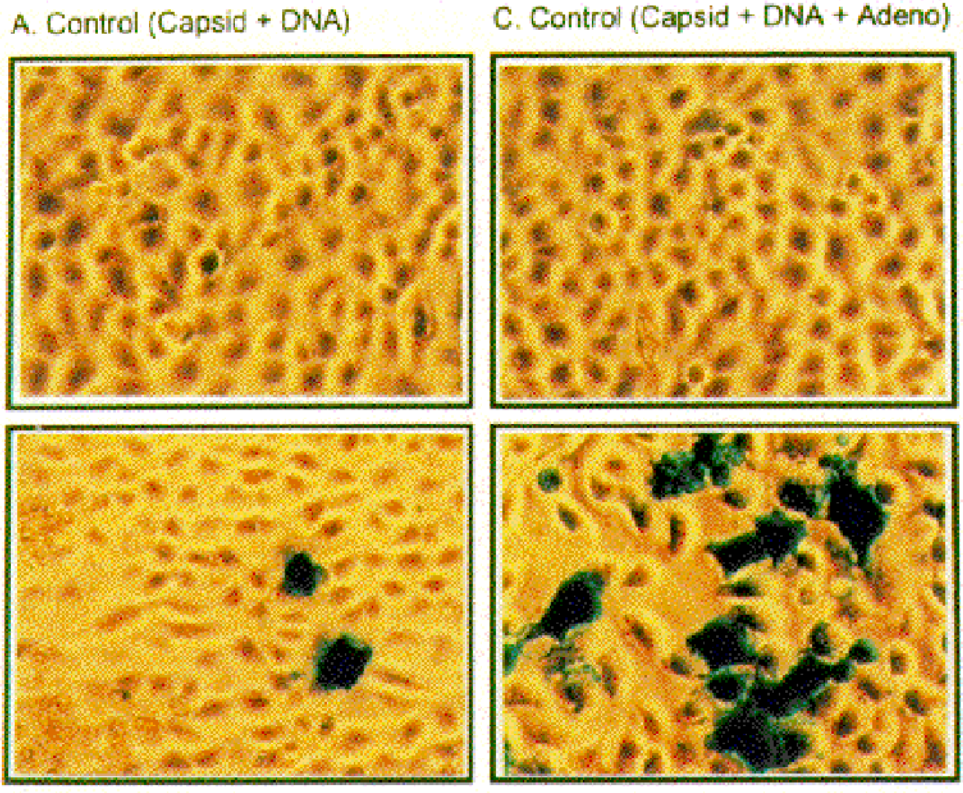
A. Control (Capsid + DNA)
C. Control (Capsid + DNA + Adeno)
B. Capsid-DNA Complex
D. Capsid-DNA Complex + Adeno