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Two Amino Acid Residues Confer Type Specificity to a Neutralizing, Conformationally Dependent Epitope on Human Papillomavirus Type 11

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Characterization of virus binding by neutralizing antibodies is important both in understanding early events in viral infectivity and in development of vaccines. Neutralizing monoclonal antibodies (MAbs) to human papillomavirus type 11 (HPV11) have been described, but mapping the binding site has been difficult because of the conformational nature of key type-specific neutralization epitopes on the L1 coat protein. We have determined those residues of the L1 protein of HPV11 which confer type specificity to the binding of HPV11-neutralizing MAbs. Binding of three HPV11-specific neutralizing MAbs could be redirected to HPV6 L1 virus-like particles in which as few as two substitutions of corresponding amino acid residues from HPV11 L1 have been made, thus demonstrating the importance of these residues to MAb binding through the transfer of a conformationally dependent epitope. In addition, a fourth neutralizing MAb could be distinguished from the other neutralizing MAbs in terms of the amino acid residues which affect binding, suggesting the possibility that it neutralizes HPV11 through a different mechanism.

Human papillomavirus types 6 and 11 (HPV6 and HPV11) are the major etiological causes of condylomata acuminata (for a review, see reference 9). A panel of monoclonal antibodies (MAbs) which neutralize HPV11 virions in the athymic mouse xenograph neutralization assay has been described (4, 6, 18). These antibodies were generated against whole HPV11 virions. They also bind virus-like particles (VLPs) composed of ca. 360 polypeptides of the major coat protein L1 which assemble, upon recombinant expression in a number of eukaryotic systems, into a structure morphologically very similar to that of whole virions (2, 10, 11, 16, 17, 22, 24). Binding of these MAbs is type specific to HPV11, and the MAbs do not bind monomeric unassembled L1 protein. Type specificity and conformational (VLP) dependence of binding are also observed for MAbs which neutralize cottontail rabbit papillomavirus (CRPV) and bovine papillomavirus (5, 21) and may be general requirements for a papillomavirus-neutralizing response (12–14).

The type specificity of the HPV11-neutralizing MAbs does not extend even to VLPs derived from the genetically closely related HPV6 L1 gene (3), which is identical to that of HPV11 in all but 39 out of 501 amino acid residues (which include the Y insertion at position 132 of HPV11 L1 [Fig. 1]) (8, 23). Therefore, residues responsible for type-specific conformational epitopes to HPV11 must be contained among these 39 amino acid differences.

We postulated that an analysis of the contributions by each of these 39 residues to binding of HPV11 L1 VLPs by the neutralizing MAbs would identify residues critical for type-specific binding. The essential nature of these residues could be established further by substitution of such critical residues into the HPV6 L1 sequence and demonstration of binding by HPV11-specific MAbs to a minimally modified HPV6 L1 VLP. To accomplish this, single amino acid replacements of the naturally occurring HPV6 L1 residues were introduced into the corresponding HPV11 L1 sequence at all 39 differing positions by site-directed mutagenesis. Because the replacement residues occur naturally in the HPV6 L1 sequence, we postulated that these substitutions were not likely to grossly perturb VLP formation. The individually substituted L1 genes were subcloned into baculovirus transfer vector pVL1393 and expressed in Sf9 cells, and extracts were prepared as previously described (1, 20). The extracts were evaluated for binding by HPV11-specific, conformationally dependent, neutralizing MAbs (6), and the results are summarized in Table 1.

The HPV6 and HPV11 L1 genes were cloned from clinical isolates (by L. Schultz and S. W. Ludmerer, Merck Research Laboratories) by using primers designed from the published sequences (8, 23). Mutagenesis was performed with the Sculptor in vitro mutagenesis system (Amersham International plc, Buckinghamshire, England), and mutations were confirmed by DNA sequencing. MAbs were obtained as ascites stock from Neil Christensen (Pennsylvania State University, Hershey) and diluted 10^{-5} (in 1.0% bovine serum albumin–phosphate-buffered saline) prior to use.

Normalized affinity values are shown in Table 1 and were determined as follows. Antibody binding, measured by enzyme-linked immunosorbent assay (ELISA), was adjusted to the total level of L1 protein in the sample with MAb H6.C6, a non-VLP-dependent MAb which cross-reacts with both HPV6 L1 and HPV11 L1. The values were then normalized to the L1-adjusted binding observed with native HPV11 L1 VLPs. Thus, a normalized affinity value at or near 1 indicates no detectable effect on binding as a result of the particular substitution. Values less than 1 result if the substitution significantly diminishes binding. An HPV11 prototype transfection was included in every set of transfections so that normalization was performed with fresh material. ELISAs were performed at excess antibody concentration, so the scale is not linear with respect to bound antibody.

