Analysis of type-restricted and cross-reactive epitopes on virus-like particles of human papillomavirus type 33 and in infected tissues using monoclonal antibodies to the major capsid protein

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A panel of six monoclonal antibodies recognizing at least three different antigenic regions has been raised against the L1 major capsid protein of human papillomavirus type 33 (HPV-33), which is associated with cervical carcinoma. The antigenic sites defined by these antibodies have been mapped and classified as type-restricted or broadly cross-reactive using bacterially expressed L1 fusion proteins of a variety of HPV types. Conformational and linear epitopes have been distinguished using native and denatured virus-like particles. HPV infection of genital lesions has been analysed using both monoclonal antibodies and DNA amplification by PCR. The antibodies obtained should be useful to probe the structure of HPV capsids and to develop a general assay for the detection and classification of productive HPV infections.

Introduction

Papillomaviruses are highly species-specific and can cause squamous epithelial and fibroepithelial tumours in their hosts. They infect exclusively the basal layer of epithelia and need differentiating cells to multiply. Human papillomaviruses (HPVs) are associated with benign or malignant hyperproliferations of a wide variety of clinical manifestations. More than 70 HPV types have been recognized, and each has a genome of about 7900 bp of circular dsDNA. Despite an overall similarity in genetic organization, their tissue specificity and malignant potential are rather diverse. HPV type 1 (HPV-1) is associated with deep plantar warts, HPV-8 is among the group of HPVs found in lesions of patients suffering from epidermodysplasia verruciformis, and HPV-6 and -11 have been isolated from condylomata acuminata. The strong association of several HPV types, notably HPV-16, -18, -31, -33, -35 and -39, with cervical carcinoma has focused much interest on this HPV subgroup (for review see de Villiers, 1989).

Attempts to understand the biology of HPV infection and the mechanisms involved in virion morphogenesis have been hampered by the lack of an efficient in vitro tissue culture system for the propagation of these viruses. By expression of the major capsid protein (L1) in eukaryotic cell lines, either alone or together with the minor capsid protein (L2), virus-like particles of HPV-1 (Hagensee et al., 1993), HPV-11 (Rose et al., 1993) and HPV-16 (Kirnbauer et al., 1993) have been obtained. Using a baculovirus expression system, we have previously produced virus-like particles of HPV-33 (Volpers et al., 1994), which is associated with malignant genital lesions (Cole & Streeck, 1986). These particles are composed of capsomers and have the same density and icosahedral symmetry as empty papillomavirus capsids. The possibility to generate such non-infectious, virus-like particles in high yield offers an experimental system for the induction and detection of clinically relevant immune responses to HPVs.

Many observations have indicated the importance of immune response in papillomavirus infections. Antibodies to the capsid proteins of various HPV types have previously been studied using bacterially expressed fusion proteins or chemically synthesized peptides. As a prerequisite to such studies, the type of infecting HPV has to be identified unambiguously. Until recently, diagnosis of an HPV type in a tissue required nucleic acid hybridization assays (Syrjänen, 1990). As a more specific and a more sensitive method amplification of HPV DNA by PCR has been developed both for general detection and for the identification of HPVs, particularly in genital infections (Manos et al., 1989; Snijders et al., 1990; van den Brule et al., 1990). One difficulty with this procedure
is the problem of quantification, the difficulty being to distinguish between latent subclinical infections and obvious clinical lesions.

As an alternative to hybridization and PCR, immunological detection of viral capsid antigens can be used for the diagnosis of productive HPV infections. However, the polyclonal antisera to disrupted bovine papillomavirus (BPV-1), which is widely used, is broadly cross-reactive and thus of little value for typing HPV infections (Gupta et al., 1987; Alonso et al., 1992). More recently, monoclonal antibodies (MAbs) to the capsid proteins of HPVs have been obtained which exhibit either broad cross-reactivity (Iwasaki et al., 1992) or more restricted specificity (McLean et al., 1990; Shepherd et al., 1992).

To investigate the potential of immunocytochemistry for the identification of individual HPV types and to compare the antigens detectable in infected tissues with those on virus-like particles, we have now raised MAbs to the major capsid protein of HPV-33. The specificities of these antibodies have been determined and the antigenic sites mapped. In this work we have used the MAbs for the characterization of epitopes on HPV-33 virus-like particles and for the identification of HPVs in genital lesions.

