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Interaction of Papillomaviruses with the Cell Surface

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To initiate an investigation of the initial step in papillomavirus infection, we have examined the interaction of bovine papillomavirus type 1 (BPV) virions with C127 cells by two assays, binding of radioiodinated BPV virions to cell monolayers and BPV-induced focal transformation. Under physiological conditions, the labeled virions bound to the cell surface in a dose-dependent manner within 1 h. Antibody studies indicated that the interaction was specific and related to infectivity: polyclonal sera raised to BPV virions or to baculovirus-expressed BPV L1 virus-like particles (VLPs) inhibited BPV binding and focal transformation, while sera to denatured BPV virions, to denatured BPV L1, or to human papillomavirus type 16 (HPV-16) VLPs were not inhibitory. An exception was that antisera to BPV L2 were neutralizing but did not inhibit binding. Unlabeled BPV virions and BPV VLPs competed with binding to the cell surface in a concentration-dependent manner. Binding to the cell surface appeared to depend primarily on L1, since BPV VLPs composed of L1 alone or of L1/L2 were equally effective in inhibiting binding and focal transformation. VLPs of HPV-16 also inhibited BPV binding and BPV transformation of C127 cells, suggesting that they interact with the same cell surface molecule(s) as BPV virions. Radiolabeled BPV bound specifically to several mammalian cell lines of fibroblastic and epithelial origin, as well as to a human schwannoma and melanoma lines, although some lines bound up to 10 times as many counts as others. Radiolabeled HPV-16 VLPs bound to both human keratinocytes and mouse C127 cells. The results suggest that papillomaviruses bind a widely expressed and evolutionarily conserved cell surface receptor.

Numerous studies have demonstrated a close association between human papillomavirus (HPV) infection and certain human cancers, particularly those of the cervix, vulva, and penis (19, 24, 28, 34). Considerable efforts are therefore being made to prevent the spread of disease by developing a prophylactic vaccine and to devise effective treatments of HPV-induced lesions (4, 10). Specific cell surface receptors involved in the initial interaction with the virus are a primary determinant for tropism of many viruses (18, 39). The early events of papillomavirus infection such as binding and entry into susceptible cells could provide potential targets for preventing the spread of these viruses. However, the nature and tissue distribution of putative cell surface receptors for papillomaviruses and the virion protein(s) responsible for binding have yet to be elucidated.

Difficulties in generating papillomavirus in vitro has hindered study of the pathway of infection. Although the implantation of infected tissue under the renal capsules of nude mice, raft cultures producing HPV capsids, and vaccinia virus-based in vitro encapsidation in cell culture represent useful advances, these approaches have generated relatively small amounts of virus (11, 25, 29, 40). Studies of papillomavirus biology have also been hampered by the paucity of infectious models (12, 37). Focal transformation of mouse fibroblasts provides a simple quantitative in vitro assay for infection solely for bovine papillomavirus (BPV) (12), whereas infection by other papillomaviruses can only be assessed in vivo (5, 7).

Papillomaviruses are nonenveloped double-stranded DNA viruses about 55 nm in diameter with an approximately 8-kb genome in the nucleohistone core (1). The capsids are composed of two virally encoded proteins, L1 and L2, that migrate on sodium dodecyl sulfate (SDS)-polyacrylamide gels at approximately 55 and 75 kDa, respectively (30). L1, which is the major capsid protein, is arranged in 72 pentamers which associate with T=7 icosahedral symmetry. The function and position within the virion of L2 are unclear (1). The L1 protein has the capacity to self-assemble so that large amounts of virus-like particles (VLPs) may be generated by expression of the L1 protein from a number of species of papillomavirus in the baculovirus and vaccinia virus-based systems (17, 20, 22, 33). Although not required for assembly, L2 is incorporated into VLPs when coexpressed with L1 (L1/L2 VLPs) in insect or mammalian cells. Immunization of rabbits with native virions or L1 VLPs, but not with nonassembled L1 expressed in *Escherichia coli*, induces high titers of neutralizing serum antibodies (5, 20, 31, 35). Immunization with *E. coli*-expressed L2 also generates neutralizing antibodies, but the titers are several orders of magnitude lower than those induced by L1 VLPs (8, 27, 31). The polyclonal and monoclonal antibodies generated against native particles recognize predominantly type-specific conformational epitopes (6–8).

