VIROLOGY 243, 275–282 (1998)
ARTICLE NO. VY979011

Type Specific and Genotype Cross Reactive B Epitopes of the L1 Protein of HPV16 Defined by a Panel of Monoclonal Antibodies

Jerzy K. Kulski,*,‡ John W. Sadleir,† Stephen R. Kelsall,*,[1] Maria S. Cicchini,*,[2] Geoffrey Shellam,*,§ Shi Wen Peng,‖ Ying Mei Qi,‖ Denise A. Galloway,** Jian Zhou,‖ and Ian H. Frazer‖,[3]

*Departments of *Microbiology and †Biochemistry, ‡Royal Perth Hospital, and §University of Western Australia, Perth, Western Australia 6000; ‖Centre for Immunology and Cancer Research, University of Queensland, Princess Alexandra Hospital, Woolloongabba, Queensland 4101, Australia; and **Fred Hutchinson Cancer Research Center, Seattle, Washington*

Received April 18, 1997; returned to author for revision June 3, 1997; accepted December 19, 1997

Mouse monoclonal antibodies (mAbs) were raised against the major capsid protein, L1, of human papillomavirus type 16 (HPV16), produced in *Escherichia coli* with the expression plasmid pTrcL1. Epitope specificity could be assigned to 11 of these 12 antibodies using a series of linear peptides and fusion proteins from HPV16. One mAb (MC53) recognized a novel linear epitope that appears to be unique to the HPV16 genotype. A further 11 mAbs were characterized as recognizing novel and previously defined linear and conformational epitopes shared among more than one HPV genotype. The apparently genotype specific mAb could be useful for the development of diagnostic tests for vegetative virus infection in clinical specimens. © 1998 Academic Press

INTRODUCTION

Human papillomavirus type 16 (HPV16) is one of more than 70 different genotypes of papillomavirus known to infect the skin and mucosa of man. Although about 30 HPV genotypes have been identified in the anogenital tract, HPV16 is the most frequently detected in high-grade cervical intraepithelial neoplasia and SCC, (Koutsky and Kiviat, 1993; Munoz and Bosch, 1992). Detection and identification of HPV in clinical specimens depends primarily on the use of DNA hybridization methods. Immunological detection methods based on the L1 capsid protein and specific for HPV16 might allow differentiation between vegetative and latent infection and would facilitate epidemiological studies on the natural history and spread of infection.

Monoclonal antibodies (mAbs) have been produced that recognize linear epitopes located within the central position of the HPV16 L1 protein (Cason *et al.,* 1989; McLean *et al.,* 1990) and conformational and linear epitopes derived after immunization with L1 virus-like particles (VLPs) (Christensen *et al.,* 1996). However, those that recognize linear L1 epitopes likely to be present in denatured tissues either are genotype cross-reactive or are of unknown genotype specificity. In this paper, we describe the production of mAbs to an unfused, full-length L1 protein of HPV16 L1 and the characterization of several linear epitopes, one of which is likely to be type-specific for HPV16.

RESULTS

Reactivity of a panel of HPV16 L1-specific mAbs with regions of the HPV16 L1 protein

A panel of monoclonal antibodies reactive with the L1 capsid protein of HPV16 was raised by immunizing a mouse with PAGE-purified whole length recombinant HPV16 L1, expressed in unfused form in *Escherichia coli.* MAbs reactive with HPV16 L1 protein were further characterized by immunoblot, using a series of recombinant proteins incorporating distinct regions of HPV16 L1 (Table 1, Fig. 1). Antibodies were also characterized by their reactivity on immunoblot with a characteristic series of degradation products of recombinant HPV16 L1 (Fig. 1) resulting from activity of OmpT and other proteases (Kelsall and Kulski, 1995). One mAb (MC12) reacted with a recombinant polypeptide derived from the 172 amino-terminal amino acids (aa's) of HPV16 L1. Six mAbs (MC7, -14, -18, -39, -40, and -53) reacted only with fusion proteins including the 48 carboxyl-terminal aa's. Four antibodies (MC10, -15, -25, and -34) reacted with fusion proteins which included aa's 172–410 of the L1 molecule, and each of these mAbs showed patterns of reactivity with L1 degradation products distinct from the L1-spe-

[1] Current address: Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, Pennsylvania 19111.

[2] Current address: Dental School, Perth, Western Australia, 6000, Australia.

[3] To whom correspondence and reprint requests should be addressed at Centre for Immunology and Cancer Research, Department of Medicine, Princess Alexandra Hospital, Woolloongabba 4012, Queensland, Australia. Fax: +61 7 3240 2048. E-mail: ifrazer@medicine.pa.uq.edu.au.

0042-6822/98 $25.00
Copyright © 1998 by Academic Press
All rights of reproduction in any form reserved.

