Characterization of Murine Polyclonal Antisera and Monoclonal Antibodies Generated against Intact and Denatured Human Papillomavirus Type 1 Virions

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Human papillomavirus type 1 (HPV1) virions, both as intact virion particles (IVP) and as detergent-denatured virions (DDV), were used to prepare polyclonal antisera and monoclonal antibodies (MAbs) in BALB/c mice. Anti-IVP antiserum contained type-specific HPV1 L2-reactive antibodies and no detectable HPV1 L1-reactive antibodies. Anti-IVP MAbs recognized a linear epitope between L2 amino acids 102 and 108 (PIDVDP). Anti-DDV antiserum contained type-specific HPV1 L1-reactive and HPV1 L2-reactive antibodies. An anti-DDV MAb recognized a linear epitope between L1 amino acids 127 and 133 (AENPTNY). HPV1a L1- and L2-encoded polypeptides expressed in *Saccharomyces cerevisiae* and by in vitro translation were equivalent in size to the major and minor virion capsid proteins, respectively.

Human papillomavirus (HPV) virions are nonenveloped icosahedral particles which are 55 nm in diameter (21). Virion capsids are composed of at least two viral proteins, a major capsid protein (53 to 57 kDa) and a minor capsid protein (76 to 86 kDa). At least some if not all of the major and the minor capsid proteins are encoded by the HPV L1 and L2 open reading frames (ORFs), respectively, because they react specifically with antisera generated against L1- and L2-encoded recombinant proteins, respectively (7). The predicted size of an L1-encoded polypeptide is 57 kDa, which is consistent with the observed mobility of the major capsid protein upon sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The predicted size of an L2-encoded polypeptide is 55 kDa. However, the observed mobility of the minor capsid protein has been reported to be between 76 and 86 kDa. It is unclear whether the observed mobility of the minor capsid protein is due to an inherent property of the L2-encoded polypeptide which causes it to migrate aberrantly upon SDS-PAGE or whether it is due to the addition of high-molecular-weight moieties to the L2 polypeptide through posttranslational modification.

To date, well-characterized monoclonal antibodies (MAbs) generated against HPV virions have not been described. Such reagents would be helpful in studies of virion capsid structure, HPV late-gene expression, and virion-cell interactions. In this report, we describe the characterization of murine polyclonal antisera and MAbs which were generated both against intact HPV type 1 (HPV1) virions and against SDS-denatured HPV1 virions. We also expressed the HPV1 L1 and L2 coding sequences in the yeast *Saccharomyces cerevisiae* and by in vitro translation to determine whether L1- and L2-encoded polypeptides were equivalent in size to the major and minor capsid proteins of HPV1 virions, respectively.

HPV1 virions were extracted from a single large plantar

wart (19). Intact HPV1 virion particles and SDS-denatured HPV1 virions were used to prepare polyclonal antisera and MAbs in BALB/c mice (10). Polyclonal antisera were prepared from the blood of each mouse at the time of sacrifice. Hybridoma culture supernatants were screened for antibody reactivities to intact HPV1 virions in an enzyme-linked immunosorbent assay (ELISA) and for reactivity with nuclear antigens in HPV1-containing plantar wart sections by immunohistochemical staining. Antibody specificities were further defined by testing the antibodies in Western immunoblot assays for reactivities with HPV L1- and L2-encoded polypeptides expressed in *Escherichia coli* (8, 13). The locations of antibody-reactive epitopes were mapped by using nested sets of deleted HPV polypeptides (14) and by using overlapping sets of synthetic oligopeptides (9).

Forty-three of approximately 1,000 viable hybridomas prepared against intact HPV1 virion preparations produced antibodies which reacted with intact HPV1 virion preparations in an ELISA. Twenty-three of the ELISA-positive clones reacted with plantar wart epithelial cell nuclei and did not react with epithelial cells in sections of normal skin. Twelve of the 23 clones gave unambiguous reactions upon immunoglobulin isotyping, and these clones were selected for further study. These 12 MAbs were all immunoglobulin G2a (IgG2a) containing kappa light chains (mouse MAb isotyping kit; Amersham Corp., Arlington Heights, Ill.).

Approximately 1,000 hybridomas prepared against disrupted virions were tested as described above. Twenty-nine clones were ELISA positive, but only one of these clones (MAb DW45) gave strong nuclear staining of HPV1-containing tissue. MAb DW45 was IgG2b containing kappa light chains.