In this report, we examine the initial interaction of papillomaviruses with the cell surface, using BPV infection of C127 cells and binding of radioiodinated virions and VLPs to monolayers of cells as model systems.

MATERIALS AND METHODS

Cells and antisera. The origins of C127 cells, primary human foreskin keratinocytes, and human schwannoma ST88-14 cells have been described previously (2, 12, 13). Other lines were

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obtained from the American Type Culture Collection. Cells were maintained in the medium suggested by the American Type Culture Collection at 37°C in a humidified 5% CO_2–95% air atmosphere and used in binding assays as they approached confluence. Antisera were generated by three inoculations at 2-week intervals of New Zealand White rabbits with 0.3 mg of protein in Freund's complete adjuvant initially and Freund's incomplete adjuvant for subsequent injections, with bleeds taken 2 weeks after the last inoculation.

Preparation of BPV. Bovine papillomas, experimentally induced with BPV-1, were washed in phosphate-buffered saline (PBS), homogenized for 3 min in a Waring blender in 200 ml of PBS, and centrifuged (10,000 × *g*, 10 min). The pelleted material was homogenized for 3 min in 100 ml of PBS and centrifuged (10,000 × *g*, 10 min). The supernatants were combined and centrifuged (SW28 rotor, 25,000 rpm, 150 min, 4°C). The pellets were resuspended by gentle sonication in a total of 13 ml of 30% (wt/wt) CsCl in PBS and centrifuged (70Ti rotor, 55,000 rpm, 24 h, 18°C). The viral bands were drawn through a needle, reisolated on a second gradient, and dialyzed extensively with PBS.

Purification of papillomavirus VLPs and iodination. VLPs were prepared as described previously, dialyzed with PBS (three changes) for 3 days, and centrifuged (10 min, 600 × *g*, 4°C) before use (22). VLPs derived from the 114/K clone of HPV-16 were prepared for iodination by centrifugation (10 min, 10,000 × *g*, 4°C), followed by fast protein liquid chromatography separation on a Superose 6 (Pharmacia) column (22). The fraction eluting with the void volume was used for iodination. BPV and HPV-16 VLPs were iodinated by one of two methods. Aliquots equaling 10 μg of L1 were labeled with 500 μCi of diiodo-Bolton and Hunter reagent (~4,000 Ci/mmol; Amersham) and extensively dialyzed against PBS (3). Greater quantities of VLPs (~100 μg) were labeled in the presence of iodogen (Pierce) as instructed by the manufacturer, and free iodine was removed by gel filtration on bovine serum albumin (BSA)-coated PD-10 columns (Pharmacia) in PBS (14). The two methods produced similar results.

Radiolabeled papillomavirus binding studies. Sixty nanograms of radiolabeled BPV or HPV-16 L1/L2 VLPs (~10^6 cpm/μg of L1) per sample was diluted into a total volume of 1 ml of medium containing 10% fetal calf serum and the appropriate reagents and incubated for 1 h at ambient temperature. Each sample was then plated onto confluent cells in a 48-well plate (Costar), incubated at 37°C for the time indicated, and washed twice with PBS. The cells were harvested in two 1-ml washes of 1% SDS–0.1 M NaOH, and the associated radiolabel was determined in a Beckman gamma counter. The percentage of specific binding was calculated as $(M - N)/(C - N)$, where M is counts per minute bound in sample, C is counts per minute bound in the presence of preimmune sera (1:100), and N is counts per minute bound in the presence of sera to BPV L1 VLPs (1:100).

BPV infectivity assay. BPV virions (200 to 300 focus-forming units per plate [~60 ng]), mixed with VLPs or antibody in a total volume of 1 ml of Dulbecco modified Eagle medium with 10% fetal calf serum, were plated onto subconfluent 60-mm-diameter petri dishes of mouse C127 cells for 1 h at 37°C. The cells were washed twice with 5 ml of medium and maintained for 3 weeks. They were refed every 3 to 4 days with 10% fetal calf serum in Dulbecco modified Eagle medium. The plates were stained with 0.5% (wt/vol) methylene blue–0.25% (wt/vol) carbol fuschin in methanol, and the number of foci was scored (12).

