Identification of B epitopes in human papillomavirus type 16 E7 open reading frame protein

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Human papillomavirus (HPV) type 16 is implicated in the aetiology of anogenital dysplasia which may progress to malignancy. HPV-16 DNA is actively transcribed in cervical carcinomas, the most abundant transcripts being from the E6 and E7 early open reading frames. The E7 protein has been shown to have transforming activity in vitro. In this report we define four immunodominant B epitopes within the protein corresponding to the E7 gene, using a panel of murine monoclonal antibodies. Three epitopes are linear and lie within the N-terminal region of the molecule, and are unique to the HPV-16 E7 protein. One epitope is non-linear and presumed to be conformational. At least three of the four epitopes of the E7 protein are detectable by immunoprecipitation from an HPV-16-infected cervical carcinoma cell line. The demonstrated immunogenicity of the E7 protein allows us to deduce that this molecule may be a potential candidate for incorporation in a vaccine against cervical cancer.

Introduction

Human papillomavirus (HPV) genome types 6, 11, 16, 18, 31, 33, 35, 39 and 42 constitute a subgroup of HPV infective for anogenital epithelium (Gissmann, 1984; zur Hausen & Schneider, 1987). Types 16 and 18 produce flat warts or acetowhite mucosal lesions which in an estimated 5% of cases progress to squamous cell carcinoma (SCC) of the cervix (Syrjanen et al., 1985). Up to 90% of cervical carcinomas in some series have HPV-16 and -18 DNA sequences integrated into chromosomal DNA (Dürst et al., 1985; Gissmann et al., 1983). Non-integrated (episomal) HPV-16 and -18 can be identified in premalignant abnormalities of the anogenital epithelium (cervical, vaginal, vulval and penile intraepithelial neoplasia).

In vitro transformation of mouse fibroblasts (Yasumoto et al., 1986), rat epithelial cells (Matlashewski et al., 1987) and primary human keratinocytes (Schlegel et al., 1988; Pirisi et al., 1987) with gene fragments of HPV-16 have implicated E7 and E6 open reading frame (ORF) gene products in cell immortalization. Cooperation with an active ras oncogene leads to full transformation (Matlashewski et al., 1987). The E7 protein is the most abundant viral protein in the HPV-16-containing CaSkI and SiHa SCC cell lines and in HPV-18-containing HeLa and C4-1 lines (Seedorf et al., 1987; Banks et al., 1987).

Host factors are important in determining the outcome of genital HPV infection (Singer et al., 1984) as demonstrated by a striking increase in SCC of the cervix but not of control organs such as breast or rectum in immunosuppressed allograft recipients (Sheil & Flavel, 1986), and an increased incidence of proneoplastic HPV-associated lesions in homosexual men immunosuppressed by human immunodeficiency virus infection (Frazer et al., 1986). The congenital B immunodeficiency diseases and the consequences of the use of T immunosuppressive drugs suggest that cellular immune responses are important for the control of the phenotypic expression of HPV infection (Kirschner, 1986).

Little information is available on the immunogenicity of the HPV genotypes implicated in transformation of anogenital epithelium. Standard immunovirological approaches to the study of HPV have been hampered by the lack of a suitable animal model and of an in vitro epithelial cell culture system permissive for HPV. E7 protein is recognized as foreign by the immune system, as anti-E7 antibodies can be detected in the serum of approximately 20% of patients with HPV-16-associated cervical lesions (Jenison et al., 1988; Jochmus-Kudielka et al., 1989; our unpublished data).

Together, these observations suggest that the E7 protein merits consideration as a candidate vaccine antigen. Virtually nothing is known of the antigenicity of E7 at the molecular level. Here we describe three linear B epitopes and at least one conformational B epitopic region of the HPV-16 E7 transforming protein, defined
by a panel of mouse monoclonal antibodies (MAbs). The amino acid composition of each of the linear B epitopes is unique to HPV-16. The MAbs defining the linear epitopes do not react, or have considerably reduced reactivity, with the corresponding analogous epitopes from other HPV types. The conformational epitope is shared with, or at least the antibodies are cross-reactive with, an epitope on HPV-18 E6.