TABLE 1

Reactivity of a Series of HPV16 L1 Protein-Specific mAbs with Various Truncated and Complete HPV16 L1 Proteins

Recombinant L1 plasmid	Encoded protein: Predicted M_r	Encoded protein: HPV16 L1 aa sequence	Reactivity with recombinant protein: mAb 12	mAbs 10, 15, 25, 34	mAbs 7, 14, 39, 40, 53, 18	CAMVIR1
TrcL1	56	1–505	+	+	+	+
GEXL1	83	1–505	+	+	+	+
GEXL1TE	65	1–395	+	+	−	+
GEXL1TS	67	1–~410	+	+	−	+
HX-1	136	1–172	+	−	−	−
HX-2	154	172–505	−	+	+	+
HX-3	125	385–457	−	−	−	−
GEX	28	0	−	−	−	−

cific mAb CAMVIR1 that also reacts with this region of L1, as exemplified for MC25 in Fig. 1, lane B10, and for CAMVIR-1 in Fig. 1, lane D10. These 11 mAbs (MC7, -10, -12, -14, -15, -18, -25, -39, -40, and -53) were therefore, together with CAMVIR-1, selected for further analysis. The antibodies were tested for reactivity against a series of overlapping peptides corresponding to the entire predicted HPV16 L1 ORF. One antibody, MC25, reactive with L1 fusion proteins comprising the central portion of HPV16 L1 by immunoblot (Fig. 1) did not react with any of the linear peptides. With this exception all mAbs mapped to linear epitopes within the regions predicted from the fusion protein studies, the 10 reactive mAbs reacting with five distinct peptides (Table 2).

Reactivity of mAbs with recombinant L1 proteins from other papillomaviruses

The reactivity of the panel of HPV16L1-specific mAbs with the L1 proteins of HPV6b, -16, and -18, each expressed as authentic sequence recombinant proteins in SF9 cells using L1 recombinant baculoviruses, and with HPV18, HPV31, HPV33, and BPV1 L1 expressed in CV1 cells using a recombinant Vaccinia virus, was determined by immunoblot analysis (Fig. 2). Four mAbs recognized the peptide comprising aa's 454–468 of HPV16. Of these antibodies, two (MC18 and MC39) reacted strongly with all other tested L1 proteins (Figs. 2B and 2G) and two (MC14 and MC40) did not react with HPV6

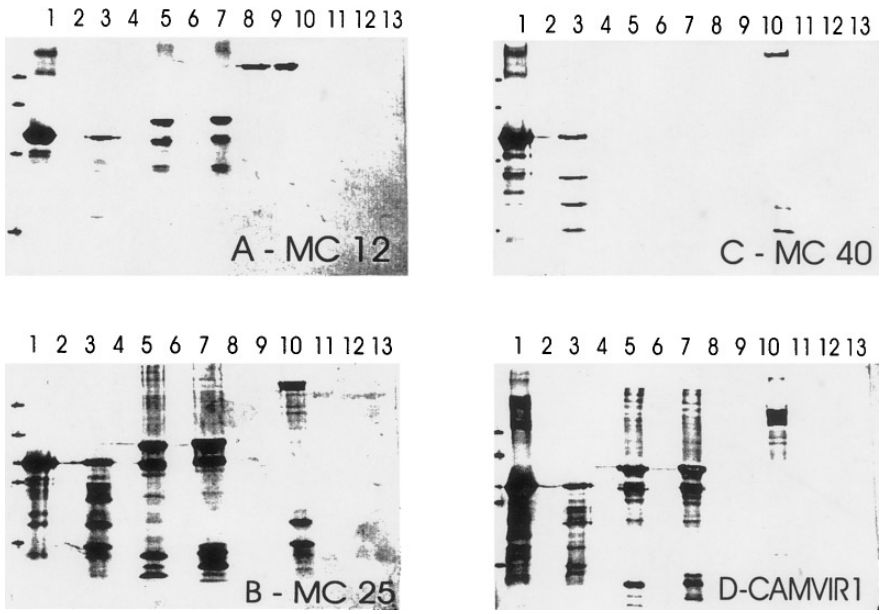

FIG. 1. Reactivity by immunoblot of HPV16L1-specific mAbs with a series of bacterial recombinant proteins containing HPV16L1 sequences. (A) Reactivity with MC-12; (B) MC-25; (C) MC-40; (D) CAMVIR-1. In each panel recombinant proteins were expressed using bacterial expression vectors as described in the legend to Fig. 1. Lanes: (1) pTrc HPV16 L1; (2) pGEXL1 uninduced; (3) pGEX L1 induced; (4) pGEXL1TE uninduced; (5) pGEXL1TE induced; (6) pGEXL1TS uninduced; (7) pGEXL1TS induced; (8 and 9) pHX-1; (10) pHX-2; (11) pHX-3; (12) pGEX; (13) pEX-1. Molecular weight markers (97.4, 69, 46, and 30 kDa) are indicated on the left in each panel.

