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Immunology

A "public" T-helper epitope of the E7 transforming protein of human papillomavirus 16 provides cognate help for several E7 B-cell epitopes from cervical cancer-associated human papillomavirus genotypes

(antibody/T-cell–B-cell cooperation/subunit vaccine/synthetic peptides)

ROBERT W. TINDLE*†, GERMAIN J. P. FERNANDO*, JANE C. STERLING‡, AND IAN H. FRAZER*

*Lions Human Immunology Laboratories, University of Queensland, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia; and ‡Department of Virology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, England

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ABSTRACT **We have identified a major T-cell epitope, amino acids 48–54 (DRAHYNI, in one-letter code) in the E7 open reading frame protein of human papillomavirus (HPV) type 16. Lymph node cells from mice immunized with synthetic peptides containing DRAHYNI proliferated and produced interleukin when challenged *in vitro* with peptide or whole HPV-16 E7 fusion protein. The T epitope was recognized in association with all five major histocompatibility complex class II I-A and I-E alleles tested. Synthetic peptides consisting of DRAHYNI linked to major B-cell epitopes on the E7 molecule formed immunogens capable of eliciting strong antibody responses to HPV-16 E7. The T epitope could provide help for the production of antibody to several B epitopes simultaneously, including a B epitope of HPV-18 E7 protein. Mice immunized with a peptide containing DRAHYNI and B epitope and, at a later date, infected with recombinant vaccinia E7 virus, displayed secondary antibody responses to E7. Because E7 has a role in cell transformation and is the most abundant viral protein in HPV-associated neoplastic cervical epithelial cells, the data have implications for vaccine strategies.**

Circumstantial evidence implicates host immune mechanisms in the control of human papillomavirus (HPV)-associated tumors of the anogenital epithelium (1), and there is an increased risk of squamous cell carcinoma of the cervix and vulva but not of control organs, such as breast and rectum, in immunosuppressed allograft recipients (2). E7 is the most abundant viral protein in HPV-16-containing CaSki and SiHa squamous carcinoma cell lines and in HPV-18-containing HeLa and C4-1 lines (3). DNA-transfection experiments implicate the E7 open reading frame protein in *in vitro* transformation of mouse fibroblasts (4), rat epithelial cells (5), and primary human keratinocytes (6). Cooperation with an active *ras* oncogene leads to full transformation (5), and there is a requirement for continued expression of the *E7* gene to maintain the transformed phenotype (7). The E7 protein may be immunogenic after infection with HPV, as anti-E7 antibodies have been described in the serum of ≈20% of patients with HPV-16-associated cervical lesions (8, 9).

These observations suggest that the E7 protein merits consideration as a candidate antigen for a potential vaccine against HPV infection. We have recently described the major immunodominant B epitopes in HPV-16 E7 (10) and HPV-18 E7 (11) defined by monoclonal antibodies. In this study we have defined, using synthetic peptides, a major T helper (T_h) epitope in HPV-16 E7.

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MATERIALS AND METHODS

Synthetic Peptides. Peptides were synthesized by using derivatized *N-tert*-butoxycarbonyl (t-Boc) amino acids on benzhydryl resin (12) or using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry on an Applied Biosystems 431A peptide synthesizer. The amino acid composition, toxicity, and mitogenicity of all peptides were checked. Peptides 8Q and GF15 were amino acid sequenced.

HPV-16 E7 Protein. HPV-16 E7 protein was produced as MS2 fusion protein from a heat-inducible phage promoter in a pPLc 24 expression vector (provided by L. Gissmann, DKFZ, Heidelberg) in *Escherichia coli* 600/537 by sequential urea extraction, as described (3). Fusion protein was dialyzed against phosphate-buffered saline, pH 7.2, (PBS), for use in proliferation assays.

Lymph Node Cell (LNC) Proliferation Assays. Mice were immunized s.c. with 20–50 μg of peptide emulsified in complete Freund's adjuvant (CFA) (H37 Ra.CFA, Difco). Eight to ten days later a suspension of draining LNCs was prepared and a 4-day proliferation assay (13) was done in peptide-coated microtiter plates. Proliferation was quantified by [^{3}H]thymidine incorporation.

Mice. Inbred mouse strains were obtained from the University of Queensland animal breeding facility or from Animal Resources (Perth, Australia). Mice were used at 8–24 weeks of age. Genetic purity was checked routinely by isoenzyme analysis.

Assay for Cytokine Production. Supernatants from LNC proliferation assays were harvested at 3 days and tested for induction of proliferation of interleukin 2- and interleukin 4-dependent HT-2 cells (14).

Peptide ELISA Assay. Peptide–bovine serum albumin conjugates (15) were bound to microtiter plates by incubation at 50 μg/ml in bicarbonate binding buffer, pH 9.6. Remaining binding sites were blocked with PBS/5% bovine serum albumin. ELISA assays on these coated plates and on HPV-16 E7/MS2 fusion protein-coated plates were conducted, as described (10).