Methods

Antigens. HPV E-ORF peptides (HPV-16 E6, E7, E4, L112 and L1232 and HPV-18 E6, E7 and E1) were produced as MS2 replicase fusion proteins (FPs) produced from heat-inducible phage promoters in pPLc24 expression vectors (provided by L. Gissmann) in Escherichia coli C600/S37. FPs were partly purified from lysozyme-disrupted bacteria by Triton X-100 and sequential urea extraction as described (Seedorf et al., 1987). Purification was monitored on PHAST (Pharmacia) SDS-PAGE. Preparations containing 60 to 90% pure FP, judged by appropriately sized major bands on gels, were obtained as 8- to 10 M-urea extracts.

Cell lines. CaSki (HPV-16), SiHa (HPV-16), HeLa (HPV-18) cervical carcinoma lines and the non-HPV-containing melanoma line M20096 (obtained from Dr P. Smith, Royal Brisbane Hospital) were maintained in RPMI 1640 with penicillin and streptomycin, HEPES buffer, glutamine and 10% foetal bovine serum (Flow Laboratories).

MAbs. BALB/c mice were immunized three times at 2-weekly intervals with 20 to 100 μg HPV-16 E7/MS2 FP intraperitoneally in complete Freund’s adjuvant and boosted intravenously 4 days prior to removal of spleen cells for fusion with SP2/0 myeloma cells using standard techniques (Campbell, 1980). Twelve of 200 hybridomas positive for E7/MS2 but negative for MS2 alone in ELISA screening, were twice cloned at limiting dilution, and the resulting MAbs were isotyped (Misostest, CSL). Ascites were raised in Pristane- (Sigma) primed mice.

ELISAs, radioisotope binding assays (RIAs). The specificity of the MAbs was determined in two ways. First, ELISA or RIA was employed, using peroxidase or 125I-conjugated anti-mouse Ig (Amersham) as second step reagents against the various HPV ORF peptides (Flow Laboratories) at 10 to 100 μg/ml. Plates were blocked with 5% bovine serum albumin (BSA) in 0.1 M-phosphate-buffered saline (PBS) pH 7.4 for 1 h at room temperature, prior to sequential addition of first and second antibody at 37 °C for 45 min, with appropriate washing steps. The reaction was quantified following the addition of orthophenylenediamine (Sigma) substrate, on a Titertek ELISA reader (for peroxidase second antibody) or by autoradiography of whole plates (for 125I second antibody). The second method was by immunolocalization on nitrocellulose following Western blot transfer of ORF peptides separated by 12% SDS-PAGE (see below).

Western blots. FPs or cell lysates were separated by 12% SDS-PAGE and transferred to nitrocellulose according to standard procedures. Blots were blocked with 5% skim milk powder in PBS (blocking buffer), and probed with MAb ascitic fluid diluted 10–2 to 10–5 in blocking buffer, for 3 h at room temperature. Following washing, specific bands were visualized with 125I-labelled Protein A (Amersham) or anti-mouse Ig (Amersham) and subsequent autoradiography on X-OMAT film (Kodak).

Competitive binding assay. Hybridomas were cultured for 3 to 5 h in methionine-free RPMI medium with 10% foetal bovine serum and 100 μCi [35S]methionine (Amersham). Supernatants were used at a dilution of 1:5 in PBS plus 0.1% BSA as a source of [35S]-labelled antibody for competition in solid-phase binding assays against HPV-16 E7/MS2 with three dilutions of unlabelled actes MAbs shown by previous titration to cause approximately 5, 50 and 90% saturation of E7 antigen-binding sites respectively. All tests were done in duplicate and experiments were repeated at least three times.

Labelled and unlabelled antibodies were added concomitantly and incubated for 1 h at 37 °C. Reactions were carried out in microtitre plates which were cut into individual wells and bound antibody was quantified by scintillation counting. Each member of a pair of competing antibodies was tested reciprocally. The positive control was complete inhibition of 35S binding of unlabelled homologous antibody; the negative control was absence of inhibition by irrelevant ascites antibody. Results are expressed as inhibition of binding of 35S-labelled antibody by non-radioactive competing antibody, and calculated as the percentage of total inhibition by saturating amounts of unlabelled homologous antibody.