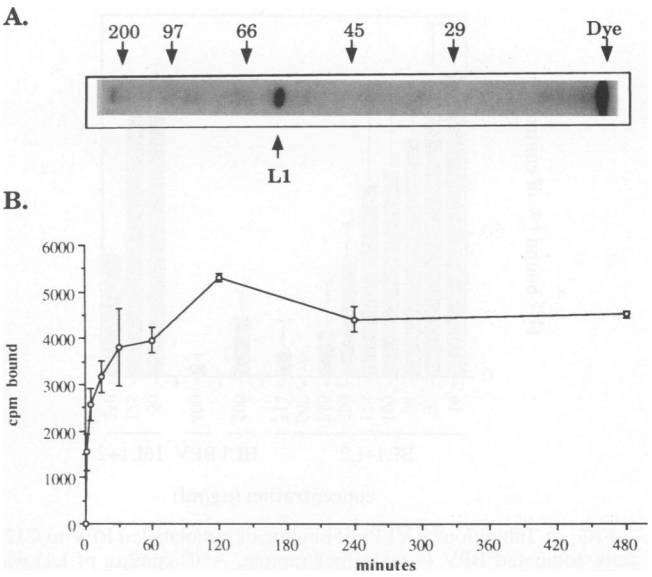

FIG. 1. Time course of binding of radiolabeled BPV to C127 cells. (A) Iodinated BPV (60 ng) was separated on an SDS–10% polyacrylamide gel and subjected to autoradiography (2 h, room temperature). Sizes are indicated in kilodaltons. (B) C127 cells in 48-well plates were incubated at 37°C for the times indicated with iodinated BPV (~60 ng per sample, ~10^6 cpm/μg of L1) and washed twice in PBS, and the associated radiolabel was counted and shown as the mean ± standard deviation (n = 3).

RESULTS

Interaction of radiolabeled BPV with mouse fibroblasts. Although not derived from the natural host, mouse C127 cells are susceptible to infection and subsequent transformation by BPV-1 (12). This system was chosen for examining the interaction of papillomavirus with the cell surface because C127 cells should possess the putative cell surface receptors for BPV-1 and focal transformation provides a quantitative assay for virus infectivity. BPV virions, purified by isopycnic density gradient centrifugation from bovine papillomas, were ^{125}I radiolabeled to a specific activity of approximately 10^6 cpm/μg of L1. When the iodinated virion preparation was subjected to SDS-polyacrylamide gel electrophoresis analysis and autoradiography (Fig. 1A), 24% of the radiolabel was found to be associated with a 55-kDa band, consistent with the molecular mass of L1 protein. Most of the remaining radiolabel comigrated with the dye front. Although labeled L1 appeared to be intact, the iodination procedure abolished the focus-forming activity of the BPV when assayed 2 days postradiolabeling (data not shown).

The time course of binding of radiolabeled virus to C127 monolayers was examined. The majority of binding occured within 1 h at 37°C, with half-maximal binding in 10 min (Fig. 1B). Binding was not inhibited by the presence of 0.1% (wt/vol) azide, 10 mg of BSA per ml, or undiluted nonspecific rabbit serum (not shown). The binding of radiolabeled virus to C127 cells was dose dependent (not shown). Binding was competed for by unlabeled BPV virions, BPV L1 VLPs, or L1/L2 VLPs in a concentration-dependent manner, indicating that the majority of binding was specific and that L2 is not required for initial binding to the cell surface (Fig. 2). Binding of labeled BPV to the cell surface was also competed for by HPV-16 L1/L2 VLPs (Fig. 2).

It has previously been shown that antisera to native BPV

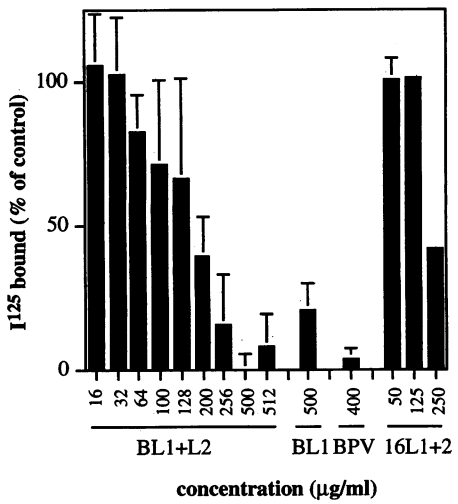

FIG. 2. Inhibition by VLPs of binding of radiolabeled BPV to C127 cells. Iodinated BPV (~60 ng per sample, ~10^6 cpm/μg of L1) was bound to C127 cells for 4 h in the presence of purified BPV L1 (BL1) or BPV L1/L2 (BL1+L2), HPV-16 L1/L2 (114/K isolate) VLPs (16L1+L2), or BPV virions at the concentrations indicated. Binding is plotted as percentage of specific binding that occurs in the absence of VLPs (mean ± standard deviation, n = 3).