TABLE 2

Reactivity of the HPV16L1-Specific mAbs with HPV16 Synthetic Peptides and Other HPV Proteins

Antibody	Reactive HPV16 L1 peptide[a]	Location on HPV16 L1 protein[b]	Reference	Reactivity with other HPV genotypes						Epitope designation
				BPV1	HPV6	HPV11	HPV18	HPV31	HPV33	
MC-12	KLDDTENASAYAANA	124–138		−	+/−	−	+/−	+	+	E4
MC-10	GTVGENVPDDLYIKG	264–278		−	+/−	−	+	+	+	E9
MC-34	GTVGENVPDDLYIKG	264–278		n.d.[c]	−	n.d.	−	+	+	E2
MC-15	QIFNKPYWLQRAQGH	304–318		+	+	+	+	+	+	E3
MC-14	FSADLDQFPLGRKFL	454–468		n.d.	−	n.d.	−	n.d.	n.d.	E5
MC-40	FSADLDQFPLGRKFL	454–468		n.d.	−	n.d.	−	n.d.	n.d.	E5
MC-18	FSADLDQFPLGRKFL	454–468		+	+	+	+	+	+	E6
MC-39	FSADLDQFPLGRKFL	454–468		+	+	+	+	+	+	E6
MC-7	KAKPKFTLGKRKATP	474–488		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	E7
MC-53	KAKPKFTLGKRKATP	474–488		−	−	−	−	−	−	E7
MC-25	None detected	−		+	+	+	+	+	+	E8
CAMVIR-1	GFGAMDF	203–209	McLean *et al.* (1990)	+	+	+	+	+	+	E1
8C4, 5A4, 1D6,	ENVPDDLYIKGSGSTA	268–283	Cason *et al.* (1989)							
3D1, 1C6	MVTSDAQIFNKPYWL	298–312	Cason *et al.* (1989)							

[a] The sequence of the L1 peptide is given with single letter amino acid code. Bold residues are the minimal reactive epitopes.
[b] Relative to second methionine in the L1 ORF of HPV16.
[c] n.d., not determined.

or HPV18 and were not tested against the other types. As aa's 454–468 of HPV16 L1 are relatively conserved across PV genotypes (Table 3), these data allow the prediction that mAbs 18 and 39 recognize an epitope which is tolerant of substitutions to alanine at position 456 and that mAbs 14 and 40 recognize an epitope which either is not tolerant of substitutions at position 456 or includes the phenylalanine at position 461. MC15 reacted strongly with all tested L1 sequences, suggesting that it reacts with an epitope within the conserved sequence 306–313 (FNKPYWLQ), previously demonstrated antigenic by Cason *et al.* (1989). MC34 reacted with HPV31 and HPV33 L1 but not with other L1 proteins less closely related to HPV16, suggesting that it reacted with the epitope LYIKG from aa 274–278, another epitope defined by Cason *et al.* (1989). MC10 also reacted with the linear peptide from aa 264 to 278, but, in contrast to MC34, reacted with HPV18 L1 and weakly with HPV6b L1 as well as with HPV31 and HPV33 L1 protein. MAb MC12, the only antibody recognizing a linear epitope in the N-terminal $\frac{1}{3}$ of the L1 protein reacted with HPV 31 L1, and HPV 33 L1 but did not react with the L1 proteins of HPV 6b, HPV11, HPV18, or BPV-1 (Fig. 2D), indicating that the recognized epitope is likely to lie within the conserved sequence (KLDDTE) from aa 128 to 134 and is tolerant of substitutions of the lysine residue. MC53 reacted with a protein of apparent M_r corresponding to L1 only in the HPV16 preparation (Fig. 2I). Reactivities at higher M_r were demonstrated inconsistently with some rVV cell lysates, including wt rV V and were presumed to represent cross reactivity with cellular components. This antibody and MC7, which was not further characterized, each recognized an epitope in a nonconserved region of the nuclear localization signal of HPV16 L1. MC25, the only antibody for which a linear epitope was not identified by peptide scanning, reacted strongly with all tested L1 proteins, suggesting that it recognizes a conserved B epitope, which from the pattern of reactivity with the truncated L1 recombinant proteins should lie in the region from aa 172 to 395. The failure of this antibody to react with linear overlapping peptides in repeated testing suggests that this antibody recognizes a conformational epitope: reactivity with L1 protein derivatives by immunoblot is not inconsistent with this conclusion as some renaturing of proteins occurs when SDS is removed.