Immunoprecipitation. The protocol for immunoprecipitation has been described (Burkhardt et al., 1987). Near-confluent monolayer cultures (10 cm plates) of CaSki and HeLa cells were labelled with 1 mCi [35S]cysteine for 4 to 5 h in approx. 3 ml serum-free Dulbecco’s modified Eagle’s medium without cysteine. Following two washes in PBS, the cells were extracted with RIPA buffer (20 mM-MOPS, 150 mM-NaCl, 1 mM-EDTA, 1% NP40, 1% deoxycholate and 0.1% SDS, pH 7.0) and the supernatant was immunoprecipitated with anti-E7 MAbs followed by goat anti-mouse Ig conviently linked to agarose beads.

Linear epitope mapping. As previously described (Geyser et al., 1987), a series of octapeptides each overlapping by seven amino acids was synthesized by the t-butylxycarbonyl deprotection technique, on 96 rods in the format and spacing of a microtitre plate. ELISA was carried out on the rods with MAbs and conjugate solution in microtitre trays as described. Ninety-one octapeptides were synthesized, their amino acid composition having been translated from the published HPV-16 E7 ORF DNA sequence (Seedorf et al., 1985), to cover the entire length of the putative 97 amino acid E7 protein. The ELISA reaction was carried out by dipping the tips of the peptide-coated rods into 1:1000 dilutions of MAb ascitic fluid, washing and visualizing the reaction by addition of peroxidase-conjugated anti-mouse Ig (Kirkegaard and Perry) followed by 2,2-azino-bis(3-ethylbenzothiazolinesulphonic acid) substrate and spectrophotometric absorbance measurement at 450 nm against the reference wavelength of 492 nm. Linear epitopes were identified by noting consensus amino acid sequences of all sequential peptides with (1988) programs. In later experiments, tetra- and pentapeptides corresponding to HPV-16 E7 linear epitopes and to some homologous regions of HPV-18, -33, -6b and -11 were synthesized in duplicate on rods and reacted in ELISA with MAbs shown to recognize HPV-16 E7 linear epitopes by octapeptide analysis (see above). Reactivity was measured as the percentage of the absorbance read-out of the corresponding HPV E7 peptide.

Amino acid sequence analysis and comparisons. Alignment and translation of DNA sequence data obtained from a DB $ GenBank Sequence Library computer search allowed comparison of HPV-16 E7 peptide with putative translational products of other HPV genotypes. Secondary structure predictions of HPV-16 E7 peptide were made using the PREDICT program (Department of Biophysics, University of Leeds, U.K.). T epitope predictions were made using the AMPHI (De Lisi & Berzofsky, 1985) and Rothbard motif (Rothbard & Taylor, 1988) programs.

Immunofluorescence. CaSki, HeLa or M20096 cells were washed,
MAbs 3B, 7B and 10F, taken as examples of the panel of 12, specifically immunoprecipitated a protein of approximately 20K from CaSki cells, which contain multiple copies of integrated HPV-16 (Fig. 2b), but no corresponding protein from HeLa cells which contain HPV-18 (Seedorf et al., 1987; Yee et al., 1985). This corresponds to the observed Mr of the E7 protein discussed by others (Smotkin & Wettstein, 1987). In another experiment MAbs 8F and 6D precipitated an identical band from CaSki cells but not from C33-A cells (HPV-16-negative cervical carcinoma cells; M. Stanley, personal communication). MAbs 3B, 7B and 10F also specifically immunoprecipitated a 25K protein from CaSki but not HeLa cells (Fig. 2b).

Competitive binding assays

Competitive binding assays were carried out using the panel of MAbs in order to delineate the epitopic sites on HPV-16 E7/MS2 FP and with HPV-18 E6/MS2 FP. No other ORF peptides were recognized. A summary of reactivities is given in Table 1.
Fig. 2. (a) Solid-phase RIA of the panel of 12 MAbs selected for binding to HPV-16 E7/MS2 replicase FP but not MS2 alone, compared to ORF/MS2 FP's of HPV-16 and HPV-18. ORF/MS2 proteins were bound to the wells of microtitre plates and reacted sequentially with ascites MAbs at 10⁻³ dilution and ¹²⁵I-labelled anti-mouse Ig, with appropriate washing steps. The microtitre plates were subsequently autoradiographed overnight. A map showing the locations of MAb additions to the microtitre plates is given. Each MAb was reacted in duplicate. The plus symbol denotes mouse polyclonal anti-MS2 serum as positive control; IRR denotes irrelevant ascites MAb as negative control. (b) CaSki (C) and HeLa (H) cells were labelled with [³⁵S]cysteine, solubilized and immunoprecipitated with undiluted ascites MAbs or with a rabbit polyclonal antiserum (R) generated against a trypE-HPV-16 E7 FP. (Immunoprecipitation was performed by Drs M. Hubbert and H. Barbosa, NIH.)