Methods

Construction of expression plasmids. To construct pRIL1-340, a BalI–PstI fragment of pHV33 (Cole & Streeck, 1986) (nucleotides (nt) 5731 to 6746), was cloned into BamHI–PstI-cut pRT2T (Nilsson et al., 1985) of which the BamHI site had been filled in by Klenow polymerase treatment. pG33L1-47/339 was obtained by cloning a BalI–ScaI fragment of pHV33 (nt 5731 to 6610) into XmaI-cut pGEXXIX (Smith & Johnson, 1988) after filling in of the vector by Klenow polymerase. Corresponding fragments of the L1 open reading frame (ORF) of HPV-1 (nt 5855 to 6673), HPV-8 (nt 5909 to 7077), HPV-11 (nt 5886 to 7076), HPV-16 (nt 5593 to 7463), HPV-18 (nt 5731 to 6933), and HPV-39 (nt 5760 to 6829) were cloned in-frame to the glutathione S-transferase (GST) gene of pGEXXIX to generate plasmids pG11L1, pG8L1, pG11L1, pG16L1, pG18L1, and pG39L1, respectively. The L1 sequences of the various HPV types present in the fusion proteins correspond to amino acids 148 to 420 (HPV-1), 48 to 439 (HPV-8), 39 to 436 (HPV-11), 46 to 531 (HPV-16), 102 to 501 (HPV-18), and 48 to 395 (HPV-39). pBR322-based plasmids with the complete sequence of HPV-1 (Danos et al., 1982), HPV-8 (Fuchs et al., 1986), HPV-11 (Dartmann et al., 1986) or HPV-16 (Seedorf et al., 1985) were kindly provided by E. M. de Villiers (Deutsches Krebsforschungszentrum, Heidelberg, Germany), pHPV18 (Cole & Danos, 1987) and pHPV39 (Volpers & Streeck, 1991) were a gift from G. Orth (Institut Pasteur, Paris, France). Subfragments of the L1 gene of HPV-33 were cloned following RsaI digestion of a BamHI–EcoRI fragment from pG33L1-47/339. The fragments obtained (nt 6122 to 6274, 6275 to 6388, 6420 to 6610) were fused in-frame to the GST gene of pGEXXIX to obtain pG33L1-177/227, pG33L1-228/265, and pG33L1-276/339, respectively. Construction of deletion mutants via PCR was carried out as described before (Volpers et al., 1993).

Expression and purification of fusion proteins. The Protein A–L1 fusion protein RIL1-340 encoded by pRIL1-340 was expressed in Escherichia coli N4830-1 and purified using IgG-Sepharose 6FF according to the supplier's instructions (Pharmacia) except that the fusion protein was eluted with 25 mM-HEPES pH 3.0, and neutralized by addition of sodium hydroxide. The GST–L1 fusion proteins were expressed in E. coli DH5 for 3 to 5 h following addition of IPTG. The bacteria were lysed by two subsequent sonications in PBS supplemented with 1% Triton X-100. The pellet containing the fusion protein was resuspended in sample buffer (Laemmli, 1970) and boiled. To purify fusion protein G33L1-47/339 encoded by pG33L1-47/339, bacterial extract was subjected to preparative 10% SDS–PAGE (Laemmli, 1970). Protein bands were visualized as potassium dodecyl sulphate precipitates, cut out and eluted from the gel by diffusion. Protein was concentrated in a Speed Vac and dialysed against several changes of PBS for 48 h to remove dodecyl sulphate.

