Expression of Human Papillomavirus Type 11 L1 Protein in Insect Cells: In Vivo and In Vitro Assembly of Viruslike Particles

ROBERT C. ROSE,^{1,2*} WILLIAM BONNEZ,¹ RICHARD C. REICHMAN,^{1,2} AND ROBERT L. GARCEA³

*Infectious Diseases Unit, Department of Medicine,¹ and Department of Microbiology and Immunology,²
University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, and
Division of Pediatric Oncology, The Children's Hospital and Dana Farber
Cancer Institute, Boston, Massachusetts 02115³*

Received 2 November 1992/Accepted 12 January 1993

The L1 coat protein of human papillomavirus type 11 (HPV-11) was expressed in Sf-9 insect cells with the recombinant baculovirus vector Ac11L1. Viruslike particles (VLPs) were identified by electron microscopy in the nucleus and cytoplasm of Sf-9 cells infected with Ac11L1. The L1 protein was purified from Ac11L1-infected insect cells. The purified protein spontaneously assembled in vitro into various aggregates, including particles appearing similar to empty virions. Reaction of VLP-containing insect cell extracts with antisera directed against either denatured or nondenatured capsid epitopes in Western blot (immunoblot) and immuno-dot blot assays suggested that conformational epitopes present in native HPV-11 infectious virions were also present on the baculovirus-produced HPV-11 VLPs. Immuno-dot blot assays using human sera obtained from individuals with biopsy-proven condyloma acuminatum correlated closely with results previously obtained in HPV-11 whole virus particle-based enzyme-linked immunosorbent assays. These morphologic and immunologic similarities to native HPV-11 virions suggest that recombinant VLPs produced in the baculovirus system may be useful in seroepidemiology and pathogenesis studies of genital HPV infection and that they may also be potential candidates for vaccine development.

Human papillomaviruses (HPVs) infect cutaneous, genital, and respiratory epithelia in a tissue-specific manner, and infection with these viruses has been associated closely with the development of both benign lesions and malignancies (26). For example, HPV type 1 (HPV-1) is present in plantar warts and HPV-6 or HPV-11 is present in condylomata acuminata (anogenital warts), while HPV-16 or HPV-18 is common in premalignant and malignant lesions of the cervical squamous epithelium (9, 18, 40). The inability to propagate HPV in vitro has led to the development of alternative approaches to antigen production for immunologic studies. Investigators have expressed recombinant capsid protein coding sequences in prokaryotic systems and used them in Western blot (immunoblot) analyses of sera obtained from individuals with HPV infection of the genital tract. Results from most of these studies (5, 12, 22, 33, 35) have suggested that antibodies to denatured, i.e., linear, epitopes of HPV capsid proteins can be detected in the sera of some infected individuals.

Whole virus particles have also been used to detect antibodies in human sera, including antibodies directed against conformational epitopes. These studies have been difficult to conduct because most naturally occurring HPV-induced lesions produce few particles. However, whole virus particles can be obtained from HPV-1-induced plantar warts (15, 25, 33) and experimentally induced HPV-11 athymic mouse xenografts (19, 20) in amounts sufficient to conduct immunologic assays. Particles derived from these sources have been used in enzyme-linked immunosorbent assays (ELISAs) to detect antibodies in human sera that react with nondenatured capsid epitopes (2, 8, 33). In

addition, neutralization of HPV-11 has been demonstrated for the athymic mouse model with antisera directed against nondenatured whole virus particles (4, 6–8). Successful development of papillomavirus vaccines may therefore be dependent upon the use of nondenatured, conformationally correct capsid antigens.

Traditional methods of virus propagation have not yet been adapted to the study of papillomaviruses, and in vivo propagation of HPV-11 in the athymic mouse system is expensive and labor intensive. Thus, immunologic studies and characterization of the neutralization domain would be facilitated by the development of an alternative method of producing conformational epitopes of the HPV capsid. Several recent reports have described recombinant baculovirus expression of noninfectious viruslike particles (VLPs) for a variety of viral pathogens (11, 24, 29, 37). In some cases, protective immunity induced by these preparations has been demonstrated for the natural host (23, 28, 30). In this report, we describe the production and purification of HPV-11 VLPs that resemble native infectious HPV-11 virions by both morphologic and immunologic criteria.

MATERIALS AND METHODS

Source of HPV-11 viral DNA and pVL11L1 construction. HPV-11 genomic DNA was obtained from virus particles which were purified from experimentally induced athymic mouse xenografts as previously described (27). The L1 coding sequence was cloned by polymerase chain reaction amplification of purified genomic DNA, with primers designed to introduce *Bgl*II and *Eco*RI restriction enzyme sites at the 5' and 3' ends, respectively. The forward and reverse primer sequences were 5'-CGC AGA TCT ATG TGG CGG CCT AGC-3' and 5'-CAT ATG AAT TCC CAC AAC ACA

* Corresponding author.