The average affinity value for all singly substituted HPV11 L1 clones was 1.02 ± 0.2, with individual values much greater than 1.0 not observed because binding was conducted at saturating antibody concentration (data not shown). HPV6 VLPs gave values in the range of 0.1 to 0.2 and established the

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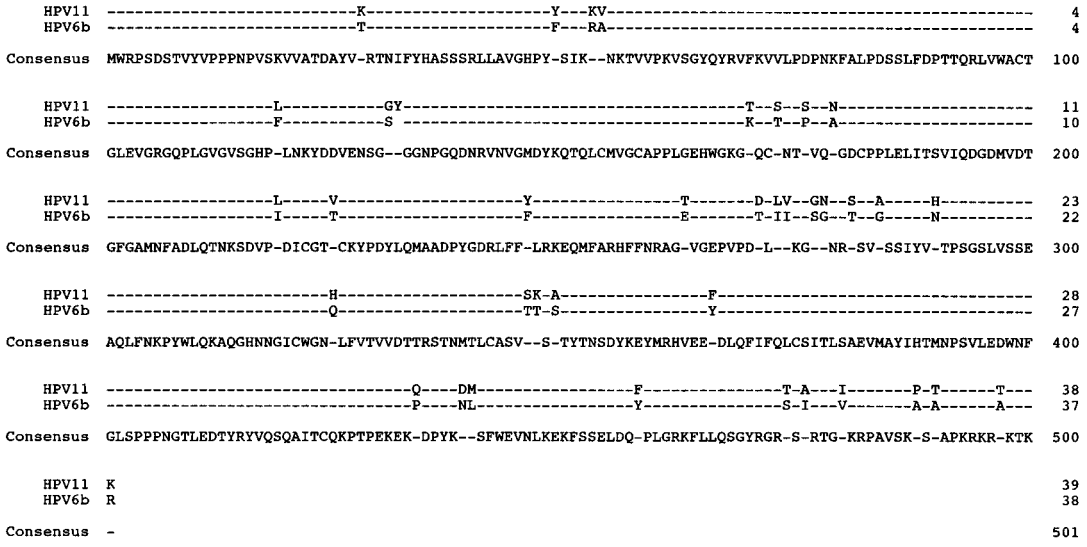

FIG. 1. Alignment of the L1 sequences of HPV6 and HPV11. The amino acid sequences were obtained from the EMBL gene bank and translated and aligned by using the GCG package and the prettyplot program. HPV11 L1 accession no., M14119; HPV6 accession no., X00203.

baseline for complete loss of type-specific recognition. Only five positions were identified in which substitution lowered the normalized affinity value greater than 1 standard deviation from the norm, and we limited further analysis to these five positions (Table 1). We operationally define these positions as "critical," although the present analysis does not distinguish direct effects on binding from indirect effects of subtle conformational changes induced on adjacent sites.

Amino acid substitutions define two binding patterns for the HPV11-neutralizing MAbs. Binding by neutralizing MAbs H11.B2, H11.F1, and H11.G5 was reduced to levels comparable to that for HPV6 VLPs both by substitution at residue 131 and by deletion of residue 132 (Table 1). Substitutions at residues 246 and 278 also resulted in strong and weak effects, respectively, on the binding of the same three MAbs. Binding by neutralizing MAb H11.H3 was markedly reduced upon substitutions at residues 346 and 246, and a more limited effect was observed upon deletion of residue 132. In contrast to the other three MAbs, no substitution reduced binding of MAb H11.H3 to levels comparable to that for nonrecognized HPV6 VLPs. In particular, no effect on H11.H3 binding was observed upon substitution at 131, a substitution which resulted in near complete loss of binding for the other three neutralizing MAbs. With the exception of position 246, all substitutions yielded VLPs with no detectable effect on binding by at least one VLP-dependent MAb (Table 1 and data not shown).

TABLE 1. Normalized affinity values for amino acid positions critical for binding of HPV11-specific VLP-dependent antibodies[a]

Substitution	Affinity				
	H11.A3.2	H11.B2	H11.F1	H11.G5	H11.H3
G131S	0.93	0.20	0.11	0.12	0.96
Δ132	1.00	0.36	0.08	0.11	0.64
G131A	0.99	0.11	0.08	0.10	1.03
Y132A	1.18	0.74	0.67	0.79	0.93
Y246F	0.48	0.33	0.52	0.45	0.32
N278G	0.63	0.78	0.76	0.65	0.92
S346T	0.87	0.80	0.88	0.85	0.41
HPV6	0.14	0.18	0.20	0.14	0.11

[a] Antibody binding was adjusted to total levels of L1 protein bound by antibody H6.C6, an anti-HPV6 MAb cross-reactive with HPV11 that recognizes L1 in both assembled and denatured forms. The L1-adjusted value then was normalized to the L1-adjusted binding with HPV11 prototype VLPs. The average of all HPV11 clones was 1.02 with a standard deviation of 0.2. Positions where replacement resulted in values less than 0.80 were selected for further evaluation. Values obtained for HPV6 VLPs, which are not cross-reactive with the HPV11 MAbs, are also shown. MAbs H11.B2, H11.F1, H11.G5, and H11.H3 are all type-specific VLP-dependent antibodies which neutralize HPV11 in the xenograph neutralization assay (6). MAb H11.A3, also type specific, is a nonneutralizing VLP-dependent antibody.

