Serological differentiation of human papillomavirus types 11, 16 and 18 using recombinant virus-like particles

Robert C. Rose,1,2* William Bonnez,1 Caroline Da Rin,1 Dennis J. McCance2,3 and Richard C. Reichman1,2

1 Infectious Diseases Unit, Department of Medicine, 2 Department of Microbiology and Immunology and 3 Cancer Center, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, U.S.A.

The L1 major capsid protein-coding sequences of human papillomavirus (HPV) types 11, 16 and 18 were expressed in the baculovirus system. Virus-like particles (VLPs) were purified from recombinant-infected Spodoptera frugiperda Sf9 cells and cell-free culture supernatants. Rabbits immunized with purified VLPs developed antibodies that reacted only with the specific VLP type used as the immunogen. In addition, rabbit antibodies raised against infectious HPV-11 virions only reacted with HPV-11 L1 VLPs and not with VLPs derived from either HPV-16 or HPV-18. These results suggest that HPV-11, HPV-16 and HPV-18 virions are antigenically distinct from one another. This observation should be considered in future studies of immune responses to HPV.

Human papillomaviruses (HPVs) cause warts and other epithelial hyperplasias (Reichman & Bonnez, 1990). These viruses have the same genetic organization and are differentiated from one another if they have less than 90% nucleic acid sequence identity within each of the E6, E7 and L1 open reading frames (ORFs). By this method, 70 different HPV genotypes have been identified (zur Hausen, 1994). A subset of these viruses cause most cervical intraepithelial neoplasias (CIN) and carcinomas (Lorincz et al., 1992; Schiffman et al., 1993). The antigenic properties of HPV virions have not been adequately characterized, owing to lack of availability of virus particles for most HPV types. To provide alternative sources of native antigen for immunological studies, investigators have expressed HPV capsid coding sequences in eukaryotic expression systems (Rose et al., 1990, 1993; Xi & Banks, 1991; Zhou et al., 1991; Kirnbauer et al., 1992; Hagensee et al., 1993). Previously, we have shown that HPV-11 recombinant virus-like particles (VLPs) retain the antigenic characteristics of HPV-11 whole virions (Rose et al., 1993). These immunological similarities include reciprocal cross-reactivity of non-denatured capsid epitopes (Rose et al., 1993, 1994), the ability to elicit the formation of neutralizing antibodies (Rose et al., 1994) and the ability to detect specific antibodies in sera of patients with condyloma acuminatum (Rose et al., 1993, 1994). HPV-1 L1 and L2 VLPs have also been used to detect antibodies in sera from individuals with planter warts (Carter et al., 1993, 1994). These observations suggest that VLPs may be reliable substitutes for whole virions in immunological studies.

To investigate the capsid antigenic relationships among some of the most prevalent HPV types, we produced recombinant HPV-16 and HPV-18 L1 VLPs by the same method used for the production of HPV-11 L1 VLPs (Rose et al., 1993). In this study, we report that antibodies raised against non-denatured HPV-11, HPV-16 and HPV-18 L1 VLPs, and antibodies raised against HPV-11 whole virions, do not cross-react with heterotypic L1 VLPs.

The source of HPV-11 genomic DNA (Bonnez et al., 1991) and construction of the Ac11L1 recombinant baculovirus (Rose et al., 1993) have been described previously. The HPV-16 L1 sequence derived from the prototype strain (Seedorf et al., 1985) was found to be deficient in VLP assembly (R. C. Rose et al., unpublished results). A tentative explanation for this deficiency has been described by Kirnbauer et al. (1993). Therefore, the HPV-16 L1 recombinant baculovirus (Ac16L1) used in the present study was constructed by standard methods from an alternative strain of HPV-16 that was recovered from a CIN III lesion (P. M. Chesters & D. J. McCance, unpublished results). The HPV-18 L1 sequence was amplified by PCR from the HPV-18 prototype (kindly provided by H. zur Hausen, German Cancer Research Centre, Heidelberg, Germany) and used to construct Ac18L1 by the same procedure used for the construction of Ac11L1 (Rose et al., 1993).

Recombinant VLPs were purified as described (Rose et al., 1994). Single bands containing purified HPV-11, HPV-16 or HPV-18 VLPs were removed from CsCl density gradients by syringe, diluted with buffer A
Short communication

Fig. 1. Western immunoblot analysis of HPV-11, HPV-16 and HPV-18 purified L1 VLP preparations (lanes 1 to 3). Recombinant L1 proteins were detected with PVL1 common antigen serum (Strike et al., 1989) as described in the text.