CTG ACA CAC-3', respectively. Restriction sites (underlined) were introduced proximal to the putative L1 start codon (boldface), and approximately 30 nucleotides downstream from the putative L1 stop codon, by primer-directed mutagenesis. Amplification was performed essentially as previously described (4), with 500 ng of each primer and 2 U of *Taq* DNA polymerase (Amplitaq; Perkin-Elmer Cetus Corp., Norwalk, Conn.). After amplification, the polymerase chain reaction product was digested with *Bgl*II and *Eco*RI. The 1,539-bp digestion product, which contained the entire HPV-11 L1 open reading frame, was purified by agarose gel electrophoresis (27) and cloned into the corresponding sites of a baculovirus transfer vector, pVL-1392 (generously provided by M. D. Summers, Texas A&M University, College Station). The resulting construct, pVL11L1, was used to cotransfect Sf-9 cells with *Autographa californica* nuclear polyhedrosis virus (AcNPV) genomic DNA by standard methods (36). Recombinant baculoviruses were recovered by visual examination and selection of occlusion-negative plaques and were subjected to two further rounds of plaque purification (36). Protein expression from isolated virus stocks was determined by Western blot (see below).

SDS-polyacrylamide gel electrophoresis and Western blot detection of recombinant L1 expression in Sf-9 cells. Infected Sf-9 cell cultures were grown in 150-cm² tissue culture flasks and prepared for analytical sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and Western blot assay. Nonrecombinant or recombinant L1-infected cells were collected from flasks by resuspending with a Pasteur pipette, and equal numbers of wild-type or recombinant L1-infected cells were centrifuged at 500 × *g* for 10 min at 4°C. Supernatants were removed, and cell pellets were transferred to ice, immediately resuspended in 1 ml of lysis buffer (30 mM Tris [pH 7.6], 10 mM MgCl₂, 1 mM CaCl₂, 1 mM phenylmethylsulfonyl fluoride, leupeptin [10 µg/ml], 1% Nonidet P-40 [NP-40]), and allowed to stand at room temperature for 15 min with periodic vortexing. After centrifugation at 500 × *g* for 2 min at 4°C, the NP-40-soluble fraction contained in the supernatant was removed and diluted 1:1 with 2× Laemmli sample buffer (21) and heated to 95°C for 3 min. The NP-40-insoluble pellet (containing nuclear material) was washed once with cold phosphate-buffered saline (PBS) (1 mM phenylmethylsulfonyl fluoride, 10 µg of leupeptin per ml) and solubilized by boiling and vortexing in 1× Laemmli buffer. Electrophoresis of samples in SDS-10% polyacrylamide gels was followed by Coomassie blue staining (Fig. 1A) or blotting (Fig. 1B) to an Immobilon-P membrane (Millipore Corp., Bedford, Mass.) as described previously (27). Immunologic detection of recombinant L1 is described below.

Sample preparation of nonrecombinant and recombinant L1 stock solutions for comparative Western blot and immuno-dot blot assays. These assays were performed with dilutions of clarified (high-speed) supernatant stock solutions prepared from extracts of either AcNPV- or Ac11L1-infected insect cells. Suspension cultures (100 ml) of Sf-9 cells infected either with AcNPV or with Ac11L1 at an approximate multiplicity of infection of 10 PFU per cell were incubated at 27°C for 72 h. Cultures were then centrifuged at 1,000 × *g* for 10 min at 4°C, and cell pellets were resuspended in 20 ml of homogenization buffer (lysis buffer with 1 M NaCl) and subjected to Dounce homogenization (50 strokes) on ice. Homogenates were transferred to cold 30-ml screw-cap Corex tubes and centrifuged at 3,000 × *g* for 10 min at 4°C. Low-speed supernatant fractions were then

FIG. 1. SDS-polyacrylamide gel electrophoresis and Western blot of wild-type AcNPV- and recombinant Ac11L1-infected Sf-9 cell lysates. Nonrecombinant (lanes 1 and 2) and recombinant L1-infected (lanes 3 and 4) Sf-9 cell lysates were fractionated into insoluble (lanes 1 and 3) and soluble (lanes 2 and 4) fractions as described in Materials and Methods and electrophoresed on 10% polyacrylamide gels. (A) Coomassie blue-stained SDS-polyacrylamide gel electrophoresis. (B) Western blot probed with a rabbit polyclonal antiserum specific for the HPV L1 common epitope (35). Molecular weight reference (*M_r*) markers (in thousands) are displayed at the left; the arrowhead at the right indicates the position of recombinant L1 (~55 kDa).

transferred to a clean tube and centrifuged at 100,000 × *g* for 30 min at 4°C. Total protein concentrations of high-speed supernatant fractions were measured by spectrophotometric absorption at 280 nm (34) and adjusted to equivalence with fresh homogenization buffer (protein concentration, ~30 mg/ml). Glycerol was added to a concentration of 10% (vol/vol), and stock solutions were aliquoted and stored at -20°C.