the E7 protein (Fig. 3). The strategy was to allow each antibody to compete reciprocally with every other to obtain two-way blocking data. This was not possible in every case since some antibodies consistently labelled poorly with [³⁵S]methionine, and in these cases one-way blocking data only were available. An example is given in Fig. 3(a); unlabelled 10F MAb clearly blocks ³⁵S-6D as effectively as ³⁵S-10F, but completely fails to block ³⁵S-8F. ³⁵S-1F is partly blocked. This result is confirmed in the reciprocal experiment (Fig. 3b) in which MAb 6D blocks ³⁵S-10F as effectively as ³⁵S-6D but not ³⁵S-8F. The overall results are summarized in Fig. 3(c). The results indicate four epitopic regions within the E7 peptide, three of which, delineated by (I) 8F, (II) 4F and (III) 10F and 6D, were clearly defined and non-overlapping. MAbs recognizing the fourth region (IV) were a mutually cross-blocking group consisting of two subgroups: 3B and 1F which partially blocked 6D of epitopic region III, and 9A, 7B and 11G which did not.

Table 1. Summary of reactivity of the panel of anti-HPV-16 E7 MAbs with HPV-16 and -18 ORF/MS2 fusion peptides

<table>
<thead>
<tr>
<th>MAb</th>
<th>Isotype</th>
<th>HPV-16 E7</th>
<th>HPV-18 E6</th>
<th>Other ORF peptides tested*</th>
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<tbody>
<tr>
<td>8F</td>
<td>IgG1κ</td>
<td>+</td>
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<td>-</td>
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<tr>
<td>4F</td>
<td>IgG1κ</td>
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<td>10F</td>
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<td>6D</td>
<td>IgG1κ</td>
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<td>3B</td>
<td>IgG1κ</td>
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<tr>
<td>9A</td>
<td>IgG1κ</td>
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<td>7B</td>
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<tr>
<td>1F</td>
<td>IgAκ</td>
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<td>11G</td>
<td>IgG2κ</td>
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<td>11F</td>
<td>IgG1κ</td>
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<td>1C</td>
<td>IgG2κ</td>
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* HPV-16 E4, E6, L112 and L1232 and HPV-18 E1 and E7.

Antibodies 1C, 11F and 4E also clearly fell in the epitopic region IV category, but cross-blocking results with 6D were inconclusive.

Epitope characterization

In order to characterize the epitopes further, the panel of MAbs was reacted with a series of overlapping octapeptides spanning the entire length of the predicted amino acid sequence of E7. This multiple length scanning or 'window' approach identifies the location of linear epitopes and defines their boundaries (GeySen et al., 1987). Four (8F, 4F, 6D and 10F) of the panel of MAbs reacted with three distinct 'nests' of linear octapeptides (Fig. 4). An epitope is defined by amino acids common to all members of an antibody-reactive 'nest' of octapeptides. Thus 8F reacts with amino acid sequence EYMLD (epitope I), 4F with IDGP (epitope II), and 6D and 10F with QAEPD (epitope III) (Fig. 4). The remaining eight MAbs failed to react with any linear octapeptide.

The omission of the N-terminal glutamic acid (E) from EYMLD (epitope I) defined by 8F reduced but did not abolish reactivity (octapeptides 10 and 11; Fig. 4a), but further omission of N- or C-terminal amino acids (Y, tyrosine; D, aspartic acid) did. The minimal reactive epitope recognized by 8F is thus the four amino acid sequence YMLD. The sequences recognized (four amino acid IDGP and five amino acid QAEPD respectively) are also the minimal reactive epitopes (Fig. 4b, c). In a separate experiment, 8F, 4F and 6D and 10F reacted with their corresponding epitopes when presented as tetra- or pentapeptides respectively rather than in the context of octapeptides (data not shown). The non-overlapping and
B epitopes in HPV-16 E7

Fig. 3. Competitive binding assays define E7 peptide epitopic regions. (a, b) Reciprocal competitive binding assays. Inhibition of binding of \(^{35}\)S-labelled MAb with limiting amounts of unlabelled MAb (ascites dilutions). (a) Unlabelled ascites MAb 6D used to block \(^{35}\)S-6D (△), \(^{35}\)S-10F (○) and \(^{35}\)S-8F (□). (b) Unlabelled ascites MAb 10F used to block \(^{35}\)S-6D, \(^{35}\)S-10F and \(^{35}\)S-8F. Symbols as in (a). (c) Summary of results of reciprocal competitive binding assays: + denotes complete blocking of \(^{35}\)S-MAb binding; \pm denotes partial blocking of \(^{35}\)S-MAb binding; − denotes no blocking; ND, not done; INC, inconclusive result.