Polyclonal antisera were tested in Western immunoblot assays for IgG and IgM reactivities to HPV1a L1 and HPV1a L2 polypeptides expressed as *trpE*-HPV fusion proteins in *E. coli* (3, 5, 6, 13, 20) (Table 1 and Fig. 1). The results are shown in Fig. 2. The polyclonal antiserum generated against intact HPV1 virions contained IgG antibodies which reacted

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TABLE 1. HPV expression plasmids

ORF (HPV type)	Expression plasmid	Restriction sites	Vector
L1			
1a	pY1L1	----(5431) ----(6939) ^a	pBS24.1 (<i>Bam</i> HI- <i>Sal</i> I)
1a	p1L1	----(5431) ----(6939) ^a	pATH11 (<i>Eco</i> RI- <i>Sal</i> I)
1a	p1L1BB1	<i>Bgl</i> II (5582)- <i>Bgl</i> II (6358)	pATH11 (<i>Bam</i> HI- <i>Bam</i> HI)
1a	p1L1BH1	<i>Bgl</i> II (6358)- <i>Hpa</i> II (7308)	pATH11 (<i>Bam</i> HI- <i>Clal</i> I)
5	p5L1HH1	<i>Hpa</i> II (5969)- <i>Hpa</i> II (7571)	pATH10 (<i>Clal</i> - <i>Clal</i> I)
6b	p6L1XH1	<i>Xba</i> I (5903)- <i>Hpa</i> II (7862)	pATH11 (<i>Xba</i> I- <i>Clal</i> I)
11	p11L1XN1	<i>Xba</i> I (5885)- <i>Nsi</i> I (232)	pATH11 (<i>Xba</i> I- <i>Pst</i> I)
16	p16L1BN1	<i>Bst</i> NI (5529)- <i>Nsi</i> I (253)	pATH11 (<i>Sma</i> I- <i>Pst</i> I)
18	p18L1XX1	<i>Xba</i> I (5730)- <i>Xba</i> I (321)	pATH11 (<i>Xba</i> I- <i>Xba</i> I)
31	p31L1HH1	<i>Hinc</i> II (5519)- <i>Hinc</i> II (215)	pATH10 (<i>Sma</i> I- <i>Sma</i> I)
33	p33L1HH1	<i>Hpa</i> II (6011)- <i>Hpa</i> II (689)	pATH10 (<i>Clal</i> - <i>Clal</i> I)
L2			
1a	pY1L2	----(3897) ----(5420) ^a	pBS24.1 (<i>Bam</i> HI- <i>Sal</i> I)
1a	p1L2	----(3897) ----(5420) ^a	pATH11 (<i>Eco</i> RI- <i>Sal</i> I)
1a	p1L2SB1	<i>Ssp</i> I (4016)- <i>Bgl</i> II (5582)	pATH11 (<i>Eco</i> RI- <i>Bam</i> HI)
6b	p6L2NX1	<i>Nco</i> I (4421)- <i>Xho</i> II (6013)	pATH11 (<i>Eco</i> RI- <i>Bam</i> HI)
11	p11L2HH1	<i>Hind</i> III (4557)- <i>Hpa</i> II (6552)	pATH10 (<i>Hind</i> III- <i>Clal</i> I)
16	p16L2OB1	<i>Oxa</i> NI (4335)- <i>Bam</i> HI (6151)	pATH11 (<i>Eco</i> RI- <i>Hind</i> III)
18	p18L2OO1	<i>Oxa</i> NI (4342)- <i>Oxa</i> NI (5800)	pATH11 (<i>Hind</i> III- <i>Eco</i> RI)

^a The HPV DNA inserts in these expression plasmids were generated by using the polymerase chain reaction.

FIG. 1. Map of HPV1a DNA fragments present in bacterial and yeast expression plasmids. The HPV1a L1 and L2 ORFs are represented as boxes. The nucleotide coordinates of the 5' ORF boundaries, the translation initiation codons (ATG), and the translation termination codons (TAA or TAG) are indicated (3, 5, 6, 20). The boxes labeled A, B, and C mark the locations of coding sequences for the HPV1 L2 MAb-reactive epitope, the HPV1 L1 MAb-reactive epitope, and a rabbit anti-BPV1 antibody reactive region, respectively. The DNA fragments present in the expression plasmids which defined the immunoreactive regions are indicated as lines below the ORFs. The nucleotide coordinates of the ends of the fragments are indicated (see Table 1 for a complete description of the expression plasmids and Fig. 3 and 4 for details of the mapping of the immunoreactive regions).

with the HPV1a L2 fusion protein. No reactivities to the HPV1a L1 fusion protein were detected. Polyclonal antiserum generated against SDS-disrupted HPV1 virions contained IgG and IgM antibodies which reacted with the HPV1a L1 fusion protein and IgG antibodies which reacted with the HPV1a L2 fusion protein. Both the L2 and the L1 polyclonal antibody reactivities appeared to be relatively HPV type specific because they did not react with homologous L2 and L1 fusion proteins encoded by HPV type 6b, 11, 16, or 18 (Table 1 and Fig. 2).