Western blot and immuno-dot blot assays. Western blot and immuno-dot blot assays were used to determine linear and conformational epitope antibody specificities in rabbit antisera and human sera. Western blot assays (see Fig. 5A and 6) were performed with 2 µl (~60 µg of total protein) of recombinant L1 stock solution diluted 1:100 with 1× Laemmli sample buffer, which contains protein denaturation reagents (21), and heated to 95°C for 3 min. The denatured sample was loaded in a single 100-mm-wide sample well, electrophoresed in an SDS-10% polyacrylamide gel, and blotted to an Immobilon-P membrane. After blocking with a 2% bovine serum albumin solution (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) for 2 h at 37°C, the membrane was sliced into 24 4-mm-wide strips, each containing ~2.5 µg of total protein. Strips were probed with antisera as described below (see legends to Fig. 5 to 7).

For immuno-dot blot analysis, nonrecombinant or recombinant L1 stock solutions were diluted 1:1,000 with cold PBS (1 mM CaCl₂) and 100-µl aliquots (containing ~3.0 µg of total protein) were dotted onto an Immobilon-P membrane.

Protein denaturation reagents were omitted from the immuno-dot blot sample preparation to preserve the native conformation of recombinant L1. Blocking, primary and secondary antibody diluent solutions, washes, and substrate used have been described previously (35). Primary antibody incubations were performed overnight at 4°C; secondary antibody incubations were done at room temperature for 90 min. For immuno-dot blots, all solutions except the substrate solution contained CaCl_2 at 1 mM. Primary antibody dilutions were 1:2,000 for rabbit antisera and 1:1,000 for human sera. Specifically bound antibodies were detected with affinity-purified anti-rabbit (Kirkegaard & Perry) or anti-human (TAGO Immunodiagnostics, Burlingame, Calif.) immunoglobulin G alkaline phosphatase conjugates used at dilutions of 1:2,000 and 1:5,000, respectively, with 5-bromo-4-chloro-3-indolylphosphate toluidinium (salt)-Nitro Blue Tetrazolium (Kirkegaard & Perry) as substrate. Immuno-dot blot reactions were assessed by visual comparison of nonrecombinant and recombinant L1 dot intensities. A reaction was considered positive if the color intensity of the recombinant L1 dot was greater than the color intensity of the nonrecombinant control dot present on the same strip.

Antisera. The two denatured L1 antisera used in this study have been described previously as anti-pEX215 and anti-pEX480 (35). Briefly, these antisera were produced by rabbit immunizations with gel-purified bacterially expressed β -galactosidase carboxy-terminal fusion proteins (32) that contained coding sequences derived from defined regions of the HPV-6b L1 open reading frame (35). pEX215 expresses a 70-amino-acid amino-terminal HPV-6b L1 peptide, and pEX480 expresses a 160-amino-acid sequence derived from the middle region of the HPV-6b L1 open reading frame. The pEX480 construct encodes the linear papillomavirus L1 common antigen (35). The rabbit whole virus particle antiserum used has also been described previously (4) and was produced by immunization of rabbits with purified nondenatured HPV-11 virions, which were obtained from athymic mouse foreskin xenografts (4, 19). Patient sera were obtained from individuals with biopsy-proven condyloma acuminatum. Serum specimens previously found positive by HPV-11 whole virus particle-based ELISA (2) were used to maximize our ability to detect antibodies directed against conformational capsid antigenic determinants. Control sera were obtained from nuns who professed no sexual contact in their lifetimes. These sera were negative for HPV-11 antibodies as determined by the HPV-11 particle-based ELISA (2).

Purification of recombinant L1 protein. Sf-9 cells (0.5-liter suspension culture) infected with the Ac11L1 recombinant baculovirus were pelleted, washed once in PBS, and frozen. All subsequent steps were performed at 4°C. Cells were lysed by thawing, resuspension in 30 ml of buffer A (40 mM Tris [pH 7.9], 250 mM NaCl, 15 mM 2-mercaptoethanol, 2 mM MgCl_2 , 1 mM phenylmethylsulfonyl fluoride), and Dounce homogenization 15 times with a B pestle. The homogenate was centrifuged at $10,000 \times g$ for 20 min, and the pellet was reextracted with 30 ml of buffer A plus 0.05% sodium deoxycholate. After centrifugation, the supernatants were combined and precipitated by the addition of ammonium sulfate to 35% saturation. The ammonium sulfate precipitate was resuspended in 15 ml of buffer B (20 mM Tris [pH 7.2], 100 mM NaCl, 0.1 mM EDTA, 5% glycerol, 15 mM 2-mercaptoethanol), the insoluble material was removed by centrifugation at $10,000 \times g$ for 20 min, and the supernatant was dialyzed against buffer B. The soluble dialysate was then chromatographed on a DE-52 cellulose column in buffer

B, and the flowthrough plus 1 column volume was collected and precipitated with ammonium sulfate to 35% saturation. The precipitate was resuspended in buffer B, dialyzed against buffer B, and applied to a P-11 phosphocellulose column. The column was washed with buffer B plus 250 mM NaCl, and the L1 protein was eluted with buffer B plus 1 M NaCl.