Separate identity of the linear epitopes I, II and III defined by octapeptide binding analysis, and recognized by MAbs 8F, 4F, and 6D and 10F respectively is supported by the competitive binding data for these MAbs (above). The position of the three epitopes on the HPV-16 E7 peptide is shown in Fig. 4(d). All three are in the N-terminal half of the molecule.

By aligning and translating DNA sequences, it was possible to compare regions of predicted amino acid sequence of other HPV genotypes corresponding to the HPV-16 E7 epitopes. The amino acid sequences of the latter epitopes are unique among the genotypes for which nucleotide sequence data are available (Fig. 5). The reactivity of MAb 8F recognizing EYMLD (epitope I) was reduced to 22% on the corresponding HPV-33 E7 sequence where the single isofunctional amino acid substitution valine (V) for methionine (M) has occurred in the EYVLD pentapeptide (data not shown). Similarly the reactivity of MAb 4F which recognizes IDGP (epitope II) was reduced to 33% on the corresponding HPV-18 E7 sequence where the single amino acid substitution valine (V) for proline (P) has occurred in IDGV. MAb 4F did not react with LDRP of HPV-33 or VDGQ of HPV-6b. MAbs 6D and 10F recognizing QAEPD (epitope III) did not react with QHLPA, QAQPA or DAQPL of HPVs-18, -33 and -11 respectively.

Eight of the MAb panel of 12 did not recognize any of the linear octapeptides. We assume that these MAbs recognize a discontinuous conformational epitope(s), not seen when E7 is presented as a series of 91 component linear octapeptides. These antibodies all cross-block and thus recognize one (or possibly two closely overlapping) determinants (discussed above). All eight cross-react with HPV-18 E6 in RIA (Fig. 2a) and Western blots (data not shown), whereas none of the four MAbs recognizing linear determinants do (data summarized in Table 1).

The MAbs were unable specifically to detect HPV-16 E7 protein in CaSki cells by indirect immunofluorescence using fixed or unfixed cell monolayers, or by flow cytometry using permeabilized cells. Results (not shown) indicated that the antibodies fell into two groups as regards reactivity with CaSki cells. Group 1 comprised those MAbs recognizing the conformational epitope, and 8F recognizing the EYMLD peptide, and showed...
Fig. 4. Reactivity of MAb with individual octapeptides spanning HPV-16 E7 in ELISA. (a) MAb 8F. (b) MAb 4F. (c) MAb 6D (MAb 10F shows an identical pattern of reactivity). Each octapeptide is identified in the histogram by both its number in the sequence and the single-letter code of its amino-terminal residue. Consensus amino acid sequences of octapeptides that react with MAb are denoted white-on-black, and define the linear epitopes. (d) Positions of putative T epitopes as predicted by Rothbard (r) and Berzofsky (b) algorithms respectively (see Discussion).

![Epitope Diagram](image)

Fig. 5. Alignment of predicted amino acid sequences of the E7 ORFs of HPV types 18, 33, 11, 1A, 8 and 6b corresponding to the three linear epitopes in HPV-16 E7 defined by MAb 8F (EYMLD), 4D (IDGP), and 6D and 10F (QAEPD). Amino acids positioned identically to those of HPV-16 are boxed.

Discussion

We have defined three linear epitopes (I, II and III) and one non-linear epitopic region (IV) within the HPV-16 E7 protein with a panel of MAb raised against an E7 bacterial fusion protein.

In the multiple length scanning approach we used, in order to be detected by antibody the length of the peptide must be equal to or larger than the sequential epitope. The present consensus is that sequential peptide epitopes are four to eight residues long (Geysen et al., 1985, 1988).

We consider it unlikely that we have missed any epitopes on the HPV-16 E7 molecule in our experiments. The following points merit discussion: the specificity of MAb, their ability to recognize eukaryotic E7 by immunoprecipitation but not immunofluorescence and the possibility that the epitopes may be immunogenic in man.