DISCUSSION

This study has confirmed two previously described B epitopes within the HPV16 L1 sequence (E2, E3—Table 2) and defined at least five novel linear epitopes (E4–E7, E9), one of which appears HPV16 specific (E7). Immunization of mice with a full-length L1 protein that was purified from bacterial lysates by PAGE appears to be comparable to immunization using disrupted viral particles. SDS- and heat-treated BPV1 particles produced seven mAbs against 4 epitopes located between aa position 198 and 420 and a polyclonal Ab against more than 15 epitopes located between aa position 108 and 468 of the BPV1 L1 protein (Lim *et al.,* 1990). By comparison, we obtained 80 hybridomas that were reactive with the L1 protein expressed by pTrcL1. Individual mAbs raised against the recombinant L1 protein recognized 6 epitopes between aa position 124 and 488 of the L1

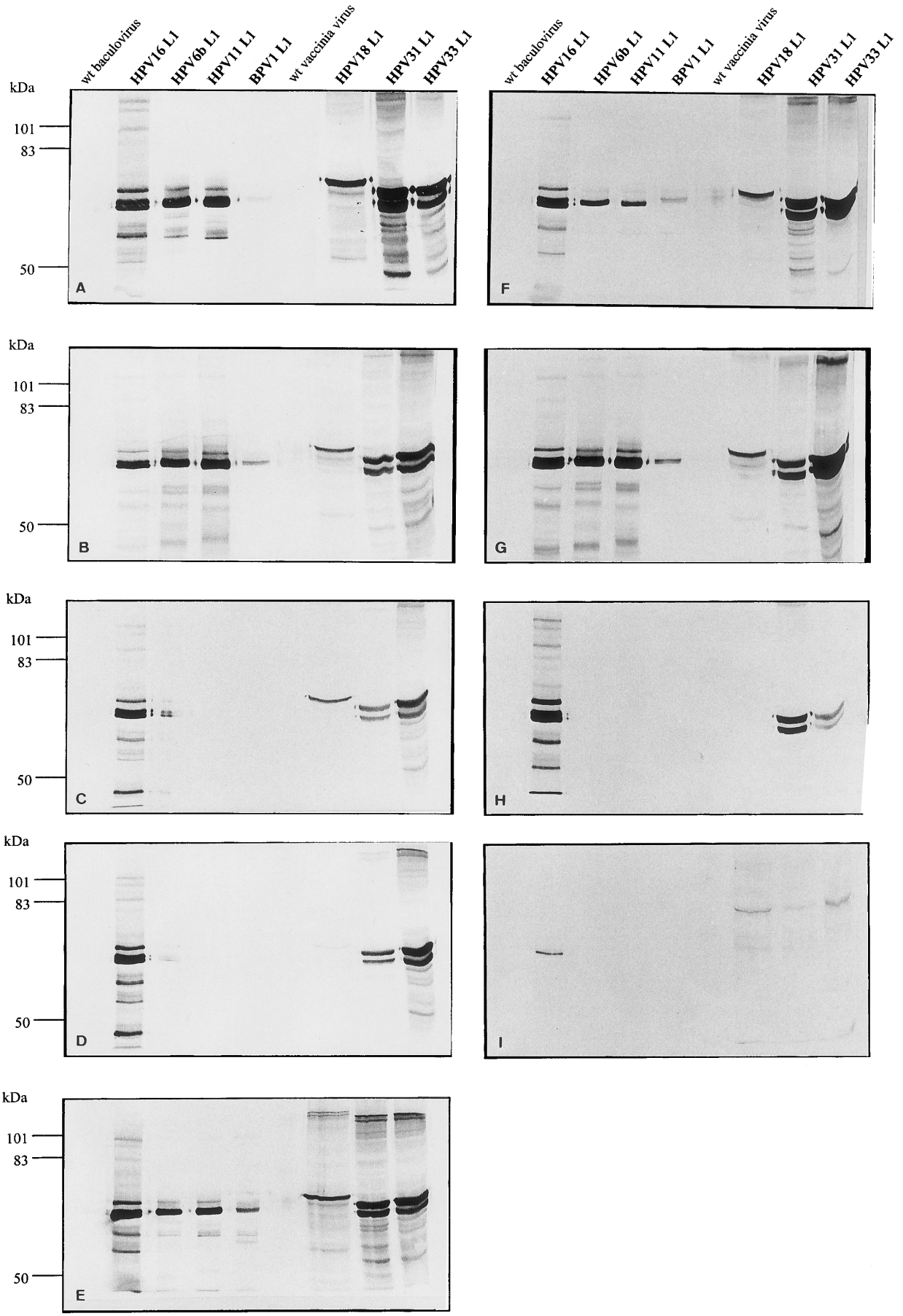
wt baculovirus
HPV16 L1
HPV6b L1
HPV11 L1
BPV1 L1
wt vaccinia virus
HPV18 L1
HPV31 L1
HPV33 L1
kDa
101
83
50
A
B
C
D
E
F
G
H
I