virions or to assembled BPV VLPs are strongly neutralizing for BPV infection of C127 cells but that antisera made to denatured BPV virions (DAKO) or denatured L1 are not neutralizing (16, 20). Furthermore, the neutralizing antibodies are type specific, since antiserum to assembled HPV-16 L1 VLPs did not neutralize BPV. To determine whether neutralizing sera would inhibit BPV binding to the cell surface, binding studies were performed after preincubation with neutralizing and nonneutralizing sera (Fig. 3). The interaction of BPV with the C127 cells was inhibited approximately 80% by antisera to native BPV virions or to BPV VLPs. This degree of inhibition

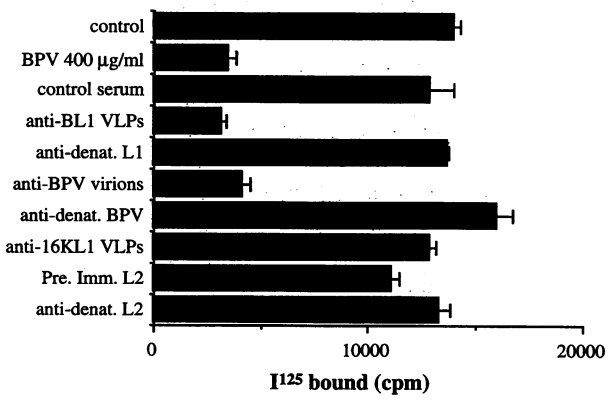

FIG. 3. Binding of iodinated BPV to C127 cells in the presence of antisera to capsid proteins or VLPs. Iodinated BPV (~60 ng per sample, ~10^6 cpm/μg of L1) was bound to C127 cells for 4 h, and nonspecific binding was determined by competition with 400 μg of unlabeled BPV per ml (a 6,000-fold excess). A number of rabbit sera to capsid proteins (1:100 dilution, except for anti-BPV L2 sera, which were diluted 1:10) were preincubated with labeled BPV for 1 h, and their effects on its binding to cells were tested (mean ± standard deviation, n = 3).

by the sera was similar to that seen in the presence of 400 μg of unlabeled BPV per ml.

These results support the conclusion that the majority of cell surface binding is specific and suggest that nonspecific interactions are responsible for the relatively small residual association observed under these conditions. Binding of radiolabeled BPV to the cells was not significantly affected by the presence of similarly diluted control sera, including antisera to denatured BPV virions, to denatured BPV L1, or to HPV-16 L1 VLPs. Rabbit antisera generated to a fusion protein of glutathione *S*-transferase and full-length BPV L2, which exhibited a neutralizing titer of 50, did not inhibit binding of ^{125}I-labeled BPV to C127 cells at a 1:10 dilution. Similar data were obtained with antiserum to BPV L2 fused to a His_6 tag (not shown).

Inhibition of BPV infection of C127 cells by papillomavirus VLPs. The ability of BPV L1 VLPs and HPV-16 VLPs to interfere with the binding of iodinated BPV to C127 cells suggested that their infection by BPV would similarly be inhibited by any papillomavirus VLPs and would not require L2. To test this hypothesis, purified VLPs consisting of BPV L1 alone or L1/L2 as well as L1/L2 VLPs of HPV-11 and HPV-16 were examined for their capacity to block BPV-1-induced transformation of C127 cells (22). Approximately 200 focus-forming units of BPV virions (60 ng of protein) were mixed with various concentrations of each of these VLP preparations (final volume, 1 ml) and incubated with monolayers of C127 cells for 1 h at 37°C. The cells were washed twice with medium and maintained for 3 weeks.

Both BPV L1 and L1/L2 VLPs competed with BPV virions for infection of C127 cells in similar concentration ranges (Fig. 4). This effect titrated with the concentration of VLPs. However, addition of BPV L1/L2 VLPs 1 h after infection with virions (for 1 h) did not significantly reduce the number of foci (Fig. 4A). Consistent with the negative results of the binding experiments, there was no significant inhibition of focal transformation when BPV was incubated with extracts from BPV L2-infected Sf9 cells treated in the same way as gradient-purified VLPs or from 20 μg of full-length BPV L2 (affinity purified from *E. coli* under denaturing conditions using a C-terminal His_6 tag [32a]) per ml (data not shown).