The MAb recognizing linear epitopes I, II and III are specific for the HPV-16 E7 protein within the context of reactivity with (i) the HPV-16 and -18 MS2/ORF proteins and (ii) homologous oligopeptides of HPV-18, -33, -11 and -6b. The reactivities of MAb defining the HPV-16 E7 linear epitopes are greatly reduced or completely prevented by amino acid substitutions seen in corresponding regions of the putative E7 peptides of other HPV genotypes. That we could not demonstrate...
MAb 4F binding to whole HPV-18 E7 FP (see Table 1), although it bound weakly to the HPV-18 E7 tetrapeptide IDGV may indicate conformational constraints imposed by sequences outside IDGV (Geysen et al., 1985). To determine fully the importance of individual residues in HPV-16 E7 linear epitopes requires exhaustive amino acid replacement analysis and will be reported elsewhere (R.W.T., unpublished).

Eight of 12 MAbs did not react with any linear octapeptide and were assumed to recognize a discontinuous conformational epitope(s). That some (3B and 1F) but not others are cross-blocked by MAb 6D suggests that this region may consist of two closely overlapping conformational epitopes, one which depends on a contribution from the epitope III (QAEPD) region, and one which does not. Note that 3B and 1F do not recognize QAEPD when it is presented in the form of a linear octapeptide. In other systems, as here, the majority of the B cell response is directed to conformational sites (Geysen et al., 1986). The cross-reactivity of the eight conformation-dependent MAbs with HPV-18 E6 may reflect the 38% homology evident when HPV-16 E7 and HPV-18 E6 proteins are aligned (GenBank Sequence Library search using SEQA program, data not shown). That these MAbs fail to immunoprecipitate a band corresponding to E6 from HPV-18-containing HeLa cells (Banks et al., 1987) may indicate their low affinity for HPV-18 E6. Homology also exists between HPV-16 E7 and HPV-16 E6 proteins (Cole & Danos, 1987) and between HPV-16 E7 and HPV-18 E7 although none of our antibodies react with HPV-16 E6 or HPV-18 E7. Oltersdorf et al. (1987) in their panel of six anti-HPV-16 E7 MAbs found one that recognized HPV-18 E7. Studies from our laboratory defining the B epitopes of HPV-18 E7 protein will be reported elsewhere by L.A.S.

At least five of the 12 MAbs recognizing three of the four epitopes (I, III and IV) react with a protein corresponding in M₆ to eukaryotic HPV-16 E7 in immunoprecipitation of CaSki cells, although further work is needed to elucidate the nature of the additional 25K band specifically precipitated by MAbs 3B, 7B and 10F from CaSki cells. Furthermore, MAb 8F reacting with epitope I (EYMLD) specifically binds to mammalian cells transfected with a vaccinia virus–HPV-16 E7 construct as detected by immunofluorescence (data not shown). It is likely therefore that the epitopes we have identified using prokaryotic E7 protein and defined using synthetic peptides have significance in HPV-16-infected eukaryotic cells.

The MAbs either failed to detect E7 by indirect immunofluorescence in CaSki or SiHa cells, or cross-reacted with cytoplasmic components masking any detection of cytoplasmic E7 which may have been occurring. Since E7 constitutes <0.01% of total cellular protein (Oltersdorf et al., 1987; Seedorf et al., 1987) it should be amenable to conventional immunodetection. Possibly, masking of the N-terminal epitopes by glycosylation, membrane insertion, or DNA-binding (Mallon et al., 1987) precludes immunodetection.

To what extent we have detected the major immunogenic sites of HPV-16 E7 is a question which can only be asked in the context of the species and major histocompatibility complex haplotype of the host animal (Berzofsky, 1988). Recognition of E7 epitopes in man invokes questions pertaining to the availability of E7 protein in infected cells, presentation to the afferent arm of the immune response, B and T cell repertoires, and generation of appropriate T help. Application of T epitope algorithms (Rothbard & Taylor, 1988; De Lisi & Berzofsky, 1985) predicts a number of putative T epitopes in the HPV-16 E7 protein (Fig. 4d), one of which corresponds precisely to the EYMLD B epitope we have described here. Experiments are under way to investigate T cell responses to potential T epitopic sites on the E7 molecule.

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