Immunization of mice and isolation of MAbs. Six-week-old BALB/c mice were immunized with 20 μg of RIL1-340 in complete Freund's adjuvant followed by one boost with RIL1-340 in incomplete Freund's adjuvant and three boosts each with 20 μg G33L1-47/339 in incomplete Freund's adjuvant injected intraperitoneally at 5 to 6 week intervals. Five days after the final boost a mouse was sacrificed and the spleen removed. A single cell suspension was obtained by pressing the spleen through a 60-mm sieve. The spleen cells were fused to myeloma cell line X63Ag8.653 maintained in Iscove's modified Eagle medium (Gibco) supplemented with 10% fetal calf serum (Gibco) and 50 mM-f-mercaptoethanol. The fusion was performed in polyethylene glycol 2500 (Boehringer Mannheim) using standard protocols (Galffe et al., 1977). Cells were distributed into 1000 wells and selected with hypoxanthine and azaserine (Sigma). After 6 to 8 days the cell culture supernatants were screened for secretion of antibodies directed against the L1 portion of the fusion protein by ELISA using gel-purified G33L1-47/339 as antigen. Immunoresponses were visualized by use of horseradish peroxidase-conjugated goat anti-mouse IgG (H + L) (Jackson ImmunoResearch) and 1,2-phenyldiamine dihydrochloride. Positive clones were further screened by ELISA using GST and other non-L1 GST fusion proteins as antigen to exclude antibodies directed against the GST portion of the fusion protein or E. coli contaminants. Positive clones were subcloned twice by limited dilution.

Preparation of virus-like particles. Insect cells (Sf9) maintained in TNM-FH medium were co-infected with recombinant baculoviruses bac33L1 and bac33L2 for 5 days. Virus-like particles were solubilized from isolated nuclei and purified by two subsequent caesium chloride density gradients exactly as described (Volpers et al., 1994).

Biopsy specimens and DNA isolation. Five snap-frozen parts of biopsies of CIN I, three of CIN II and seven biopsy parts of condylomata acuminate were used for DNA isolation to perform HPV PCR analysis. If available, an additional biopsy part-fixed in 4% buffered formalin and embedded in paraffin was used for immunohistochemical examination. Snap-frozen samples were serially sectioned (10 to 15 sections) on a cryostate. The first and last sections (5 μm) were used for haematoxylin–eosin staining for histological examination. The sections in between (20 μm) were used for DNA isolation. DNA was extracted according to standard procedures (Walboomers et al., 1988).

HPV detection and typing. The general primers GP5 (5'-TTT GTT ACT GTG GTA GAT AC-3') and GP6 (3'-ACT AAA TGT CAA ATA AAA AG-5'), which span a region of 140 to 150 bp within the HPV L1 ORF, were used in the general primer-mediated PCR (GP-PCR) described previously to detect a broad spectrum of HPV types (Snijders et al., 1990). The type-specific PCR was performed using combinations of HPV-6, -16, -33- and HPV-11, -18, -31-specific cloning site-flanking primers as described (van den Brule et al., 1990). GP-PCR-positive samples were identified by Southern blot analysis of the PCR products using a cocktail of HPV-6, -11, -16, -18, -31 and -33-
specific probes under moderate stringency conditions for hybridization. The analysis of HPV type-specific PCR products was carried out using HPV-6, -11, -16, -18, -31 and -33-specific 32P-end-labelled oligonucleotides (van den Brule et al., 1990).

**Immunohistochemistry.** Paraffin-embedded sections on coated slides (0.1% poly-L-lysine) were deparaffinized with xylene, rehydrated, and treated three times for 5 min each at 95 °C in citric buffer pH 6.0. Endogenous peroxidase was blocked by incubating for 30 min with methanol containing 0.3% H2O2. After washing repeatedly in PBS, sections were preincubated with normal rabbit serum (1:50) for 10 min. Subsequently, incubation was performed with the MAbs at room temperature for 60 min at the indicated dilution. MAbs were detected with biotinylated rabbit anti-mouse Fab antibody fragment (1:500) (Vector Lab). Detection was performed by incubation with horseradish peroxidase coupled to an avidin-biotin complex (1:500) (Vector Lab) for 60 min. The complex was visualized using diaminobenzidine and H2O2. Slides were counterstained with haematoxylin, dehydrated and mounted in Depex. A similar protocol was used for frozen sections except that the deparaffinization step and the citric buffer step were omitted and the sections were fixed for 3 min in acetone and stored at -20 °C before use.

**Results**

**Specificity of MAbs to the L1 major capsid protein**

To generate MAbs to the major capsid protein of HPV-33, fragments of the L1 ORF of the HPV-33 genome encoding amino acids 47 to 384 or 47 to 339 were expressed in *E. coli* as fusion proteins with the IgG-binding domain of Protein A or with GST, respectively. The purified Protein A fusion protein was used to inoculate mice, and the GST fusion protein was used for boosting to stimulate the immune response to L1. Hybridomas obtained using standard procedures were screened with the GST-L1 fusion protein, and six independent isolates were established as cell lines secreting MAbs directed against L1. All antibodies were of the IgG1 subtype except one (MAb 33L1-14) which was of the IgG2a subtype.