Electron microscopy of thin sections. Cells were prepared for electron microscopy as described previously (24). Briefly, monolayers were fixed in 2% paraformaldehyde-0.1% glutaraldehyde in PBS for 1 h at 4°C. After fixation, the cells were washed three times in Sabatini's solution, post-fixed with 1% osmium tetroxide for 1 h, and washed three times with Sabatini's solution. The samples were then passed through a graded alcohol series, infiltrated with acetone, and embedded in Epon 812. Ultrathin (60-nm) sections true to the plane of the dish were cut with a diamond knife on an MT2 Ultramicrotome (Sorvall), stained with uranyl acetate and lead citrate, mounted on 300-mesh grids, and examined with a Philips 300 electron microscope. Preparation of grids of negatively stained specimens was performed as previously described (24).

RESULTS

Expression and immunologic detection of recombinant HPV-11 L1 protein in Sf-9 cells. SDS-polyacrylamide gel electrophoresis analysis of total Sf-9 cell proteins from insect cells infected with the recombinant virus Ac11L1 demonstrated a novel 55-kDa protein seen by Coomassie blue staining in Ac11L1-infected cells (Fig. 1A, lane 3). This protein is not present in wild-type AcNPV lysates and comigrates with a protein that is immunoreactive (Fig. 1B, lanes 3 and 4) with a rabbit antiserum prepared against the linear HPV L1 common antigen (35). Lower-molecular-weight L1-immunoreactive bands were also detected and may be derived from degradation of the full-length L1 product (Fig. 1B, lanes 3 and 4). Although the predominant portion of L1 produced in this system appeared in the NP-40-insoluble fraction, approximately 25 to 30% was present in the NP-40-soluble fraction (Fig. 1B, lane 4). Maximal L1 accumulation occurred at 72 h postinfection.

Electron microscopy of recombinant L1-infected Sf-9 cells. Electron micrographs of thin sections of Ac11L1-infected Sf-9 cell monolayers fixed at 72 h postinfection (Fig. 2A) showed distinct VLPs. No such structures were seen in mock- or AcNPV-infected Sf-9 cells. Although the majority of the particles were identified in the nucleus of the cells, VLPs could be detected infrequently in the cytoplasm. Particle diameters determined by direct measurement of the VLPs in Fig. 2B were approximately 50 nm (scaled by magnification standards and uncorrected for dehydration shrinkage of the specimen [approximately 10%]). This measurement is consistent with the diameter of isolated papillomavirus virions (17). In rare cells, paracrystalline arrays of VLPs (Fig. 2C) that were similar in appearance to those seen in papillomavirus-infected tissue (10) could be identified. These structures were distinguished from baculovirus inclusions seen in cross section in that the VLP arrays were not surrounded by a membranelike structure and individual particles which had a greater diameter did not stain centrally in comparison with inclusions. The lack of central staining indicated that nucleic acid was not encapsidated. In view of the amount of protein detected by Coomassie blue staining of the SDS-polyacrylamide gel, the particles were not abundant in monolayer sections. Thus, assembly into VLPs may

FIG. 2. Electron micrograph of Sf-9 cells infected with Ac11L1. Infected cell monolayers were prepared for microscopy as described in Methods and Materials. (A) Cell 72 h postinfection with capsidlike particles present in the nucleus. (B) A separate field at a higher magnification demonstrating the relative uniformity of particle size. (C) Cell with paracrystalline arrays of particles. n, nucleus; nm, nuclear membrane; c, cytoplasm. Bar = 100 nm.

FIG. 3. SDS-polyacrylamide gel electrophoresis of L1 protein purified from Ac11L1-infected Sf-9 cells. Shown are Coomassie blue-stained gels of soluble whole-cell lysate starting material (lane 1), soluble fraction before DEAE chromatography (lane 2), fraction after DEAE chromatography (lane 3), and phosphocellulose-purified L1 (lane 4). Lane S, molecular weight standards. Weights (in thousands) appear on the left.

be inefficient in Sf-9 cells, although biochemical fractionation suggested that a majority of the L1 protein is localized in the nucleus. This inefficient *in vivo* assembly may explain our current inability to purify VLPs directly from the infected cells by using techniques previously described (24).

Purification and *in vitro* self-assembly of recombinant HPV-11 L1. The L1 protein was purified from the Ac11L1-infected Sf-9 cells (Fig. 3) by a protocol modified from that used to purify the polyomavirus major capsid protein, VP1, after expression in insect cells (24). Approximately 0.25 mg of near-homogeneous L1 (Fig. 3, lane 4) was obtained from 0.5 liter of infected cells, after phosphocellulose chromatography.

To test whether the purified recombinant L1 protein could assemble *in vitro*, the protein obtained after elution from the P-11 column was incubated at 4°C for 7 days in 2 M NaCl (in buffer A) and examined by electron microscopy after negative staining. High-ionic-strength conditions were used because they have previously been shown to induce the *in vitro* self-assembly of the polyomavirus VP1 protein. As shown in Fig. 4, doughnutlike structures resembling the pentameric capsomeres of L1 could be identified, as well as a variety of polymorphic capsomeric aggregates. Some aggregates ap-

peared similar to VLPs in shape and size. The relative heterogeneity in size of the *in vitro*-assembled L1 aggregates contrasts with the homogeneous particles identified in infected cells (Fig. 2).