(1 mM-MgCl₂, 1 mM-CaCl₂, 1 mM-PMSF, in PBS) to 12 ml and sedimented at 100,000 g for 90 min at 4 °C. Pellets were resuspended in 200 µl of buffer A containing 50% glycerol, quantified by spectrophotometry (A₂₈₀) and stored at −20 °C. Recombinant L1 proteins were analysed by SDS-PAGE and Western blot immunoassays as previously described (Rose et al., 1993). Samples containing 5 µg of purified HPV-11, HPV-16 or HPV-18 VLPs were separated by electrophoresis, blotted and probed with papillomavirus L1 (PVL1) common antigen rabbit antiserum as previously described (Strike et al., 1989; Rose et al., 1993). Predicted coding capacities of the HPV-11, HPV-16 and HPV-18 L1 ORFs are 501 amino acids (Dartmann et al., 1986), 505 amino acids (Seedorf et al., 1985) and 507 amino acids (Cole & Danos, 1987) respectively. An L1-immunoreactive band of the expected size (approximate M₉ value of 55K) appeared in each of the three sample preparations tested by Western blot immunoassay (Fig. 1). The PVL1 common antigen rabbit antiserum exhibited no background seroreactivity against material pelleted at 100,000 g from non-recombinant Autographa californica nuclear polyhedrosis virus (AcMNPV)-infected Sf9 cell suspension culture supernatant (data not shown). Lower M₉ L1-immunoreactive proteins were also detected by Western blot immunoassay of the CsCl-purified VLP preparations (Fig. 1) and are likely to be degradation products of full-length L1 proteins, as relative amounts of these proteins varied in subsequent analyses (data not shown). However, the major 55K L1-immunoreactive bands in each of the samples did not vary, either in their mobilities or their relative amounts (data not shown). Electron microscopy of purified samples (negatively stained with 2% phosphotungstic acid) confirmed VLP formation in HPV-11 (Fig. 2a), HPV-16 (Fig. 2b) and HPV-18 (Fig. 2c) VLP preparations.

HPV-11, HPV-16 and HPV-18 L1 VLP rabbit immune sera were prepared by immunizing two New Zealand...
Fig. 3. Antigenic specificities of HPV-11, HPV-16 and HPV-18 L1 VLPs measured by \( A_{405} \) values (see text). The antigens used were HPV-11 L1 VLPs (□), HPV-16 L1 VLPs (■) and HPV-18 L1 VLPs (▲). The antisera used were (A) PVL1-common antigen rabbit antiserum (Strike et al., 1989), (B) HPV-11 whole virion rabbit antiserum (Bonnez et al., 1992b), (C and D) HPV-11 L1 VLP rabbit antiserum, (E and F) HPV-16 L1 VLP rabbit antiserum and (G and H) HPV-18 L1 VLP rabbit antiserum.

White rabbits intramuscularly at two sites with each of the VLP preparations (six rabbits in total), using previously described methods (Bonnez et al., 1992b; Rose et al., 1994). Rabbit anti-PVL1 common antigen (Strike et al., 1989), HPV-11 whole virion (Bonnez et al., 1992b) and HPV-11, HPV-16 and HPV-18 VLP antisera were tested by ELISA against the three recombinant VLP preparations (Fig. 3). For this ELISA, purified VLPs were diluted to a concentration of 10 ng/µl in PBS and aliquots containing approximately 1 µg of antigen or PBS alone were dispensed into alternate rows of 96-well ELISA plates. The conditions of the assay were exactly as previously described (Rose et al., 1994), except primary antisera were pre-absorbed with non-recombinant (AcMNPV) baculovirus-infected Sf9 cell lysate diluted in 2% (v/v) blocking solution prior to testing. All antisera were tested in duplicate, on numerous occasions, at dilutions ranging from 1:1000 to 1:128000. \( A_{405} \) values for all of the rabbit anti-VLP antisera shown in Fig. 3 were obtained at the optimum dilution for these antisera of 1:16000. \( A_{400} \) values for the anti-PVL1 common antigen and HPV-11 whole virion rabbit antiserum were obtained at a lower dilution (1:1000). Specific \( A_{405} \) values were determined by subtracting control values (PBS wells) from experimental values (antigen-containing wells) for each replicate. Mean \( A_{405} \) values were calculated from these.