TABLE 3

Comparison of Amino Acid Sequences of mAb-Reactive Peptides with Related Sequences of L1 Protein of Different Papillomavirus Types

			mAb reactivity								
Epitope region	Peptide sequence[a]	PV type	12	10	34	15	14	40	18	39	53
124–138	A.L.A..VNRKVTTQ	BPV	−								
	.......SN..GG.P	6	±								
	.Y..V..SGG.GG.P	11	−								
	KLDDTENASAYAANA	16	+								
	......SSH.ATS.V	18	±								
	.F.....SNR..GGP	31	+								
	.F....TGNK.PGQP	33	+								
264–278	.SEK.APTT.F.L.N	BPV		−	−						
	.E...P...T.....	6		±	−						
	.T...P.....LV..	11		−	−						
	GAVGENVPDDLYIKG	16		+	+						
	.TM.DT..QS.....	18		+	−						
	.T...S..T......	31		+	+						
	.TL..A.........	33		+	+						
304–318	....R....F....M	BPV				+					
	.L........K....	6				+					
	.L........K....	11				+					
	QIFNKPYWLQRAQGH	16				+					
	.L.......HK....	18				+					
	........M......	31				+					
	.L.............	33				+					
454–468	L.L.........R..	BPV							+	+	
	..SE...Y.......	6					−	−	+	+	
	..SE...........	11							+	+	
	FSADLDQFPLGRKFL	16					+	+	+	+	
	..L....Y.......	18					−	−	+	+	
	....	31							+	+	
	....	33							+	+	
474–488	STVR.RRISQKTSSK	BPV									−
	RGRSSIRT.VKRPAV	6									−
	RGRTSART.IKRPAV	11									−
	KAKPKFTLGKRKATP	16									+
	RR..TIGPR..S.PS	18									−
	R.R...KA...S.P.	31									−
	.....LKRAAPTS.R	33									−

[a] The single letter amino acid code is used throughout—periods represent conserved residues.

protein of HPV16. The greater diversity of epitopes recognized by mAbs in our study compared with previous studies using fusion proteins suggests an advantage to using a full-length protein in unfused form as an immunogen.

This study produced some antibodies with specificity similar to those previously defined in HPV16. Epitope E2 lies within a region (aa 264–278) shown by Cason *et al.* (1989) to contain a B epitope (DLYIKG), and four of five of their L1 specific mAbs were directed to this epitope. As DLY is found within HPV16, 31, and 33 but is not in HPV6b, 18, or BPV1 (Table 3), and our antibody MC34 reacted only with L1 proteins with all three of these residues, it is likely that this represents the minimal B epitope. Another epitope defined in the present study (E3) lies within the same region of L1 (aa's 304–318) as an epitope (FNKPYWL) previously shown reactive (Cason *et al.,* 1989) with one L1-specific mAb. As this epitope is

FIG. 2. Reactivity by immunoblot of HPV16L1-specific MAbs with L1 proteins of HPV6b, HPV11, HPV16, and BPV1 expressed as baculovirus recombinants in SF9 cells and with HPV18, HPV31, and HPV33 L1 proteins expressed as recombinant VVs. Similar amounts of purified L1 protein of HPV16, HPV6b, HPV11, and BPV1 were loaded as described in the legend to Fig. 2. Each panel represents reactivity with an individual antibody as follows: (A) CAMVIR-1; (B) MC-18; (C) MC-10; (D) MC-12; (E) MC-15; (F) MC-25; (G) MC-39; (H) MC-34; and (I) MC-53. The position of the molecular weight markers is indicated on the left. For each panel the lanes are: 1, wild type baculovirus cell lysate; 2, HPV16L1rBV; 3, HPV6L1rBV; 4, HPV11L1rBV; 5, BPV1L1 rBV; 6, wild type vaccinia virus cell lysate; 7, HPV18L1rVV; 8, HPV31 rVV; 9, HPV33 rVV.