Immunoreactivity of HPV-11 VLP-containing insect cell extracts with rabbit antisera. The immunologic properties of the recombinant L1 protein were studied by using rabbit antisera that reacted with native or denatured L1 protein epitopes. Rabbit anti-pEX480 antiserum, directed against the common papillomavirus antigen, reacted well with denatured recombinant L1 in Western blot assays but did not react with the same antigen preparation by immuno-dot blot, a type of immunoassay in which the antigen is placed on the blotting membrane under nondenaturing conditions (Fig. 5; compare strips A). In contrast to the pattern of reactivity exhibited by anti-pEX480, the rabbit polyclonal antiserum raised against HPV-11 whole virus particles did not react with recombinant L1 by Western blot but reacted strongly with recombinant L1 in the immuno-dot blot assay (Fig. 5; compare strips C). This reactivity was specific as demonstrated by lack of reactivity in the postimmune serum against the native nonrecombinant control preparation (Fig. 5B, strip C). Rabbit anti-pEX215 antiserum was included in these immunoassays to allow comparison of the relative amounts of L1 present in the two types of immunoassays. The levels of immunoreactivity of the anti-pEX215 antiserum with recombinant L1 in both formats are roughly equivalent (Fig. 5, strips B), indicating that the amounts of L1 present are approximately equal. Furthermore, the observation that this antiserum is able to react with L1 in both formats suggests that the linear immunoreactive L1 amino-terminal epitope(s) is not obscured by the adoption of higher-order L1 conformation.

Immunoreactivity of VLP-containing insect cell extracts with human sera. To determine the prevalence of antibodies in human sera directed against linear versus conformational epitopes, sera obtained from individuals with biopsy-proven condyloma acuminatum were evaluated in Western blot and immuno-dot blot assays using recombinant L1 as antigen. None of the patient or control sera were immunoreactive with denatured recombinant L1 by Western blot (Fig. 6, strips D to O [patients] and P to X [controls]). Conversely, 11 of 12 patient serum samples (Fig. 7; strips D to O were read as positive, with the exception of strip H) and 0 of 9 control serum samples (Fig. 7, strips P to X) were immunoreactive with recombinant L1 by immuno-dot blot, a highly statistically significant difference ($P = 7 \times 10^{-5}$; Fisher's exact test). This result correlates well with results previously obtained with the same sera in an HPV-11 particle-based ELISA (2).

DISCUSSION

In this report, we have shown that expression of the L1 major capsid protein of HPV-11 in the baculovirus system is sufficient for the formation of VLPs. In addition, purified recombinant L1 protein spontaneously assembled *in vitro* to form a variety of aggregates, including particles with morphologic characteristics similar to those of the VLPs that formed *in vivo*. These observations indicate that L1 has an intrinsic capacity for self-assembly and that other papillomavirus proteins are not required for VLP formation in the baculovirus system. These recombinant VLPs also possess immunologic characteristics of HPV-11 virions as defined by rabbit antisera and can be used as antigens to detect antibodies to HPV-11 in human sera.

FIG. 4. Electron micrograph of purified L1 preparation demonstrates *in vitro* self-assembly. Shown is purified L1 protein (Fig. 3, lane 4) after equilibration in 2 M NaCl for 1 week. The single field shows L1 self-assembled aggregates of various diameters. Arrowheads indicate clumps of unassembled capsomeres. Bar = 100 nm.

Zhou et al. have reported the formation of HPV-16 VLPs in CV-1 cell nuclei following infection with a vaccinia virus HPV-16 L1-L2 double-recombinant expression vector but not with a vector expressing L1 alone (39). These VLPs lacked a well-defined symmetry and were more variable in size and smaller (35 to 40 nm in diameter) than either HPV virions (55 nm) or baculovirus-produced HPV-11 VLPs (≈ 50 nm). Differences in levels of foreign gene expression between the vaccinia virus and baculovirus systems may explain why L1 alone forms VLPs in insect cells. High-level baculovirus expression of L1 protein might drive VLP assembly in the absence of L2. The relative inefficiency of VLP assembly in Sf-9 cells, in comparison with the amount

of L1 expressed, supports this hypothesis. Fundamental biological differences between HPV-16 and HPV-11 could also explain the discrepancies between the vaccinia virus and baculovirus expression systems. For example, it is known that particle formation is more common for HPV-11 than for HPV-16 in naturally infected tissues and that HPV-16 L1 mRNA is rapidly degraded (13, 14). A less likely explanation is that the L1 proteins produced in the two systems are qualitatively different because of viral or cellular differences in transcription, translation, and/or posttranslational processing mechanisms.