As shown in Fig. 3, HPV-11 whole virus particle (B) and HPV-11 VLP antisera (C and D) reacted strongly with HPV-11 VLPs, but none of these antisera reacted with the HPV-16 or HPV-18 VLP preparations. Similarly, HPV-16 (E and F) and HPV-18 (G and H) L1 VLP rabbit antiserum reacted only with homotypic VLPs. The specificities of these reactions were verified in pre-adsorption experiments, in which the immunoreactivity of each rabbit VLP antiserum was abolished by pre-adsorption with homotypic, but not heterotypic, VLPs (data not shown). None of the rabbit preimmune sera reacted with any of the VLP preparations (data not shown). The anti-PVL1 common antigen antiserum, which reacted strongly with recombinant L1 proteins by Western immunoblot (Fig. 1), reacted only weakly with native VLP preparations in the ELISA (Fig. 3). This observation suggests that epitopes normally recognized by this antiserum are masked under the conditions of the ELISA and that the L1 proteins tested in this assay are predominantly non-denatured.

The results of our study show that L1 VLP epitopes of HPV-11, HPV-16 and HPV-18 are antigenically distinct. Although L2 capsid proteins were not present in these VLP preparations, it is likely that the observed antigenic difference between HPV types also applies to virions. L2 represents approximately 10% of the total protein content of HPV particles (Doorbar & Gallimore, 1987) although its exact location in the particle has not been determined (Baker et al., 1991). Recent studies have suggested that it may be required for DNA encapsidation (Zhou et al., 1993) and that a domain present in the relatively conserved N-terminal portion of the HPV-16 L2 amino acid sequence mediates non-specific DNA
binding (Zhou et al., 1994). Although the remainder of the L2 amino acid sequence is very heterogeneous among papillomaviruses (Danos et al., 1984) and contains type-specific antigenic epitopes (Komly et al., 1986; Rose et al., 1990), it is unclear whether L2-specific antibodies react with intact virions (Komly et al., 1986; Hagensee et al., 1993). Recently, Carter et al. (1994) used HPV-1 L1 and L2 VLPs to detect capsid-specific antibodies in sera from individuals with plantar warts. Interestingly, serological responses were directed only against L1 conformational epitopes, with no detectable antibody response directed toward L2 (Carter et al., 1994). These observations suggest that L2 may be relatively inaccessible, or perhaps poorly immunogenic, when incorporated into virions. Thus, the presence of L2 may not significantly alter the antigenicity of whole virions.

Previous studies have indicated that different HPV types can be distinguished from one another using serological techniques. For example, antibodies that reacted with plantar wart virions were found much more commonly in sera from patients with plantar warts than in sera from patients with either common, flat, anogenital or laryngeal warts (Pfister & zur Hausen, 1978; Kienzler et al., 1983; Viac et al., 1990). Anisimová et al. (1990) have also shown directly by immunoelectron microscopy that HPV-1 and HPV-2 are antigenically distinct. However, it also appears that other HPV types are antigenically related. For example, we previously reported the detection of antibodies that specifically recognize HPV-11 virions in sera from patients with documented HPV-6 infection (Bonnew et al., 1991, 1992a). Owing to the lack of available virions for most HPV types, VLPs are at present the best tool available to explore antigenic relatedness among HPVs.

Antigenic differences among HPV types are likely to reflect genetic diversity within the L1 coding sequence. Chan et al. (1992) constructed a papillomavirus phylogenetic tree that was based upon genetic divergence within a defined region of the papillomavirus L1 amino acid sequence. Their work shows the relatively close evolutionary relationship between HPV-6 and HPV-11, which is consistent with potential cross-reactivity between HPV-6 and HPV-11 capsids. On the other hand, HPV-16 and HPV-18, which have diverged extensively in their L1 sequences, are expected to have little antigenic cross-reactivity with each other or with HPV-11. These predictions are consistent with the results of the present study.

The biological relevance of HPV capsid antigenic variability is unknown, but diversity of the capsid protein could account for papillomavirus tissue specificity. The availability of recombinant VLPs from a variety of papillomaviruses may prove useful in the identification of putative host- and tissue-specific cellular receptors. In addition, VLPs should play an important role in the delineation of the antigenic characteristics of HPVs and in studies of immune responses to these viruses.

We thank Sharon Bassigno for her assistance with electron microscopy. This work was supported in part by Public Health Service award AI-82509 (to R. C. Reichman).

References


\textit{Short communication} 2449