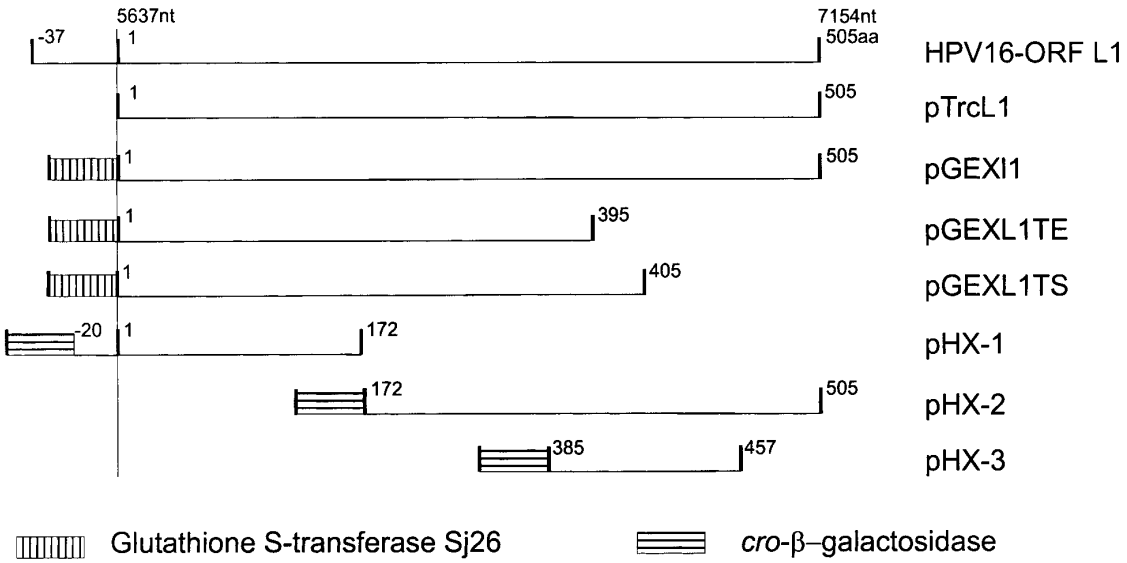

FIG. 3. Schematic representation of various prokaryotic expression constructs containing HPV16L1 sequence. For each, the expressed HPV16 L1 amino acids are followed by a designation given to the construct. The vector proteins, 26-kDa glutathione *S*-transferase (Sj26) and 117-kDa *cro*-β galactosidase (*cro*-β-gal) are fused to the amino terminus of the L1 capsid protein and are represented in the diagram for pGEX and pHX vectors by horizontal and vertical shading respectively.

conserved across all tested PV L1 proteins and our antibody MC15 was reactive with each of these, it is probable that MC15 will share this specificity. Sequences within E3 were also recognized by a mAb raised against HPV33 L1 fusion protein that had been produced in *E. coli* (Sapp *et al.,* 1994). This sequence is highly conserved (>90%) across most PV genotypes for which sequence is available and thus antibodies of this specificity will likely detect most PV L1 capsid proteins after denaturation.

Three of the novel B epitopes defined in this study (E5–E7) are clustered within a conserved C-terminal region of the HPV L1 protein. The function of this region is not known, but it is not essential for capsid formation. One epitope (E7) lies within a nuclear localization sequence region of L1 (Zhou *et al.,* 1991), which is not conserved from PV to PV genotype, and the antibody reactive with this epitope, MC53, was specific for HPV16 among the tested genotypes. Further analysis of 71 predicted L1 protein sequences from the EMBL database showed no better match for the HPV16 sequence in this region than the nonreactive HPV33 sequence, suggesting that this epitope may be HPV16 specific. The apparently conformational epitope (E8) is potentially of interest as it is conserved across six quite divergent HPV genotypes, and antibodies recognizing conformational epitopes have potential as virus neutralizing antibodies (Hines *et al.,* 1994).

Interestingly, none of the epitopes defined in this paper overlap with any of the series of seven linear epitopes defined by the mAbs raised by Christensen *et al.* (1996) using HPV16 L1 VLPs as immunogen, suggesting that the epitopes presented by VLPs to the mouse immune system are not seen as such when L1 is presented as a linear bacterial protein. Many of the B epitopes recognized in the present series are conserved across many PV genotypes, suggesting that there is little immune selection pressure on these epitopes. This could mean that these linear epitopes are neither produced nor presented in the course of natural infection, or alternatively that an immune response to these epitopes of PV L1 protein is not a determinant of the outcome of exposure to PV infection.

MATERIALS AND METHODS

Vectors and expression of full-length or fragmented L1 protein

The prokaryotic expression constructs containing HPV16 sequence that were used in this study are shown in Fig. 3. The 505 amino acids of the L1 coding sequence (nts 5637–7154) of HPV16, starting from the second initiation codon of the L1 ORF (Seedorf *et al.,* 1985) were expressed in an unfused form with the bacterial expression vector pTrcL1 (Kelsall and Kulski, 1995) and in a fused form with bacterial expression vector pGEXL1 (Kelsall and Kulski, 1994). The bacterial expression plasmids pGEXL1TE and pGEXL1TS contained a truncated L1 sequence with approximately 300 nts deleted from the 5′ end of the stop codon by *Eco*RI or S1 nuclease digestion, respectively, and expressed a protein with a deletion of approximately 105 amino acids from the carboxy-terminal end. The plasmids pHX-1 and pHX-2 expressed the amino-terminal (1 to 172 aa) and carboxy-terminal (172 to 505 aa) ends of the L1 protein, respectively, fused with *cro*-β-galactosidase of the pEX vector

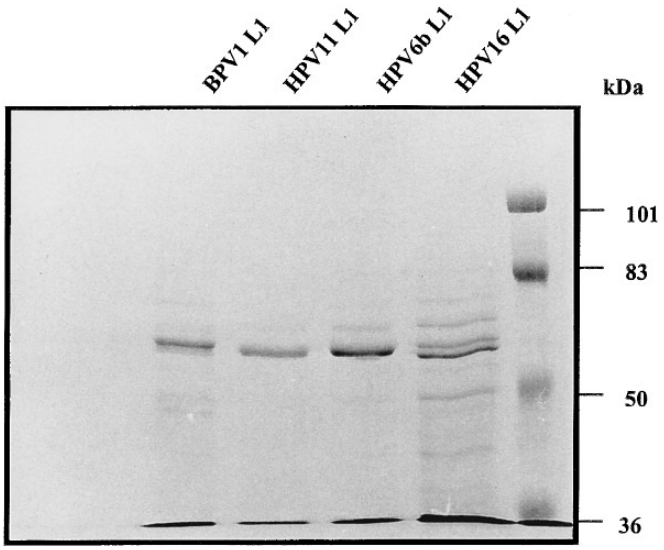

FIG. 4. Protein composition of purified L1 VLPs. L1 protein expressed using recombinant baculovirus in Sf9 cells was purified as VLPs from cell lysates by density gradient separation and dialysed against PBS buffer. Approximately 15 μg of each L1 protein was separated by SDS–PAGE and stained with Coomassie blue.