L1/L2 VLPs of HPV-16 and HPV-11 were able to compete in a dose-dependent manner for infection by BPV virions in a concentration range similar to that of BPV VLPs (Fig. 4). These results suggest the HPV VLPs and BPV may bind the same receptor on C127 cells.

Radiolabeled BPV binds to many mammalian cell lines. Having established the parameters of the BPV virion binding for C127 cells, we wished to determine if other cell types interacted with radiolabeled BPV (Fig. 5). Specific interaction was defined as the difference in binding in the presence of a control antiserum (at a 1:100 dilution) and a neutralizing antiserum (anti-BPV L1 VLPs at a 1:100 dilution). By this criterion, radiolabeled BPV specifically bound to fibroblasts and epithelial cells derived from a variety of mammalian species. Furthermore, the virus also bound to two human neural crest-derived lines; a schwannoma line (ST88-14) and a melanoma line (SKMEL-28). The total counts bound by some cell lines (C33A, MDCK, CHO, ST88-14, and SKMEL-28) was reproducibly lower despite similar cell densities. However, the binding even in these lines was always significantly greater in the presence of the control antiserum than with the neutralizing antiserum (Fig. 5).

Iodinated HPV-16 VLPs exhibit properties similar to those of BPV virions. To provide direct experimental support that HPV-16 VLPs specifically bind with C127 cells, HPV-16 VLPs