The 12 MAbs generated against intact virions reacted specifically with the HPV1a L2 fusion protein p1L2 and not with L2 fusion proteins encoded by HPV type 6b, 11, 16, or 18. These MAbs did not react with the HPV1a L1-encoded proteins or with L1 proteins encoded by the other HPV types (data not shown). The MAb (DW45) generated against SDS-disrupted virions reacted with the HPV1a L1 fusion protein p1L1 and not with L1 fusion proteins encoded by HPV type 5, 6b, 11, 16, 18, 31, or 33 (Table 1). DW45 did not react with the L2-encoded fusion proteins (data not shown).

Immunoreactive regions of the HPV1a L1 and L2 polypeptides which were recognized by the HPV1 MAbs and by rabbit polyclonal antiserum generated against SDS-denatured bovine papillomavirus type 1 (rabbit anti-BPV1 antiserum; Dako Corp., Santa Barbara, Calif.) were mapped by using nested sets of deleted expression plasmids (14). Rabbit anti-BPV1 antiserum cross-reacts with SDS-denatured virions of other animal and human papillomavirus types (16) and reacts with L1 polypeptides encoded by HPV1a, -6b, -11, -16, and -18 (14). Unidirectional 3'-to-5' deletions were made in the HPV DNA inserts of the bacterial expression plasmids by using exonuclease III and nuclease S1 (S1) according to the protocol of Henikoff (11). Plasmids derived from the exonuclease III-S1 deletions expressed nested sets of fusion proteins sequentially deleted from the carboxy toward the amino terminus of the HPV-encoded polypeptide. The deleted fusion proteins were reacted with the antibodies in a Western immunoblot assay to determine the carboxy boundary of the immunoreactive region (14).

The HPV1a L2 polypeptide segment recognized by all 12 MAbs generated against intact HPV1 virions mapped to the same location. The MAbs reacted with the p1L2Ex4222 fusion protein (3' end at HPV1a coordinate 4222) and larger fusion proteins and failed to react with the p1L2Ex4183 fusion protein (3' end at coordinate 4183) and smaller fusion proteins (Fig. 3). This placed the 3' boundary of the immunoreactive region-coding segment between nucleotide (nt) 4183 and nt4222. This DNA segment encodes HPV1 L2 amino acids (aa) 95 through 108 (measured from the first L2 methionine codon). The HPV1 L2 epitope was further localized by reacting the MAbs in an ELISA with a set of overlapping peptides (hexamers) which spanned the region from aa88 through aa118 (9, 14). The hexapeptides were synthesized on polyethylene pins by using a commercially available kit (9) (Epitope Scanning Kit; Cambridge Research Biochemicals, Wilmington, Del.). The MAbs reacted with two adjacent hexapeptides, one which included aa102 to aa107 and the other which included aa103 to aa108 (Fig. 3). Therefore, the HPV1 L2-reactive MAbs recognized a linear amino acid sequence which lies between aa102 and aa108 (Pro-Ile-Asp-Val-Asp-Pro). The HPV1a L2 amino acid sequence was aligned with the L2 sequences of BPV1 and HPV types 5, 6b, 11, 16, 18, 31, and 33 by using the hierarchical clustering algorithm of Corpet (4). The MAb-reactive epitope (aa102 to aa107) is contained within an 11-amino-acid HPV1a L2 segment (aa100 to aa110) which

FIG. 2. Antibody reactivities of murine polyclonal antisera generated against intact and denatured HPV1 virions. Immune polyclonal antisera were reacted in Western immunoblot assays with bacterial recombinant proteins encoded by the L1 and L2 ORFs of HPV types 1a, 6b, 11, 16, and 18 (see Table 1 for identification of the bacterial recombinant proteins). (A) Coomassie blue-stained SDS-12.5% polyacrylamide gel of whole bacterial lysates. (B and C) Replicate Western immunoblots which were reacted with antiserum generated against intact virions. (D and E) Western immunoblots which were reacted with antiserum generated against SDS-denatured virions. Antigen-antibody complexes were detected by using either anti-IgG antiserum (B and D) or anti-IgM antiserum (C and E). Molecular size standards (in kilodaltons) are on the left.