(Browne *et al.,* 1988). The plasmid pHX-3 contained a 216-bp *Pst*I fragment from the L1 ORF of HPV16 DNA (nts 6792–7008) that was inserted into the *Pst*I site of the bacterial expression vector pEX-1 to express a 72-aa fragment of the L1 protein (amino acid position 385 to 457) fused with *cro*-β-galactosidase.

HPV6, HPV16, or HPV18 L1 proteins were produced and purified as VLPs in insect cells (Sf9) (Fig. 4) after infection with recombinant baculoviruses encoding HPV6L1, HPV16L1, or HPV18L1 as previously described (Park *et al.,* 1993). BPV1 L1 VLPs were produced in CV-1 cells using a rVV as previously described (Zhou *et al.,* 1993). HPV18, HPV31, and HPV33 L1 proteins were produced as lysates of cells infected with the appropriate recombinant vaccinia virus. These recombinant vaccinia viruses were constructed by recombination of pMJ601-L1 plasmids into the vaccinia virus vector using methods described previously (Hagensee *et al.,* 1993). The L1 coding sequences were obtained by PCR cloning using primers containing *Sal*I and *Sma*I ends: HPV18, 5′-TGGCTTGAATTCGTCGACAGATGGCTTTGTGGCG-GCC-3′ 3′-CGAAGCGAATTCCCCGGGTTACTTCCT-GGCACGTACACG-5′; HPV31, 5′-TTGCTTGAATTCGTC-GACATGTCTCTGTGGCGGCC-3′, 3′-CGAAGCGAAT-TCCCCGGGTTACTTTTTAGTTTTTTTACG-5′; HPV33, 5′-TTGCTTGAATTCGTCGACAGATGTCCGTGTGGCG-GCC-3′, 3′-CGAAGCGAATTCCCCGGGTTATTTTTTA-ACCTTTTTGCG-5′. The PCR products were cleaved and ligated into similarly cleaved pMJ601 vector plasmid. Virus was plaque purified three times; expression of L1 protein was confirmed by immunoblot with polyclonal cross-reactive L1 sera and capsid formation was assessed by electron microscopy (G. Wipf and D. A. Galloway, unpublished results).

Production of mouse mAbs

The HPV16 L1 capsid protein expressed in *E. coli* (JM101) with the plasmid pTrcL1 was separated from bacterial proteins on 4–18% SDS–PAGE gradient gels (Kelsall and Kulski, 1995), purified, quantitated, and used to immunize BALB/c mice. The protein was highly pure as judged by the presence of only a single band in a lane of an SDS gel heavily overloaded with the purified L1 protein and stained with Coomassie blue R-250. BALB/c mice were immunized three times with 10 μg protein in Freund's adjuvant. A single-cell suspension of splenic lymphocytes was prepared from the highest responder and fused with mouse myeloma cells X63 Ag8.653 at a cell ratio of 3:5 in 50% polyethylene glycol (M_r 1000, BDH) for 90 s and then diluted in RPMI medium containing 20% fetal bovine serum and HAT (0.96 μM hypoxanthine, 0.16 μM thymidine, 0.36 μM aminopterin). The resultant hybrid cells were plated into 96-well microtiter plates (five plates per spleen) containing spleen feeder cells. Eighty hybridomas that secreted antibody against L1 protein expressed by pTrcL1 were screened by Western immunoblotting using bacterial preparations of pTrcL1.

Immunoblotting

SDS–PAGE and immunoblotting for detection of recombinant L1 proteins that had been expressed in bacteria were performed as described previously (Kelsall and Kulski, 1995). Immunoblots for detection of reactivity with HPV6b, HPV11, HPV16, or HPV18 L1 proteins expressed by recombinant baculoviruses and BPV1, HPV18, HPV31, and HPV33 L1 protein expressed using rVV were essentially as described for the bacterial proteins except that blots were blocked with 5% skim milk in PBS at 4°C overnight, goat anti-mouse alkaline phosphatase was used as a detection conjugate (Promega), and color development was with NBT-BCIP.

Peptides and linear epitope mapping

A series of 15-mer peptides, overlapping by five residues and spanning the deduced amino acid sequence of HPV16 L1 protein, was used to screen mAbs by ELISA essentially as described previously (Zhou *et al.,* 1992). To denote the position of the amino acids in the L1 protein, the putative second initiation codon was designated amino acid number 1.