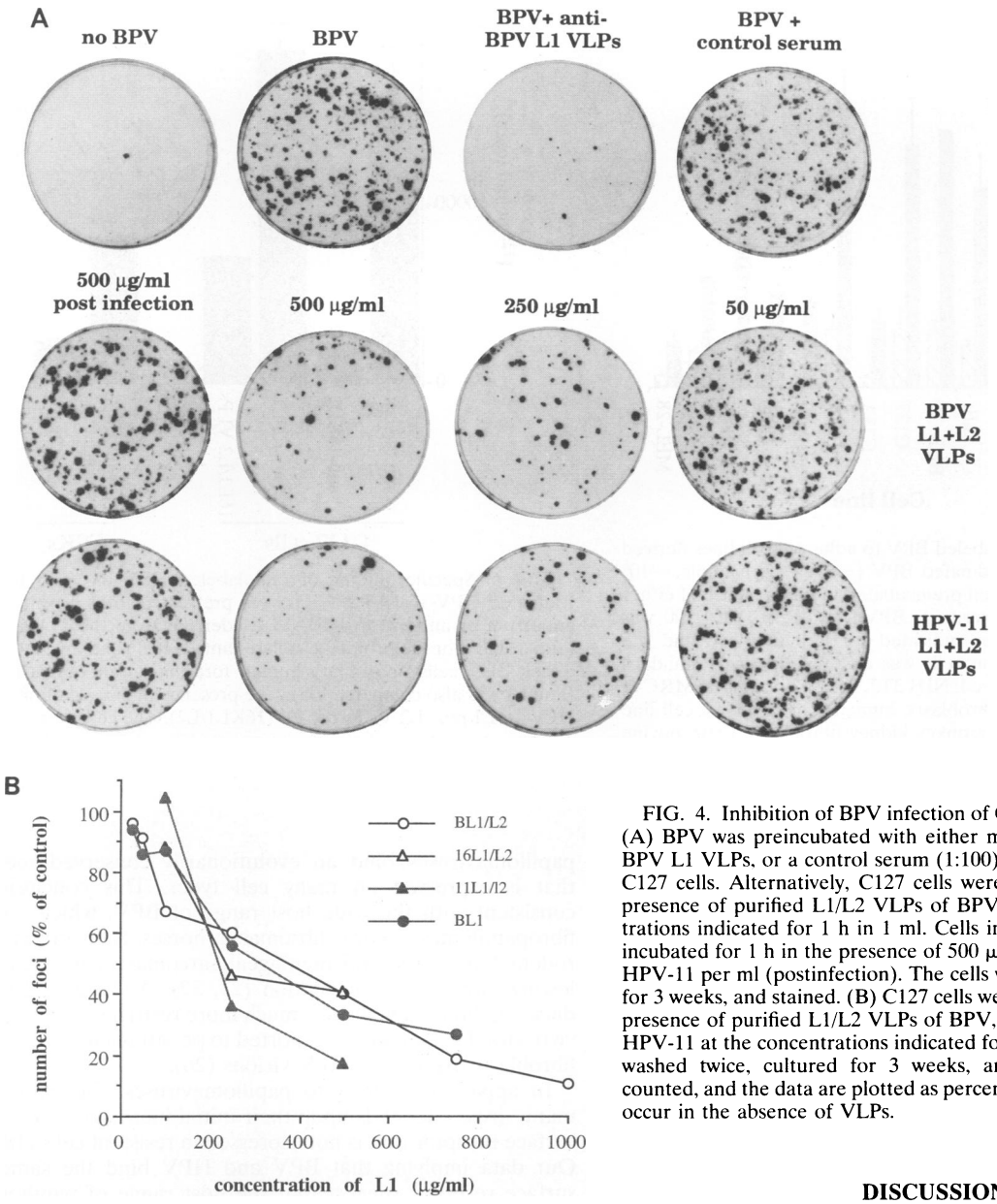

FIG. 4. Inhibition of BPV infection of C127 cells by purified VLPs. (A) BPV was preincubated with either medium alone, antiserum to BPV L1 VLPs, or a control serum (1:100) for 1 h before plating onto C127 cells. Alternatively, C127 cells were infected with BPV in the presence of purified L1/L2 VLPs of BPV or HPV-11 at the concentrations indicated for 1 h in 1 ml. Cells infected with BPV were also incubated for 1 h in the presence of 500 μg of L1/L2 VLPs of BPV or HPV-11 per ml (postinfection). The cells were washed twice, cultured for 3 weeks, and stained. (B) C127 cells were infected with BPV in the presence of purified L1/L2 VLPs of BPV, HPV-16 (114/K isolate), or HPV-11 at the concentrations indicated for 1 h in 1 ml. The cells were washed twice, cultured for 3 weeks, and stained. The foci were counted, and the data are plotted as percentages of number of foci that occur in the absence of VLPs.

were radiolabeled, and cell binding experiments analogous to those using BPV virus were performed. Iodinated HPV-16 VLPs bound to C127 cells, and the interaction was inhibited by the presence of unlabeled HPV-16 VLPs at 500 μg/ml (Fig. 6). Binding was also inhibited by rabbit antisera generated to L1/L2 VLPs derived from the wild-type 114/K isolate but not by sera to L1/L2 preparations of the prototype HPV-16 isolate, which, because it contains a point mutation, assembles 3 orders of magnitude less efficiently (Fig. 6) (20, 22).

The labeled HPV-16 VLPs also bound normal human foreskin keratinocytes in the presence of control sera, and this binding was inhibited by rabbit sera raised against assembled HPV-16 L1 VLPs. In contrast, binding was the same in the presence of a 1:10 dilution of rabbit antisera to a fusion protein of glutathione *S*-transferase and full-length HPV-16 L2 produced in *E. coli* as in the prebleed from the same rabbit (data not shown) (22).

DISCUSSION

These experiments present an initial characterization of the specific interaction of papillomavirus virions with cell surfaces. Binding of radiolabeled BPV virions occurred rapidly under physiological conditions in the presence of azide, indicating that nonspecific pinocytosis is not responsible for the association of radiolabel with the cells. Although radiolabeling of the BPV virions rendered them noninfectious, both cell surface binding by labeled BPV and the induction of focal transformation by unlabeled BPV were inhibited by VLPs of BPV and of HPV-16 as well as by neutralizing anti-BPV sera. These findings imply that the cell surface binding of the labeled virions, as measured in this assay, represents a parameter that is necessary for viral infection. Since even distantly related VLPs inhibited BPV binding, it is likely that the VLPs from BPV and HPV interact with the same cell surface receptor(s).