Other investigators have reported the expression of HPV-16 L1 and L2 coding sequences in the baculovirus

FIG. 5. Western blot and immuno-dot blot comparisons of rabbit antiserum immunoreactivities with recombinant L1. (A) Recombinant L1 was electrophoresed under denaturing conditions and blotted to an Immobilon-P membrane. Molecular weights (in thousands) are shown at left. (B) Nonrecombinant (+) or recombinant L1 (L1) stock solutions were applied to an Immobilon-P membrane under nondenaturing conditions. Strips A, postimmune rabbit anti-denatured L1 common antigen (anti-pEX480) antiserum (35); strips B, postimmune rabbit anti-denatured L1 amino-terminal (anti-pEX215) antiserum (35); strips C, rabbit anti-whole virus particle antiserum (4). Primary rabbit antisera were diluted 1:2,000.

system (38). Although VLP formation was not reported in that study, the formation of a complex between the L1 and L2 proteins was suggested by results obtained from immunoprecipitation experiments using HPV-16 L1-L2-coinfected Sf-9 cell lysates. We have conducted similar experiments in which we attempted to immunoprecipitate metabolically labelled HPV-11 L1-L2 complexes from coinfecting Sf-9 cell lysates. Although the L1 and L2 labelled proteins were successfully immunoprecipitated with cognate antisera, we obtained no evidence of L1-L2 complex formation (data not shown). This result was unexpected in view of the extremely high level of sensitivity of the radioimmunoprecipitation assay and suggests the need for further investigation of the ability of these proteins to interact with each other.

Recently, Kirnbauer et al. have reported the expression of bovine papillomavirus type 1 and HPV-16 L1 coding sequences in the baculovirus system and have reported the presence of VLPs in CsCl gradient fractions of recombinant-infected Sf-9 cell lysates (16). Although serologic analyses using HPV-16 VLPs and genital HPV-infected patient sera were not conducted, the results obtained with antibodies raised against bovine papillomavirus type 1 VLPs in a focus-forming assay suggested that these antibodies were able to inhibit the ability of bovine papillomavirus type 1 to infect C127 cells. This observation supports the hypothesis that antibodies with conformational epitope specificities are involved in the neutralization phenomenon (4, 6-8).

Papillomavirus and polyomavirus virions are both T=7 icosahedral structures composed of 72 pentameric capsomeres of either L1 (1) or VP1 (31). It is therefore perhaps not unexpected that the L1 and VP1 proteins have common biochemical and structural properties despite differences in their primary sequences. Both VP1 and L1 have similar chromatographic properties, and both form pentamers that can self-assemble in vitro into multiple larger aggregates, including assemblies resembling viral capsids. In addition, the capsidlike particles formed after expression of papillomavirus L1 and polyomavirus VP1 (24) in insect cells are more homogeneous in size and morphology than the products assembled in vitro, indicating that under physiologic conditions bonding between capsomeres appears optimized for the T=7 structure. Further studies will be necessary to determine both the protein domains and the range of buffer conditions, e.g., calcium concentrations and pH, that promote L1 in vitro self-assembly. The comparison of the interactions utilized by L1 and VP1 capsomeres in forming capsids should yield a more general understanding of the bonding strategies used in forming a common final structure from distinctly different protein subunits.

Our immunologic observations suggest that recombinant L1 adopts a native conformation. The rabbit antiserum raised against the denatured L1 common antigen was immunoreactive only with denatured recombinant L1 (i.e., by Western blot), whereas the rabbit antiserum raised against nondenatured whole virus particles reacted only with nondenatured recombinant L1 (i.e., by immuno-dot blot). Furthermore, human sera from condyloma acuminatum patients which were reactive with HPV-11 virions in an ELISA (2) also reacted with nondenatured HPV-11 recombinant L1. Therefore, it appears that VLP conformational epitopes are similar to those present in native HPV-11 virions, which are recognized by the human immune system during natural infection.

FIG. 6. Western blot assay using recombinant L1 insect cell lysate and human sera. Control strips A and B, pre- and postimmune rabbit anti-whole virus particle antisera; control strip C, postimmune rabbit anti-denatured L1 antiserum (35); strips D to O, condyloma acuminatum patient sera; strips P to X, control sera. Molecular weight markers (in thousands) are at the left, as is the arrowhead denoting the position of 55-kDa recombinant L1.

FIG. 7. Immuno-dot blot assay using insect cell lysates. Letters correspond to different primary antibodies as described in the legend to Fig. 6. Nonrecombinant (+) and recombinant L1 (L1) diluted stock solutions were used.

Several studies of papillomavirus serology demonstrate that conformational epitope antibody specificities are good indicators of papillomavirus infection (2–4, 8, 15, 33). These specificities may also play a significant role in viral pathogenesis. For instance, we and others have shown that a rabbit antiserum directed against whole HPV-11 particles neutralizes HPV-11 infectivity (4, 6). Furthermore, Christensen et al., using human sera, have reported a correlation between anti-whole HPV-11 virion antibody and serum neutralizing activity (8). Detection of such antibodies with our recombinant L1 VLP preparation may have diagnostic and functional significance.

VLPs such as those described in the present report could be used in a variety of ways to increase our understanding of HPV biology. For example, they could be employed to study HPV capsid formation. VLPs may also play a role in the identification and characterization of an HPV cell receptor and should be considered as potential candidates for vaccine development. In addition, VLPs may provide abundant reagents with which to carry out immunologic studies not only of HPV-11 but of other HPV types as well.