To map the binding sites of the MAbs (MAbs 33L1-1, -2, -4, -7, -8 and -14), L1 restriction fragments and overlapping subfragments amplified by PCR were cloned as fusions with GST and expressed in *E. coli*. Immunoreactive proteins were identified in crude extracts by Western blotting (Fig. 1). Fusions containing L1 amino acids 176 to 227 reacted with MAb 33L1-1,-2,-4,-7,-8 and -14, whereas L1 amino acid sequences 47 to 209 (not shown), 228 to 265, and 276 to 339 were unreactive (Fig. 1b). Fine mapping indicated that the epitopes of these antibodies had the L1 sequence NKSDVPID (amino acids 216 to 223) in common. The reactivity with MAb 33L1-1 is shown as an example in Fig. 1(b).

The epitope of MAb 33L1-7 was mapped to the amino acid sequence 303 to 313 (EQLFNKPYWL). A fusion
Fig. 2. Cross-reactivity with L1 proteins of other HPV types. Fragments of the L1 genes of HPV-1, -8, -11, -16, -18, -33 and -39 fused to GST were expressed in E. coli, as described in detail in Methods. Whole cell lysates were analysed by 10% SDS-PAGE and either stained with Coomassie blue (a) or transferred to nitrocellulose and immunostained with MAbs 33L1-1 (b), 33L1-7 (c) or 33L1-8 (d). G33L1 is identical to G33L1-47/339.

protein containing amino acids 305 to 313 was also reactive although very weakly (Fig. 1 c). Fusion proteins extending only to amino acid 310 were completely unreactive (data not shown).

MAb 33L1-8 was strongly reactive with fusion proteins carrying the L1 amino acid sequence 239 to 245 (SEPYGDS). However the reactivity of the antibody was dramatically reduced when L1 sequences carboxy-terminal of the epitope were included in the fusion protein, as observed for G33L1-228/265 (Fig. 1d), G33L1-239/250, -239/255 and -239/265 (data not shown). It is possible that the accessibility of the epitope to this antibody is sterically blocked by the three consecutive phenylalanine residues, amino acids 247 to 249 of the L1 protein. It should be noted that MAb 33L1-8 also reacted preferentially with minor degradation products in the antigen preparation used for immunization and screening.

To characterize further the specificity of MAbs 33L1-1 to -14, their reactivity with other HPV types was studied. To do so, the L1 proteins of HPV-1, -8, -11, -16, -18 and -39 fused to GST were assayed in Western blots (Fig. 2). As expected from the epitope mapping, MAbs 33L1-1, -2, -4 and -14 were reactive with the same fusion proteins. Fig. 2(b) shows the results obtained with MAb 33L1-1: a positive reaction with the L1 proteins of HPV-8, -11 and -33, no reaction with HPV-1, -16, -18 and -39. MAb 33L1-7 reacted with the L1 proteins of all HPV types tested except HPV-1 (Fig. 2c). MAb 33L1-8 bound only to the L1 proteins of HPV-16, -18 and -33 (Fig. 2d), again preferentially recognizing degradation products.

Characterization of epitopes on virus-like particles

We have recently obtained virus-like particles by expression of HPV-33 L1 alone or together with L2 using the baculovirus expression system (Volpers et al., 1994). To investigate whether the L1 antigenic determinants recognized by MAbs 33L1-1 to -14 correspond to linear or conformational epitopes and to test their accessibility on the virus-like particles, binding to native and denatured particles was analysed using a dot blot assay (Fig. 3). No differences in the reactivity of the MAbs with L2-positive versus L2-negative virus-like particles were observed. Surprisingly, all MAbs tested bound to untreated (native) virus-like particles on nitrocellulose indicating that the epitopes were exposed. However, only MAb 33L1-7 showed increased binding to particles that had been denatured by boiling in 0.5% SDS and 1% mercaptoethanol. The binding of MAbs 33L1-1 and -2 to denatured particles was severely reduced, whereas no binding was observed for MAbs 33L1-4, -8 and -14. The same reactivity was observed when the virus-like particles isolated from insect cells were submitted to SDS-PAGE.
Fig. 3. Reactivity with native (N) or denatured (D) virus-like particles. Empty virus-like particles purified by two successive cesium chloride gradients were diluted 1:5 in PBS and transferred either directly or after boiling for 10 min in 0.5% SDS and 1% β-mercaptoethanol to nitrocellulose membranes soaked in PBS. The membranes were blocked with PBS supplemented with 5% blotto and incubated with the indicated antibodies for 2 h. Bound antibodies were visualized after incubation with horseradish peroxidase-coupled secondary antibodies using enhanced chemiluminescence.