The HPV-16 VLPs competed for binding and infection by BPV somewhat less efficiently than either BPV or HPV-11 VLPs, but it remains to be determined whether this result

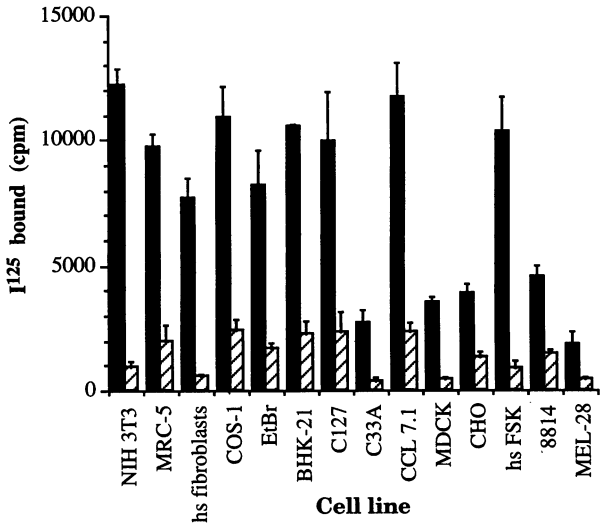

FIG. 5. Binding of radiolabeled BPV to adherent cell lines derived from a variety of species. Iodinated BPV (~60 ng per sample, $\sim 10^6$ cpm/μg of L1), which had been preincubated in the presence of either control antiserum (■) or antiserum to BPV L1 VLPs (▨) at 1:100, was incubated with the cell lines indicated for 4 h at 37°C, and the radiolabel associated with the cells was counted (mean ± standard deviation, $n = 3$). Cell lines used: NIH 3T3, mouse fibroblasts; MRC-5, human lung fibroblasts; hs fibroblasts, human skin fibroblast cell line 1634; COS-1, African green monkey kidney fibroblasts; EtBr, bovine tracheal fibroblasts; BHK-21, Syrian hamster kidney fibroblasts; C127, mouse mammary tumor cells; C33A, human cervical carcinoma epithelial cells; CCL 7.1, rhesus monkey LLC-MK2 kidney epithelial cells; MDCK, canine kidney epithelial cells; CHO, Chinese hamster ovary epithelial cells; hs FSK, primary human foreskin keratinocytes; 8814, human schwannoma cell line ST88-14; MEL-28, human melanoma cell line SKMEL-28.

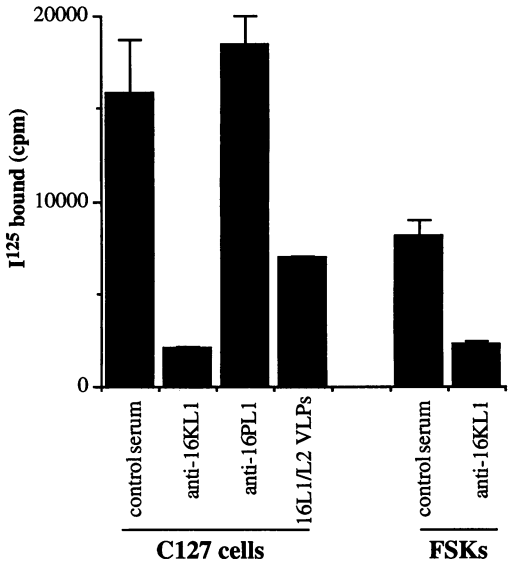

FIG. 6. Specific binding of radiolabeled HPV-16 VLPs to cells. Iodinated HPV-16 L1/L2 VLPs were pretreated for 1 h with control antiserum or antisera to HPV-16 L1 derived from the 114/K isolate (anti-16KL1) or the prototype isolate (anti-16PL1) and incubated with either C127 cells or primary human foreskin keratinocytes (FSKs). Binding was also competed for in the presence of 500 μg of unlabeled HPV-16 L1 plus L2 VLPs per ml (16KL1/L2). The cells were washed, and the associated radiolabel was counted (mean ± standard deviation, $n = 3$).

reflects a difference in binding affinity or in efficiency of particle formation. The latter possibility deserves serious consideration, since sera raised against an L1/L2 preparation of the HPV-16 prototype strain, which self-assembles much less efficiently than the wild-type HPV-16 L1/L2, failed to prevent the binding of wild type HPV L1/L2 VLPs.

Hundreds of micrograms of virions or VLPs were required to compete for both the binding of ~60 ng of radioiodinated BPV to the cell surface and infectivity, which may indicate that papillomaviruses bind to a high-copy-number receptor on the cell surface, as with sialyloligosaccharide bound by polyomavirus or heparan sulfate bound by herpes simplex virus and cytomegalovirus (9, 15, 36).