ACKNOWLEDGMENTS

This work was supported by Public Health Service awards AI-82509 (R. C. Reichman) from the National Institute of Allergy and Infectious Diseases and CA37667 (R. L. Garcea) from the National Cancer Institute, Department of Health and Human Services.

REFERENCES

- Baker, T. S., W. W. Newcomb, N. H. Olson, L. M. Cowser, C. Olson, and J. C. Brown. 1991. Structures of bovine and human papillomaviruses: analysis by cryoelectron microscopy and three-dimensional image reconstruction. *Biophys. J.* **60**:1445–1456.
- Bonnez, W., C. DaRin, R. C. Rose, and R. C. Reichman. 1991. Use of human papillomavirus type 11 virions in an ELISA to detect specific antibodies in humans with condylomata acuminata. *J. Gen. Virol.* **72**:1343–1347.
- Bonnez, W., C. DaRin, R. C. Rose, S. K. Tying, and R. C. Reichman. Evolution of the antibody response to human papillomavirus type 11 (HPV-11) in patients with condyloma acuminatum according to treatment response. *J. Med. Virol.*, in press.
- Bonnez, W., R. C. Rose, and R. C. Reichman. 1992. Antibody-mediated neutralization of human papillomavirus type 11 (HPV-11) infection in the nude mouse. Detection of HPV-11 mRNAs by the polymerase chain reaction. *J. Infect. Dis.* **165**:376–380.
- Bonnez, W., R. C. Rose, R. C. Reichman, and D. G. Strike. 1990. The *Pst*I-*Xho*II restriction fragment of the HPV-6b L1 ORF lacks immunological specificity as determined by sera from HPV 6 condyloma acuminatum patients and controls. *UCLA Symp. Mol. Cell. Biol.* **124**:77–80.
- Christensen, N. D., and J. W. Kreider. 1990. Antibody-mediated neutralization in vivo of infectious papillomaviruses. *J. Virol.* **64**:3151–3156.
- Christensen, N. D., J. W. Kreider, N. M. Cladel, S. D. Patrick, and P. A. Welsh. 1990. Monoclonal antibody-mediated neutralization of infectious human papillomavirus type 11. *J. Virol.* **64**:5678–5681.
- Christensen, N. D., J. W. Kreider, K. V. Shah, and R. F. Rando. 1992. Detection of human serum antibodies that neutralize infectious human papillomavirus type 11 virions. *J. Gen. Virol.* **73**:1261–1267.
- Crum, C. P., and R. U. Levine. 1984. Human papillomavirus infection and cervical neoplasia: new perspectives. *Int. J. Gynecol. Pathol.* **3**:376–388.
- Dunn, A. E. G., and M. M. Ogilvie. 1968. Intranuclear virus particles in human genital wart tissue: observations on the ultrastructure of the epidermal layer. *J. Ultrastruct. Res.* **22**:282–295.
- Gheysen, D., E. Jacobs, F. de Foresta, C. Thiriart, M. Francotte, D. Thines, and M. De Wilde. 1989. Assembly and release of HIV-1 precursor Pr55gag virus-like particles from recombinant baculovirus-infected insect cells. *Cell* **59**:103–112.
- Jenison, S. A., J. M. Firzlauff, A. Langenberg, and D. A. Galloway. 1988. Identification of immunoreactive antigens of human papillomavirus type 6b by using *Escherichia coli*-expressed fusion proteins. *J. Virol.* **62**:2115–2123.
- Kennedy, I. M., J. K. Haddow, and J. B. Clements. 1990. Analysis of human papillomavirus type 16 late mRNA 3' processing signals in vitro and in vivo. *J. Virol.* **64**:1825–1829.
- Kennedy, I. M., J. K. Haddow, and J. B. Clements. 1991. A negative regulatory element in the human papillomavirus type 16 genome acts at the level of late mRNA stability. *J. Virol.* **65**:2093–2097.
- Kienzler, J. L., M. T. Lemoine, G. Orth, N. Jibard, D. Blanc, R. Laurent, and P. Agache. 1983. Humoral and cell-mediated immunity to human papillomavirus type 1 (HPV-1) in human warts. *Br. J. Dermatol.* **108**:665–672.
- Kirnbauer, R., F. Booy, N. Cheng, D. R. Lowy, and J. T. Schiller. 1992. Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. *Proc. Natl. Acad. Sci. USA* **89**:12180–12184.
- Klug, A., and J. T. Finch. 1965. Structure of viruses of the papilloma-polyoma type. I. Human wart virus. *J. Mol. Biol.* **11**:403–423.
- Koutsky, L. A., D. A. Galloway, and K. K. Holmes. 1988. Epidemiology of genital human papillomavirus infection. *Epidemiol. Rev.* **10**:122–163.
- Kreider, J. W., M. K. Howett, A. E. Leure-Dupree, R. J. Zaino, and J. A. Weber. 1987. Laboratory production in vivo of infectious human papillomavirus type 11. *J. Virol.* **61**:590–593.
- Kreider, J. W., M. K. Howett, S. A. Wolfe, G. L. Bartlett, R. J. Zaino, T. V. Sedlacek, and R. Mortel. 1985. Morphological transformation *in vivo* of human uterine cervix with papillomavirus from condylomata acuminata. *Nature (London)* **317**:639–641.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680–685.
- Li, C.-C. H., K. V. Shah, A. Seth, and R. V. Gilden. 1987. Identification of the human papillomavirus type 6b L1 open reading frame protein in condylomas and corresponding antibodies in human sera. *J. Virol.* **61**:2684–2690.
- Lopez de Turiso, J. A., E. Cortes, C. Martinez, R. Ruiz de Ybanez, I. Simarro, C. Vela, and I. Casal. 1992. Recombinant vaccine for canine parvovirus in dogs. *J. Virol.* **66**:2748–2753.
- Montross, L., S. Watkins, R. B. Moreland, H. Mamon, D. L. D. Caspar, and R. L. Garcea. 1991. Nuclear assembly of polyomavirus capsids in insect cells expressing the major capsid protein VP1. *J. Virol.* **65**:4991–4998.
- Pfister, H., and H. zur Hausen. 1978. Seroepidemiological