Table 1. Immunohistochemistry of HPV-33L1 MAbs*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>HPV type</th>
<th>L1-1</th>
<th>L1-4</th>
<th>L1-7</th>
<th>L1-8</th>
<th>L1-14</th>
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<tr>
<td>(a) Frozen tissue sections</td>
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<tr>
<td>Condyloma</td>
<td>HPV-11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Condyloma</td>
<td>HPV-11</td>
<td>ND†</td>
<td>–</td>
<td>ND†</td>
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<td>–</td>
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<tr>
<td>(b) Formalin-fixed, paraffin-embedded tissue sections</td>
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<tr>
<td>CIN I</td>
<td>HPV-6</td>
<td>–</td>
<td>–</td>
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<tr>
<td>CIN I</td>
<td>HPV-6</td>
<td>–</td>
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<td>Condyloma</td>
<td>HPV-11</td>
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<td>Condyloma</td>
<td>HPV-11</td>
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<td>Condyloma</td>
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<tr>
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<tr>
<td>CIN I</td>
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<tr>
<td>CIN II</td>
<td>HPV-16</td>
<td>+ +†</td>
<td>±</td>
<td>–</td>
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<tr>
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<td>–</td>
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<td>–</td>
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<tr>
<td>CIN I</td>
<td>HPV-33</td>
<td>+ +</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CIN II</td>
<td>HPV-33</td>
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* The antibodies were used at a dilution of 1:500.
† ND, Not determined.
‡ ++, Strong nuclear staining; +, clear nuclear staining; ±, weak nuclear staining.

and assayed by Western blot, i.e. no binding of MAbs 33L1-4, -8 and -14 to the L1 protein, but a positive reaction with MAbs 33L1-1, -2 and -7 (data not shown). These data indicate that the epitopes of MAbs 33L1-7 and possibly of MAbs 33L1-1 and -2 are linear, whereas MAbs 33L1-4, -8 and -14 recognize conformational epitopes. Since MAbs 33L1-4, -8 and -14 were reactive with fusion proteins (Fig. 1 and 2) but not with full-length L1 protein in Western blots, refolding of their epitopes under these conditions is likely to be favoured by truncation of L1. Interestingly, MAbs 33L1-4, -8 and -14 bound to virus-like particles that had been treated with SDS in the absence of β-mercaptoethanol (data not shown) suggesting that the conformation of the epitopes is stabilized by disulphide bonds. Although MAbs 33L1-1 and -2 bind to the same L1 amino acid sequence as MAbs 33L1-4 and -14, the fine tuning of the interaction with their epitope is clearly different.

Identification of L1 antigens in infected tissues

We were interested to analyse whether the MAbs could be used for immunohistochemistry and whether their specificities characterized by immunoblotting were preserved under these conditions. Therefore sections of condylomata and cervical intraepithelial neoplasias (CIN I and CIN II) were used for HPV typing by PCR and for immunohistochemical examination. Both snap-frozen and paraffin-embedded samples were stained with the various MAbs. The results obtained are summarized in Table 1, and examples of the immunohistochemical staining are shown in Fig. 4.

Several samples of frozen sections of condylomata containing HPV-11 were analysed. With one of these samples, L1 antigens were detected by MAbs 33L1-1, -4, -7 and -14, whereas no reaction was obtained with MAb 33L1-8 (Table 1a), in agreement with Western blotting (Fig. 2b to d). Photomicrographs of the histochemical analyses obtained with MAbs 33L1-1, -7 and -14 of an HPV-11-containing sample are shown in Fig. 4. With a section from another condyloma containing HPV-11, no reaction was observed using MAbs L1-4, -8 or -14 (Table 1a).