FIG. 3. Mapping of the immunoreactive region recognized by MAb W275. The immunoreactive region of the HPV1a L2 polypeptide recognized by MAb W275 was mapped by using a nested set of fusion proteins which were serially deleted from the carboxy toward the amino terminus (clones A through J). (A) Coomassie blue-stained SDS-12.5% polyacrylamide gel of whole bacterial lysates which contain these fusion proteins (the positions of the fusion proteins are marked with arrows). (B) Replicate Western immunoblot which was reacted with MAb W275. The nucleotide coordinates of the 3' ends of the HPV1 L2 inserts in clones H and I are indicated (see also Fig. 1). Molecular size standards (in kilodaltons) are on the left. The immunoreactive epitope was localized further by reacting MAb W275 in an ELISA with a set of overlapping hexapeptides which spanned the region defined by exonuclease III-S1 deletion mapping (C). In panel C, the x axis indicates the assigned hexapeptide number and the y axis represents the optical density (O.D.)

does not align with the L2 sequences of the other papillomavirus types and which appears to be an inserted segment relative to the other papillomavirus types. This region of the polypeptide is not predicted to be hydrophilic based on the algorithm of Kyte and Doolittle (18), nor would this region be predicted to be antigenic based on the algorithm of Hopp and Woods (12). All 12 MAbs generated against intact HPV1 virions reacted with the same amino acid segment, and all the MAbs were IgG2a containing kappa light chains. Therefore, it is possible that all 12 clones were daughter cells derived from the clonal expansion of a single HPV1 L2-reactive B cell.

Initial mapping of the DW45-reactive region in the HPV1a L1 polypeptide localized the carboxy boundary of the coding sequence between nt5806 (pL1Ex5806) and nt5886 (pL1Ex5886) (Fig. 4), and further mapping localized the coding sequence between nt5812 and nt5851 (data not shown). This segment encodes HPV1a L1 aa127 through aa140 (measured from the second L1 methionine codon). The HPV1 L1 epitope was further localized by reacting the MAbs in an ELISA with a set of overlapping peptides (hexamers) which spanned the region from aa121 through aa147. The MAbs reacted with two adjacent hexapeptides, aa127 to aa132 and aa128 to aa133. Therefore, the HPV1 L1-reactive MAbs recognized a linear amino acid sequence which lies between aa127 and aa133 (Ala-Glu-Asn-Pro-Thr-Asn-Tyr).

The region of the HPV1a L1 polypeptide recognized by rabbit anti-BPV1 antiserum was further localized by deletion mapping to determine whether it was distinct from the DW45-reactive epitope. The carboxy-terminal boundary of the rabbit anti-BPV1 reactivity mapped to the coding sequence between nt5998 and nt6045, which encodes HPV1 L1 aa190 through aa205 (Fig. 4). None of the fusion proteins which were deleted beyond aa190 reacted with the rabbit anti-BPV1 antiserum. Therefore, the rabbit anti-BPV1 reactive region(s) lies closer to the carboxy terminus of the L1 polypeptide relative to the DW45-reactive region, and these regions do not overlap.

To determine whether L1- and L2-encoded polypeptides were consistent in size with the major and minor viral capsid proteins, respectively, we expressed the HPV1a L1 and L2 coding sequences (20) in the yeast *S. cerevisiae* (1, 2, 17) and by in vitro translation using a rabbit reticulocyte lysate. The HPV1 MAbs were used to immunoprecipitate the HPV1-encoded polypeptides from lysates of yeast cells which were grown in the presence of [³⁵S]methionine (15). The HPV1 L1-reactive MAb DW45 specifically immunoprecipitated a 57-kDa protein from yeast cells containing pY1L1, and the HPV1 L2-reactive MAb W275 specifically precipitated a 92-kDa protein from yeast cells containing plasmid pY1L2 (Fig. 5). These L1- and L2-encoded yeast polypeptides had the same apparent molecular weights on SDS-12.5% PAGE as the major and minor capsid proteins of HPV1a virions, respectively (data not shown).