This analysis delineated two distinct, albeit possibly overlapping binding patterns (Fig. 2). Binding of antibodies H11.B2, H11.F1, and H11.G5 is centered at residues 131 and 132. Although binding of H11.H3 is affected by residue 132, its binding is shifted towards the C-terminal direction of the linear L1 sequence through a strong dependence upon residue 346. A type-specific epitope to HPV6 has been mapped to residues 417 to 437, but its possible role in neutralization has not been determined (15). Also, note that the substitutions which produce the most profound effects on binding neutralizing MAbs—substitutions at residues 131, 132, and 346—have little effect on the binding of the nonneutralizing, VLP-dependent MAb H11.A3.2.

To address whether Y-132 principally affects epitope alignment, we compared the effects of alanine substitutions at positions 131 and 132 on MAb binding (Table 1). The G-131→A (G131A) substitution, like the G131S substitution, abolished

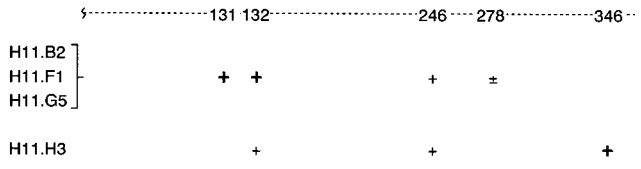

FIG. 2. Two binding patterns for HPV11-neutralizing MAbs defined by single amino acid substitutions. Four HPV11-neutralizing MAbs can be placed into one of two groups on the basis of differential effects on binding upon the shown amino acid substitutions. +, loss or near loss of binding upon amino acid substitution; +, pronounced effect but detectable binding upon substitution; ±, marginal effect upon substitution.

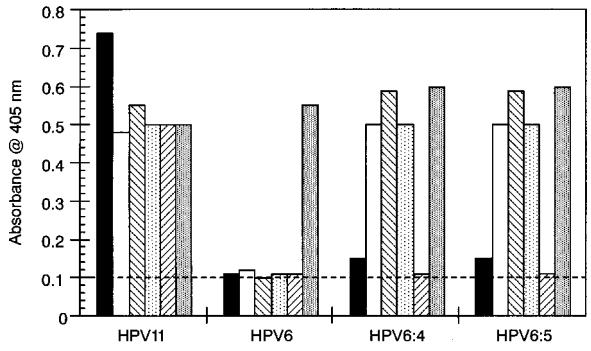

FIG. 3. Four substitutions into the HPV6 L1 sequence redirect binding of HPV11-specific neutralizing MAbs. Four substitutions were made into the HPV6 L1 sequence to generate clone HPV6:4, as described in the text. VLPs were expressed and assembled in Sf9 cells, and an ELISA was performed to evaluate antibody binding. Datum points were collected in triplicate and averaged. Clone HPV6:5, which contains an additional substitution at position 345, does not induce binding by antibody H11.H3, although VLPs produced from it do bind the other three neutralizing MAbs. MAbs, used as 10^{-5} dilution of ascites stock, are indicated as follows: ■, H11.A3.2; □, H11.B2; ▧, H11.F1; ⬚, H11.G5; ▨, H11.H3; ▩, H6.C6. Binding to HPV11 extract by MAb CRPV.5A, a CRPV-specific antibody which does not cross-react with human papillomavirus types, is shown as a dashed line.

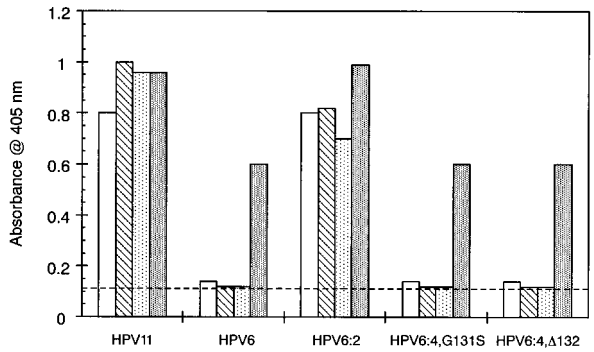

FIG. 4. Two essential substitutions into the HPV6 L1 sequence are sufficient to redirect binding of HPV11-neutralizing MAbs. Clone HPV6:2, which contains a G131S substitution followed by an additional Y residue, produces VLPs which bind three neutralizing antibodies. The essentialness of these positions to binding is demonstrated by the lack of binding to VLPs produced from clones HPV6:4,S131G and HPV6:4Δ132, which lack either substitution but contain substitutions at all other residues observed to affect binding of the MAbs. □, H11.B2; ▧, H11.F1; ⬚, H11.G5; ▩, H6.C6. Binding to HPV11 extract by MAb CRPV.5A, a CRPV-specific antibody which does not cross-react with human papillomavirus types, is shown as a dashed line.