The histochemical analyses obtained on formalin-fixed, paraffin-embedded tissue sections are summarized in Table 1b. Examples of a CIN II lesion containing HPV-16 and a CIN I lesion containing HPV-33, are shown in Fig. 4. MAb 33L1-1 was reactive with a CIN I lesion containing HPV-33 (Table 1b, Fig. 4). MAbs 33L1-4 and -14 were not reactive with the formalin-fixed samples suggesting that their conformational antigens are not preserved under these conditions. Nuclear staining with MAb 33L1-8 was absent in paraffin-embedded sections containing HPV-6 and -11 but weakly positive with samples containing HPV-16 or -33 (Table 1b, Fig. 4). MAb 33L1-7 showed the best staining throughout (Fig. 4) and revealed positivity for an HPV-11-containing condyloma, an HPV-16-containing CIN II, and an HPV-33-containing CIN I. Nuclear staining was observed only in foci within the superficial cell layers and not in the basal and parabasal cell layers of CIN containing the atypical cells. No staining was observed with paraffin-
embedded sections containing HPV-6 and -11 (Table 1b), possibly owing to the absence of the capsid antigen.

**Discussion**

This work describes the characterization of linear and conformational epitopes of the HPV-33 L1 major capsid protein and their accessibility to MAbs on virus-like particles and in productively infected tissue. MAb 33L1-7 recognizes a conserved epitope and shows broad cross-reactivity with other HPV types. A consensus sequence E/D-S/A-Q-L/I-F-N-K/R-P-Y/F-W-L can be derived for this epitope from a comparison of the L1 sequences of the seven HPV types tested with this antibody (Fig. 5). Of all HPV types analysed in immunoblotting only HPV-1 L1 was unreactive, possibly owing to substitution of the proline residue in the epitope by serine. Of the 39 HPV L1 sequences available to us, 29 (74 %) conform to the consensus sequence and eight carry conservative exchanges: T, Q or V instead of S or A at position 304, I or M instead of L at position 313. Therefore we predict that at least 74 %, and possibly 95 % (37 of 39) of all sequenced HPV types, both cutaneous and genital, have L1 proteins which should bind to MAb 33L1-7. This indicates that MAb 33L1-7 could be useful for the development of a general HPV detection assay, complementing PCR-based HPV DNA detection assays, e.g. for epidemiological screening.

The preferential binding of MAb 33L1-7 to denatured virus-like particles indicates that its epitope, amino acid sequence 303 to 313, is linear and buried inside the capsid structure. The low reactivity observed with native particles may be accounted for by traces of L1 molecules not assembled into particles that may be present in the
preparation of the virus-like particles (Volpers et al., 1994). In addition, binding of the capsids to nitrocellulose (Fig. 3) may lead to some denaturation.

The internal location of the linear, broadly cross-reactive epitope of MAb 33L1-7 is in agreement with previous studies on cross-reactive epitopes carried by virions of HPV-1 (Orth et al., 1978) and BPV-1 (Cowsert et al., 1987). Whereas immunization with intact virions yields type-specific or type-restricted antisera, antisera raised against disrupted viral particles are broadly cross-reactive. Some of the cross-reactive epitopes of BPV-1 have been mapped using epitope scanning of synthetic peptides (Lim et al., 1990; Dillner et al., 1991). MAbs directed against such epitopes have been shown to be exclusively reactive with disrupted virions (Cowsert et al., 1987). The most widely shared cross-reactive epitope of BPV-1 L1 is located at the carboxy terminus (Dillner et al., 1991) but the corresponding region was not included in the HPV-33 fusion protein used to generate the antibodies described in this work.

According to the differential binding of MAbs 33L1-1, -2, -4, and -14 to native and denatured virus-like particles their epitope, amino acid sequence 216 to 223, should be externally exposed. Although these antibodies recognize the same epitope, they clearly define different conformational states of this sequence. Whereas MAbs 33L1-1 and -2 were reactive with denatured virus-like particles albeit weakly, no binding of MAbs 33L1-4 and -14 was observed. However the reactivity was retained when reducing agents were omitted during denaturation. Similarly, MAb 33L1-8 recognizing the epitope 239 to 245 on native but not on denatured particles, retained its reactivity when the particles had been denatured under non-reducing conditions. The generation of antibodies that bind to the same epitope but with different affinities is well known and may be owing to processing or partial unfolding of the protein used for immunization (Fieser et al., 1987).