A wide variety of cell types from several mammalian genera specifically bound the labeled BPV virions. These included human schwannoma and melanoma lines, which are both of neural crest origin, as well as fibroblastic and epithelial cells. Some lines bound up to 10 times as many counts as others. However, the number of counts bound did not correlate with the species of origin in that there were human and rodent lines that bound high numbers of counts. This observation argues that the low binding activity seen with other lines did not result primarily from evolutionary divergence of the cell surface receptor(s).

A more limited survey using HPV-16 VLPs gave positive cell surface binding with the rodent C127 cells and human foreskin keratinocytes, which were the two cell types tested. Together with the BPV binding data, these results strongly suggest that papillomaviruses bind an evolutionarily conserved receptor that is expressed on many cell types. This conclusion is consistent with the wide host range of BPV, which induces fibropapillomas in cows, fibromas in horses, transformation of rodent fibroblasts, and meningeal sarcomas in hamsters following intracranial inoculation (23, 32). Although HPVs induce papillomas and have a much more restricted host range in vivo, viral DNA has been reported to persist in cultured human fibroblasts exposed to HPV virions (26).

In apparent contrast to papillomaviruses, the tropism of many viruses depends upon their initial interaction with a cell surface receptor that is not expressed in resistant cells (18, 39). Our data implying that BPV and HPV bind the same cell surface receptor suggest that the host range of papillomaviruses may be determined by requiring distinct secondary receptor(s) or by other factors within the cell such as permissiveness for viral transcription or replication. Evidence supporting the notion that papillomaviruses may be differentially regulated at the level of transcription has come from data analyzing the expression of a reporter gene placed downstream from the HPV-11 regulatory region (38). When microinjected with this construct, genital and laryngeal keratinocytes, which are the normal host cells for HPV-11, express higher levels than do microinjected cutaneous keratinocytes.

Our observations indicate that L2 protein is not required for binding. Similar concentrations of BPV VLPs composed of L1 alone or of L1/L2 were equally effective in inhibiting the binding of radiolabeled BPV to C127 cells. In addition, polyclonal antisera made against BPV L2 did not inhibit the binding of radiolabeled BPV to C127 cells, although the antisera possessed significant neutralizing activity, which indicates that the antibody does stably associate with the virions. The ability of the L2 antibodies to neutralize infectivity without

inhibiting BPV cell surface binding suggests that the L2 antibodies interfere with a step distal to binding, such as virion entry or uncoating.

Serum-mediated inhibition of BPV binding correlated with neutralizing activity directed against L1. Strongly neutralizing polyclonal antisera (titer of $>10^5$), which were generated with BPV virions or BPV L1 VLPs, prevented binding of radiolabeled BPV to the cell surface. Binding was unaffected by antisera to denatured BPV or to nonassembled BPV L1, which were not neutralizing. Therefore, neutralizing antibodies to conformational epitopes on L1 preferentially inhibited virion binding to cells.

Neutralizing antisera appear to be type specific. Although wild-type HPV-16 L1/L2 VLPs competed with cell surface BPV binding and BPV infectivity, antisera raised against wild-type HPV-16 L1 VLPs neither neutralized BPV infectivity nor inhibited BPV binding to C127 cells. However, antisera to HPV-16 L1 VLPs did prevent cell surface binding of HPV-16 L1/L2 VLPs.

Since most antivirion antibodies generated during natural infection appear to react with conformationally dependent epitopes of L1 (21), the results raise the possibility that inhibition of cell surface binding of radiolabeled VLPs might be used as a surrogate assay for neutralizing activity for viruses such as HPV-16 for which no infectivity assay is available. Compared with the recently described enzyme-linked immunosorbent assay to detect antibodies against HPV-16 VLPs, such a cell surface binding interference assay would probably be more stringent in at least two respects (21). Antibodies that bound to VLPs but were nonneutralizing would score negative in the cell surface binding assay. Neutralizing antibodies that did not interfere with cell surface binding, such as those directed against L2, would also score negative in the binding assay.

This type of assay might facilitate development of a VLP-based vaccine to prevent HPV infection. Our finding that antibodies raised in rabbits against the wild-type HPV-16 L1 VLPs inhibited binding of wild-type HPV-16 VLPs to cell surfaces, while sera raised to the L1 of the prototype HPV-16 strain, which carries a mutation that leads to inefficient self-assembly, failed to do so strongly suggests that the wild-type particles would be a more attractive candidate for such a vaccine.

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