Common Neutralization Epitope in Minor Capsid Protein L2 of Human Papillomavirus Types 16 and 6

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Studies of virus neutralization by antibody are a prerequisite for development of a prophylactic vaccine strategy against human papillomaviruses (HPVs). Using HPV16 and -6 pseudovirions capable of inducing β-galactosidase in infected monkey COS-1 cells, we examined the neutralizing activity of mouse monoclonal antibodies (MAbs) that recognize surface epitopes in HPV16 minor capsid protein L2. Two MAbs binding to a synthetic peptide with the HPV16 L2 sequence of amino acids (aa) 108 to 120 were found to inhibit pseudoinfections with HPV16 as well as HPV6. Antisera raised by immunizing BALB/c mice with the synthetic peptide had a cross-neutralizing activity similar to that of the MAb. The data indicate that HPV16 and -6 have a common cross-neutralization epitope (located within aa 108 to 120 of L2 in HPV16), suggesting that this epitope may be shared by other genital HPVs.

Human papillomaviruses (HPVs), which have been classified into more than 70 genotypes, cause a variety of proliferating epithelial lesions, including skin and cervical cancers. HPV strains are usually grouped on the basis of the pathogenicity and target tissue. Among HPV types associated with anogenital diseases, high-risk HPV16 is predominantly found in cervical cancer and low-risk HPV6 is found in benign condylomata (6, 13). An icosahedral HPV capsid with a diameter of 55 nm consists of 72 pentameric capsomeres composed of structural proteins L1 and L2 with an estimated molar ratio of 30 to 1. Both type-specific and cross-reactive antibodies binding to the capsid proteins are detectable in sera from patients positive for HPV DNAs (13). Neutralizing activities of anti-L1 antibodies have been studied by using infectious HPV pseudovirions and surrogate cell culture systems to monitor the pseudovirus infection (5, 9, 10). So far, anti-L1 antibodies against HPV6, -11, -16, -18, and -33 have been shown to have a type-specific neutralizing activity. In this study, we examined the neutralizing activity of mouse anti-HPV16 L2 monoclonal antibodies (MAbs) that recognize surface epitopes (4), by using infectious HPV16 and -6 pseudovirions generated by in vitro packaging (5).

Eleven anti-HPV16 L2 MAbs used in this study had been obtained in our previous study (4) by immunization of BALB/c mice with HPV16 L1/L2 capsids (particles self-assembled in insect Sf9 cells expressing L1 and L2). These MAbs recognize linear surface epitopes of the L1/L2 capsids. Epitopes for 7 of 11 MAbs have been localized within a region of amino acids (aa) 69 to 81 in HPV16 L2 (the entire L2 protein is composed of 473 aa residues), but the epitopes for the remaining 4 have not been determined. Besides the previously used synthetic peptide with an HPV16 L2 sequence of aa 69 to 81 (P-69/81), two peptides with aa 95 to 107 (P-95/107) and aa 108 to 120 (P-108/120) were used for the assay of MAbs binding to these peptides (Table 1). The amino acid sequences of the three peptides are conserved among genital HPVs.

Binding of MAbs to L1/L2 capsids or peptides was measured by enzyme-linked immunosorbent assay (ELISA), for which capsids in phosphate-buffered saline (PBS [pH 7.0]) or bovine serum albumin (BSA)-conjugated peptides (synthesized and conjugated by Sawady Technology, Tokyo, Japan) in carbonate buffer (pH 9.6) were fixed in the wells of an ELISA plate (Dynatech Laboratories, Chantilly, Va.). The capsids or the three peptides fixed in the plates were used as antigens after being blocked with 0.2% gelatin in PBS. Diluted ascites fluid (300 μl/well) containing MAb was added to the wells and incubated for 1 h at room temperature. Horseradish peroxidase-conjugated, goat anti-mouse immunoglobulin (IgG; Dako Corp., Carpinteria, Calif.) (1:2,000 in 1% BSA in PBS) was used as a secondary antibody. A mixture of 0.01% H2O2 and o-phenylenediamine (2 mg/ml) in 0.1 M citrate buffer (pH 4.7) was added to the wells, and the A450 was measured. Specific absorbency was calculated by subtracting the absorbency of mock wells covered with gelatin or BSA.

As shown in Table 1, two MAbs, no. 5 and 13, were found to bind to P-108/120, whereas seven MAbs, no. 2, 4, 6, 7, 9, 10, and 17, in agreement with the previous results, bound to P-69/81. Two MAbs, no. 11 and 12, bound to none of the three peptides. The data show that the epitopes for MAbs 5 and 13 are in the region of aa 108 to 120.

For the assay of the neutralizing activity, MAb5 (IgG2a), MAb13 (IgG3), and MAb6 (IgG2a) were selected from those listed in Table 1. MAb5 and MAb13 bound to the epitope(s) within aa 108 to 120, and MAb6 recognized an epitope within aa 69 to 81. For comparison, anti-HPV6 L1 and anti-HPV16 L1 antisera were tested for neutralizing activity. These antisera were obtained from BALB/c mice injected with purified HPV6 and HPV16 L1 capsids (particles self-assembled in insect Sf9 cells expressing L1 alone [7]) without adjuvant, respectively. For each type, a pool of sera from 10 mice was used.