The reactivity in Western blots of MAbs 33L1-4, -8, and -14 with fusion proteins expressed in E. coli can only be explained by a partial refolding of the epitope during transfer to nitrocellulose membranes, a refolding which is not observed with full-length L1 protein denatured under reducing conditions. This indicates that the presence of disulphide bonds within full-length L1 protein favours refolding of the conformational epitopes, possibly by separating these epitopes from interfering sequences in other domains of the protein. The conserved cysteines C-225 and C-229 could be involved in the intramolecular disulphide bonds. Truncation of L1 in the fusion proteins may have a similar effect.

Deduced from a sequence comparison, MAb 33L1-8 should cross-react with most of the sequenced HPV types associated with severe genital lesions that carry either the same epitope (e.g. HPV-16, -52 and -58) or a conservative variant (e.g. HPV-18, -45 and possibly HPV-31 and -35). Conversely, MAb 33L1-1 is expected to be mainly cross-reactive with L1 proteins of HPV types associated with benign genital lesions. Thus the MAbs described in this work have the potential to partially classify HPVs into low-risk viruses (reactive with MAbs 33L1-1 and -7), high-risk viruses (reactive with MAbs 33L1-7 and -8), and HPV closely related to HPV-33 (reactive with MAb 33L1-1, -7 and -8). However these predictions will need further verification, as sequences outside the primary epitopes can influence the reactivities of the antibodies.

MAbs directed against the major capsid protein of various HPV types have been described previously (Cason et al., 1989; Iwasaki et al., 1992; McLean et al., 1990; Shepherd et al., 1992). One of them binds to a sequence overlapping the epitope recognized by MAb 33L1-7. However this antibody did not exhibit the same degree of cross-reactivity (Cason et al., 1989). A widely cross-reactive anti-HPV MAb has been described recently but the corresponding epitope has not been mapped (Iwasaki et al., 1992).

One of the most promising aspects of this work is the potential of using the MAbs as reagents to diagnose genital HPV infections by immunohistological staining. However, more extensive screening will be required before it can be evaluated whether the antibodies are useful for routine clinical applications. In the present study (Table 1), a substantial number of thin sections from lesions that had been typed HPV-positive by PCR after extraction of DNA, reacted negatively. This is probably owing to the absence of antigen in the particular sections and not to short-comings of the antibodies, since samples that had been tested positive were always positive on retesting. Similarly, in previous studies only 60 to 80 % of lesions positive for HPV DNA were found to contain detectable amounts of the major capsid protein (Firzlaff et al., 1988; Aionso et al., 1992). The reactivity of the MAbs towards tissue sections also depended on the method of antigen preservation. The reduced reactivity of MAb 33L1-1 with fully denatured virus-like particles observed in dot blots (Fig. 3) was reflected in the immunohistochemical analysis (Table 1). Whereas staining by MAb 33L1-1 was very effective in frozen tissue sections, formalin-fixed, paraffin-embedded tissue samples were only faintly stained, probably owing to partial denaturation of the antigen. MAb 33L1-8 is likely to display the same preferential staining of frozen sections but this could not be verified since there were no HPV-16- or -33-containing frozen tissue sections available to us.

Using thin sections for immunocytochemistry may present a general problem that we have also encountered in this work, since different sections of the same biopsy
specimen may or may not contain HPV antigen. This makes an unambiguous diagnosis somewhat difficult. Undivided clinical samples, as obtained for example from lavages, may be more appropriate. Immunohistochemical testing of the MAbs using such material has now been initiated.

In summary, MAb 33L1-7 appears to be panreactive and might substitute polyclonal antisera to denatured BPV-1 for screening HPV infections. No antibodies showing specificities similar to MAb 33L1-1 and -8 are known to us. Therefore the MAbs described in this work should complement existing antibodies in the detection and classification of productive HPV infections.

We thank Petra den Otter for the immunohistochemical analyses and Margarete Hoffmann for typing of the manuscript. P.J.F.S. has been supported by a fellowship of the Royal Academy of Art and Science. R.E.S. acknowledges a research grant of the Minister of Science and Technology.

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(Received 14 June 1994; Accepted 29 July 1994)