- studies of human papilloma virus (HPV-1) infections. *Int. J. Cancer* **21**:161-165.
26. Reichman, R. C., and W. Bonnez. 1990. Papillomaviruses, p. 1191-1200. *In* G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennett (ed.), *Principles and practice of infectious diseases*, 3rd ed. Churchill Livingstone, New York.
 27. Rose, R. C., W. Bonnez, D. G. Strike, and R. C. Reichman. 1990. Expression of the full-length products of the HPV-6b and HPV-11 L2 open reading frames by recombinant baculovirus, and antigenic comparisons with HPV-11 whole virus particles. *J. Gen. Virol.* **71**:2725-2729.
 28. Roy, P., T. French, and B. J. Erasmus. 1992. Protective efficacy of virus-like particles for bluetongue disease. *Vaccine* **10**:28-32.
 29. Sabara, M., M. Parker, P. Aga, C. Cosco, E. Gibbons, S. Parsons, and L. A. Babiuk. 1991. Assembly of double-shelled rotaviruslike particles by simultaneous expression of recombinant VP6 and VP7 proteins. *J. Virol.* **65**:6994-6997.
 30. Saliki, J. T., B. Mizak, H. P. Flore, R. R. Gettig, J. P. Burand, L. E. Carmichael, H. A. Wood, and C. R. Parrish. 1992. Canine parvovirus empty capsids produced by expression in a baculovirus vector: use in analysis of viral properties and immunization of dogs. *J. Gen. Virol.* **73**:369-374.
 31. Salunke, D. M., D. L. Caspar, and R. L. Garcea. 1989. Polymorphism in the assembly of polyomavirus capsid protein VP1. *Biophys. J.* **56**:887-900.
 32. Stanley, K. S., and J. P. Luzzio. 1984. Construction of a new family of high efficiency bacterial expression vectors: identification of cDNA clones coding for human liver proteins. *EMBO J.* **3**:1429-1434.
 33. Steele, J. C., and P. H. Gallimore. 1990. Humoral assays of human sera to disrupted and nondisrupted epitopes of human papillomavirus type 1. *Virology* **174**:388-398.
 34. Stoscheck, C. M. 1990. *Methods in enzymology*, vol. 182: quantitation of proteins, p. 54. Academic Press, Inc., New York.
 35. Strike, D. G., W. Bonnez, R. C. Rose, and R. C. Reichman. 1989. Expression in *Escherichia coli* of seven DNA segments comprising the complete L1 and L2 open reading frames of human papillomavirus type 6b and the location of the "common antigen." *J. Gen. Virol.* **70**:543-555.
 36. Summers, M. D., and G. E. Smith. 1987. *A manual of methods for baculovirus vectors and insect cell culture procedures*. Texas A&M University, College Station.
 37. Urakawa, T., M. Ferguson, P. D. Minor, J. Cooper, M. Sullivan, J. W. Almond, and D. H. Bishop. 1989. Synthesis of immunogenic, but non-infectious, poliovirus particles in insect cells by a baculovirus expression vector. *J. Gen. Virol.* **70**:1453-1463.
 38. Xi, S.-Z., and L. M. Banks. 1991. Baculovirus expression of the human papillomavirus type 16 capsid proteins: detection of L1-L2 protein complexes. *J. Gen. Virol.* **72**:2981-2988.
 39. Zhou, J., X.-Y. Sun, D. J. Stenzel, and I. H. Frazer. 1991. Expression of vaccinia recombinant HPV 16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. *Virology* **185**:251-257.
 40. zur Hausen, H. 1985. Genital papillomavirus infections, p. 83-90. *In* P. W. J. Rigby and N. M. Wilkie (ed.), *Viruses and cancer*. Cambridge University Press, Cambridge.