Neutralizing activity was tested against that of in vitro-reassembled HPV pseudovirions containing an expression plasmid for β-galactosidase. HPV16 pseudovirions were produced and purified as described previously (5), and HPV6 pseudovirions
TABLE 1. Binding of MAbs to synthetic peptides

<table>
<thead>
<tr>
<th>MAb no.</th>
<th>ELISA titer (A450)</th>
<th>L1/L2 capsid</th>
<th>P-69/81</th>
<th>P-95/107</th>
<th>P-108/120</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>0.209</td>
<td>0.116</td>
<td>0.001</td>
<td>0.001</td>
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</tr>
<tr>
<td>2</td>
<td>0.148</td>
<td>0.071</td>
<td>0.000</td>
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<tr>
<td>4</td>
<td>0.110</td>
<td>0.103</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.151</td>
<td>0.077</td>
<td>0.000</td>
<td>0.000</td>
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<tr>
<td>7</td>
<td>0.172</td>
<td>0.055</td>
<td>0.000</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.100</td>
<td>0.101</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.121</td>
<td>0.110</td>
<td>0.001</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.181</td>
<td>0.001</td>
<td>0.002</td>
<td>0.115</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.198</td>
<td>0.000</td>
<td>0.000</td>
<td>0.120</td>
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<tr>
<td>11</td>
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<td>0.000</td>
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<td>12</td>
<td>0.146</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

were prepared similarly. The pseudovirions at 50 infectious units in 0.1 ml of PBS (pH 6.8) were mixed with 0.1 ml of serial dilutions of the ascerted fluid containing MAB or antisera in PBS (pH 6.8), incubated at 4°C for 1 h, and then mixed with COS-1 cells (4 × 10⁵), which had been dispersed with PBS containing EDTA (2.5 mM) and washed with Dulbecco's modified Eagle's medium before mixing, in 0.3 ml of PBS containing BSA (0.01%) for 2 h at 4°C with constant agitation. The cells were seeded into six-well plates, grown for 36 h, and fixed with glutaraldehyde. Cells expressing β-galactosidase were stained with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) as a substrate (In Situ β-Galactosidase Staining Kit; Promega Corp.). The number of blue cells was counted, and the neutralizing activity was expressed as a reciprocal of the maximum dilution of serum or ascites fluid that reduced the number of blue cells to half of that in the sample treated with PBS containing BSA.

Table 2 summarizes the results of assays for binding activity to the L1/L2 capsids and neutralizing activity of the antisera and MAbs. As shown previously (5, 9, 10), the binding and neutralizing activities of MAbsb were also type specific. In contrast, MAbsb and MAbsc were found to inhibit pseudoinfections with both HPV16 and -6, strongly suggesting that the two HPVs have a common L2 neutralization epitope.

To further confirm the presence of a type-common neutralization epitope, mouse antisera were raised by immunizing BALB/c mice with the BSA-conjugated peptides P-1/12 and P-108/120, representing HPV16 L2 sequences of aa 1 to 12 and 108 to 120, respectively. Fifty micrograms of the antigen mixed with Freund's complete adjuvant was used for each mouse for the first intracutaneous injection, which was followed by three intraperitoneal injections of the antigen mixed with Freund's incomplete adjuvant at intervals of 2 weeks. For each peptide, sera were collected and pooled from 10 mice at 2 weeks after the final immunization.

Table 2 includes neutralization data with the antisera against the synthetic peptides. Like MAbsb and -13, the anti-P-108/120 sera bound to pseudovirions of both HPV16 and -6 and inhibited pseudoinfections with both HPVs. Thus, the data clearly show that the neutralization epitope common to HPV6 and -16 is in the L2 region of aa 108 to 120 in HPV16.

The L2 region of aa 108 to 120, containing the cross-neutralization epitope for MAbsb and -13, is considered to be on the surface of virions, because the MAbs bind to intact L1/L2 capsids (Table 1). The region close to the N terminus of bovine papillomavirus type 1 (BPV1) L2 appears to be displayed on the surface of the intact virions, as shown by binding of three anti-L2 MAbs recognizing epitopes in an L2 region of aa 61 to 123 (12). Despite the lack of amino acid sequence homology in the N-terminal region, the topographical location of L2 in the virion is probably common to HPVs and BPVs. Furthermore, antibodies against the N-terminal region of BPV L2 (aa 45 to 173 for BPV1 [2, 8], aa 11 to 200 for BPV4 [3], and aa 131 to 151 for BPV4 [1]) have been reported to be involved in neutralization. It is probable, therefore, that binding of anti-L2 antibodies to the surface region of L2 inhibits infection through a common mechanism.

The amino acid sequences of the surface regions corresponding to the HPV16 L2 region of aa 108 to 120 seem to be highly conserved among different HPVs (Fig. 1). The amino acid sequence homology is higher between HPV16 and HPV33/58 than between HPV16 and HPV6. In fact, the guinea pig antiserum binding to an HPV33 L2 sequence of aa 107 to 117 is cross-reactive with the L2 proteins of HPV11, -16, and -18 (11). Although the cross-neutralization between different types remains to be examined, it is likely that this region contains a type-common cross-neutralization epitope among genital HPVs. This epitope may be used as a target for development of a type-common anti-HPV vaccine.

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REFERENCES