binding of H11.B2, H11.F1, and H11.G5 but was without effect on the binding of H11.H3. In contrast, the Y132A substitution did not result in the severe binding impairments observed with the deletion at this position and in particular was without effect on the binding of MAb H11.H3. These results strongly suggest that Y-132 principally affects alignment of the epitope but does not directly stabilize antibody interactions nearly as much as the neighboring G-131. A gross distortion of VLP structure upon deletion of Y-132 probably did not occur, as the deletion did not affect binding of VLP-dependent, nonneutralizing MAb H11.A3.2 (Table 1).

Two substitutions in the HPV6 L1 sequence redirect binding of HPV11-neutralizing MAbs. Three of four neutralizing MAbs share the same set of critical residues at amino acid positions 131, 132, 246, and 278. To test whether we could switch binding of HPV11-specific antibodies to an unrecognized HPV type, we inserted a tyrosine after residue 131 of the HPV6 L1 gene and substituted HPV11 residues at positions 131, 245, and 277 of HPV6 L1. This clone, HPV6:4, directed production of VLPs which bound HPV11-neutralizing antibodies H11.B2, H11.F1, and H11.G5 (Fig. 3). Binding by H11.H3 and nonneutralizing but VLP-dependent H11.A3.2 was not observed, thus demonstrating that these altered VLPs still lack some immunological characteristics of HPV11 VLPs. Although specific amino acids have been implicated in binding conformationally dependent antibodies (19), to our knowledge this is the first explicit demonstration of residues which define a conformational epitope by redirecting the binding of antibodies to a previously unrecognized modified surface.

A second HPV6 L1 clone, HPV6:5, which contained a substitution at residue 345 in addition to the four changes discussed above, produced VLPs which bound the same three HPV11-specific MAbs. Binding, however, did not extend to antibody H11.H3. There may be a strong surface contact composed of several residues unique in sequence to HPV11 L1, but the individual contributions of the residues are modest. Consistent with this view is the fact that no single substitution diminished binding by H11.H3 to baseline levels comparable to that for HPV6 VLPs, in contrast to what was observed for the other three neutralizing MAbs (Table 1).

Single mutations in the HPV11 L1 sequence at either residue 131 or 132 abolished binding by antibodies H11.B2, H11.F1, and H11.G5 (Table 1). Therefore, we constructed an HPV6 L1 mutant which contained only these two substitutions and tested VLPs produced from it for binding. Figure 4 shows that clone HPV6:2 produced VLPs which bound the same three neutralizing MAbs. In contrast, binding is not observed to VLPs produced from back-mutated HPV6:4 clones which lack either of these substitutions (HPV6:4,S131G and HPV6:4Δ132), even though the three other critical replacement residues are present. This result demonstrates that the epitope is principally centered at residues 131 and 132, although residues 245 and 277 may elicit subtle conformational effects which modestly impact antibody affinity.

In summary, the binding site of antibodies H11.B2, H11.F1, and H11.G5 is defined by the same set of residues, while antibody H11.H3 binds a distinct, although possibly overlapping site. This result is consistent with the previously reported observation that anti-idiotype antibodies generated against H11.B2 also bind antibodies H11.F1 and H11.G5 but not H11.H3 (7). Thus, these two sets of antibodies may define distinct steps in viral neutralization, as has previously been suggested for BPV-1 neutralizing antibodies (21). A crystal structure of the HPV11 VLP will clarify the spatial relationship of the residues which define these two overlapping sites.

Neutralization studies of HPVs have been limited because of the lack of a suitable neutralization assay for all types but HPV11. Neutralization assays do exist for both bovine and cottontail rabbit papillomaviruses, and neutralizing MAbs have been defined for these types. Because our data demonstrate gain of binding to HPV6 VLPs which harbor appropriate substitutions at residues 131 and 132, the site of binding to the neutralizing MAbs likely encompasses this region. Presently, we are introducing mutations into the CRPV L1 sequence at residues which correspond to HPV11 L1 residues 131 and 132. Effects on binding caused by these mutations may suggest that this region is an important neutralization domain common among many papillomavirus types. Such information should be useful in the future delineation of the early steps in papillomavirus infectivity and may suggest possibilities in the design of peptides which block infectivity.

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