The HPV1a L1 and L2 coding sequences were transcribed in vitro by bacteriophage T7 RNA polymerase from transcription vector pBS(+) (Stratagene, La Jolla, Calif.). The L1-encoded and L2-encoded RNAs, respectively, were

value obtained by reacting MAb W275 with that hexapeptide in an ELISA. The map displays selected hexapeptide sequences. The MAb W275-reactive hexapeptides are underlined, and the limits of the immunoreactive region are indicated on the extended amino acid sequence at the top of the map.

FIG. 4. Mapping of the immunoreactive regions recognized by MAb DW45 and by rabbit anti-BPV1 antiserum. The immunoreactive regions of the HPV1a L1 polypeptide recognized by MAb DW45 and by rabbit anti-BPV1 antiserum, respectively, were mapped by using a nested set of fusion proteins which were serially deleted from the carboxy toward the amino terminus (clones A through L). (A) Coomassie blue-stained SDS-12.5% polyacrylamide gel of whole bacterial lysates which contain these fusion proteins. (B and C) Replicate Western immunoblots which were reacted with MAb DW45 or with rabbit anti-BPV1 antiserum, respectively. The nucleotide coordinates of the 3' ends of the HPV1a L1 inserts in clones H, I, K, and L are indicated. Molecular size standards (in kilodaltons) are on the left. The immunoreactive epitope recognized by MAb DW45 was further localized by reacting the antibody in an ELISA with a set of overlapping hexapeptides which spanned the region defined by exonuclease III-S1 deletion mapping (D), as in Fig. 3C. O.D., Optical density.

FIG. 5. Immunoprecipitation of HPV1a L1- and L2-encoded polypeptides expressed in yeast cells and in vitro translation of L1 and L2 coding sequences. (A) Yeast cells containing expression vector pBS24.1 (lanes 1 and 2), HPV1a L1 containing plasmid pY1L1 (lanes 3 and 4), and HPV1a L2 containing plasmid pY1L2 (lanes 5 and 6) were incubated in medium containing [35 S]methionine. Yeast lysates were precipitated with HPV1 L1-reactive MAb DW45 (lanes 1, 3, and 5) and HPV1 L2-reactive MAb W275 (lanes 2, 4, and 6). (B) HPV1a L1-encoded and L2-encoded RNAs were transcribed in vitro from bacterial plasmids by using bacteriophage T7 RNA polymerase. The RNAs were translated in vitro by using lysates of rabbit reticulocytes in the presence of [35 S]methionine, and the radiolabeled proteins were subjected to electrophoresis through SDS-10% polyacrylamide gels. In lane 1, the in vitro translation reaction contained no added RNA. In lane 2, the translation reaction contained RNA transcribed from the HPV1a L1 coding sequences. In lane 3, the translation reaction contained RNA transcribed from the HPV1a L2 coding sequences. Molecular size standards (in kilodaltons) are on the left.

translated in vitro by rabbit reticulocyte lysate (*In Vitro* Express Translation Kit; Stratagene) in the presence of [35 S]methionine. The L1 polypeptide had an apparent molecular mass of 57 kDa and the L2 polypeptide had an apparent molecular mass of 91 kDa on an SDS-10% polyacrylamide gel (Fig. 5). The L1- and L2-encoded polypeptides are consistent in size with the major and minor viral capsid proteins, respectively. Therefore, the aberrant mobility of the minor viral capsid protein upon SDS-PAGE appears to be due to an inherent property of the L2-encoded polypeptide and is not due to the addition of high-molecular-weight moieties by posttranslational modification.

We demonstrated that a polyclonal antiserum generated in a BALB/c mouse against intact HPV1 virions contained type-specific antibodies directed against the L2-encoded minor capsid protein and contained no antibodies directed against linear epitopes of the L1-encoded major capsid protein. MABs recognized a linear epitope between L2 aa102 and aa108 (PIDVDP). These results suggest that HPV1 L2 aa102 through aa108 are located on the external viral capsid surface and that they constitute an immunodominant linear epitope in BALB/c mice. A polyclonal antiserum generated in a BALB/c mouse against detergent-denatured HPV1 virions contained both HPV1 L1- and L2-reactive antibodies, and in each case these antibodies were relatively HPV type specific. Polyclonal antisera generated in rabbits against SDS-denatured HPV virions have generally included L1-reactive antibodies which broadly cross-react with other

papillomavirus types (14, 16). The lack of such antibodies in the BALB/c mouse immunized with SDS-denatured HPV1 virions may indicate that, unlike rabbits, the BALB/c mouse did not generate an antibody response to a linear L1 epitope which is highly conserved among papillomaviruses.

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