Computer analysis of papillomavirus L1 protein sequences

Peptide sequences recognized by the mAbs were aligned with the L1 protein sequences and the var-

ious HPV L1 protein sequences were aligned using "Megalign" sequence alignment software (DNAstar).

ACKNOWLEDGMENTS

We particularly thank Dr. Peter Heenan and Cutaneous Pathology (Nedlands, Western Australia) for their generous support. This work was funded in part by grants from the Cancer Foundation of Western Australia, the National Health and Medical Research Council, the Royal Perth Hospital Medical Research Foundation, and University of Queensland Mayne Bequest.

REFERENCES

Browne, H. M., Churcher, M. J., Stanley, M. A., Smith, G. L., and Minson, A. C. (1988). Analysis of the L1 gene product of human papillomavirus type 16 by expression in a vaccinia virus recombinant. *J. Gen. Virol.* 69, 1263–1273.

Cason, J., Patel, D., Naylor, J., Lunney, D., Shepherd, P. S., Best, J. M., and McCance, D. J. (1989). Identification of immunogenic regions of the major coat protein of human papillomavirus type 16 that contain type-restricted epitopes. *J. Gen. Virol.* 70, 2973–2987.

Christensen, N. D., Dillner, J., Eklund, C., Carter, J. J., Wipf, G. C., Reed, C. A., Cladel, N. M., and Galloway, D. A. (1996). Surface conformational and linear epitopes on HPV-16 and HPV-18 L1 virus-like particles as defined by monoclonal antibodies. *Virology* 223, 174–184.

Hagensee, M. E., Yaegashi, N., and Galloway, D. A. (1993). Self-assembly of human papillomavirus type 1 capsids by expression of the L1 protein alone or by coexpression of the L1 and L2 capsid proteins. *J. Virol.* 67, 315–322.

Hines, J. F., Ghim, S. J., Christensen, N. D., Kreider, J. W., Barnes, W. A., Schlegel, R., and Jenson, A. B. (1994). Role of conformational epitopes expressed by human papillomavirus major capsid proteins in the serologic detection of infection and prophylactic vaccination [see comments]. *Gynecol. Oncol.* 55, 13–20.

Kelsall, S. R., and Kulski, J. K. (1994). *In* "Immunology of Human Papillomaviruses" (M. A. Stanley, Ed.), pp. 51–54. Plenum, New York.

Kelsall, S. R., and Kulski, J. K. (1995). Expression of the major capsid protein of human papillomavirus type 16 in *Escherichia coli*. *J. Virol. Methods* 53, 75–90.

Koutsky, L. A., and Kiviat, N. B. (1993). Genital infectious agents and invasive cervical cancer. *Lancet* 342, 184–185.

Lim, P. S., Jenson, A. B., Cowsert, L., Nakai, Y., Lim, L. Y., Jin, X. W., and Sundberg, J. P. (1990). Distribution and specific identification of papillomavirus major capsid protein epitopes by immunocytochemistry and epitope scanning of synthetic peptides. *J. Infect. Dis.* 162, 1263–1269.

McLean, C. S., Churcher, M. J., Meinke, J., Smith, G. L., Higgins, G., Stanley, M., and Minson, A. C. (1990). Production and characterisation of a monoclonal antibody to human papillomavirus type 16 using recombinant vaccinia virus. *J. Clin. Pathol.* 43, 488–492.

Munoz, N., and Bosch, F. X. (1992). *In* "The Epidemiology of Human Papillomavirus and Cervical Cancer (N. Munoz, F. X. Bosch, K. V. Shah, and A. Meheus, Eds.), pp. 251–261. IARC Sci Publ 119, Lyon, France.

Park, D. S., Selvey, L. A., Kelsall, S. R., and Frazer, I. H. (1993). Human papillomavirus type 16 E6, E7 and L1 and type 18 E7 proteins produced by recombinant baculoviruses. *J. Virol. Methods* 45, 303–318.

Sapp, M., Kraus, U., Volpers, C., Snijders, P. J. F., Walboomers, J. M. M., and Streeck, R. E. (1994). 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. *J. Gen. Virol.* 75, 3375–3383.

Seedorf, K., Krammer, G., Durst, M., Suhai, S., and Rowekamp, W. G. (1985). Human papillomavirus type 16 DNA sequence. *Virology* 145, 181–185.

Zhou, J., Doorbar, J., Sun, X. Y., Crawford, L. V., McLean, C. S., and Frazer, I. H. (1991). Identification of the nuclear localization signal of human papillomavirus type 16 L1 protein. *Virology* 185, 625–632.

Zhou, J., Stenzel, D. J., Sun, X. Y., and Frazer, I. H. (1993). Synthesis and assembly of infectious bovine papillomavirus particles in vitro. *J. Gen. Virol.* 74, 763–768.

Zhou, J., Sun, X. Y., Davies, H., Crawford, L., Park, D., and Frazer, I. H. (1992). Definition of linear antigenic regions of the HPV16 L1 capsid protein using synthetic virion-like particles. *Virology* 189, 592–599.