Competitive Binding ELISAs. Fifty microliters of serum dilutions shown by previous titration to cause 5%, 50%, and 90% saturation of HPV-16 E7-binding sites in a standard ELISA assay (above) were preincubated with free peptide at 100 μg/ml (final concentration) for 1 hr at 37°C. The serum dilutions were then transferred to microtiter plates coated

Abbreviations: HPV, human papillomavirus; T_h, T-helper; CFA, complete Freund's adjuvant; VAC–E7, recombinant vaccinia E7 virus; WR-VAC, Western Reserve vaccinia virus; pfu, plaque-forming units; LNC, lymph node cell.

†To whom reprint requests should be addressed.

with HPV-16 E7/MS2 fusion protein, and ELISA was performed.

Peptide Immunization for Antibody Production. Mice were immunized two to three times i.p. with 20–50 μg of peptide emulsified in CFA at 14-day intervals and were bled 8 days after the last injection.

Production of Recombinant Vaccinia Virus Expressing HPV-16 E7 Protein. A 1776-base pair (bp) *Pst* I–*Pst* I fragment of HPV-16 DNA containing the E7 open reading frame was cloned into pUC13, and the resulting plasmid was submitted to digestion with *Hin*cII and *Hin*dIII. The *Hin*dIII site of the released 1793-bp fragment was end-repaired with T4 DNA polymerase, and after partial digestion with *Pvu* II, a 339-bp *Pvu* II–*Hin*dIII fragment of DNA was recovered and ligated into the *Sma* I site of the vaccinia virus insertion vector pRK19. This plasmid was transfected (16) by calcium phosphate precipitation into CV-1 cells infected with wild-type Western Reserve vaccinia virus (WR-VAC). Virus plaques underwent several rounds of plaque purification to isolate recombinant vaccinia E7 (VAC–E7). Expression of the E7 protein by VAC–E7-infected cells was confirmed by immunoblot of infected cell lysates and by immunocytology, using a rabbit polyclonal antibody and a murine monoclonal antibody, both raised against an HPV-16 E7–β galactosidase fusion protein (C.J.S., unpublished data).

Carrier Priming Assay. Mice (three or four per group) were immunized i.p. with 20–50 μg of peptide 8Q or PBS emulsified in CFA. Three to five weeks later, mice were infected by tail base scarification with 10^7 plaque forming units (pfu) of VAC–E7 or 10^7 pfu of WR-VAC. Eight and thirteen days later, serum was prepared from each mouse, and anti-E7 antibodies were determined by ELISA assay against peptide 8Q or HPV-16 E7 fusion protein bound to microtiter plates.

RESULTS

A set of overlapping 15- to 20-mer peptides covering the entire predicted HPV-16 E7 protein (Fig. 1*A*) was used to locate T-cell proliferative epitopes. Four groups of C57BL/6(H-2^b) mice were immunized with mixtures of peptides 2Q–5Q, 6Q–9Q, or 10Q–12Q in CFA or with RPMI medium in CFA. Pooled LNCs from each group were challenged *in vitro* with individual peptides at 2 or 20 μg/ml. The data shown in Fig. 1*B* are representative of six assays. Peptide 8Q consistently elicited strong proliferation in LNCs from the 6Q–9Q immunized group (Fig. 1*B,b*). Peptide 7Q elicited a weaker response in this group. Peptides 8Q and 7Q share a 13-amino acid overlap at positions 44–56. Weak and inconsistent responses were seen in LNCs from 2Q–5Q-immunized mice, when challenged with peptides 4Q and 5Q (three of six experiments, Fig. 1*B,a*). LNCs from 10Q–12Q-immunized mice failed to respond to any peptide. No further peptides from the 2Q–12Q series induced proliferation in assays using LNCs from appropriately immunized B10.A(4R)(H-2^{h4}), B10.A(2R)(H-2^{h2}), or BALB/c(H-2^d) mice.

B10 congenic mice, differing only at the major histocompatibility complex class 2 locus (Fig. 2*A*), and other strains of major histocompatibility complex class 2 haplotype-defined mice, S7R(I-A^sI-E^s), S9R(I-A^sI-E$\alpha^k\beta^s$), C3H(I-A^kI-E^k), CBA(I-A^kI-E^k), DBA(I-A^dI-E^d), BALB/c(I-A^dI-E^d), C57BL/6(I-A^bI-E^b), and BL10(I-A^bI-E^b), were immunized with mixtures of peptides 8Q and 6Q. LNCs from these mice all showed strong proliferative responses to peptide 8Q but not to control peptide 6Q.

LNCs from 8Q-immunized mice were challenged *in vitro* with a series of C-terminal and N-terminal truncations of 8Q (Table 1). LNCs stimulated with peptides B3, B4, and 8Q, and B7–10 proliferated significantly, indicating that the consensus sequence DRAHYNI was the minimal proliferative epitope. LNCs from 8Q-primed B10.A(2R) and S9R mice proliferated in response to the 7-mer peptide DRAHYNI, although the stimulation indices were much lower (6.1 and 5.1, respectively).

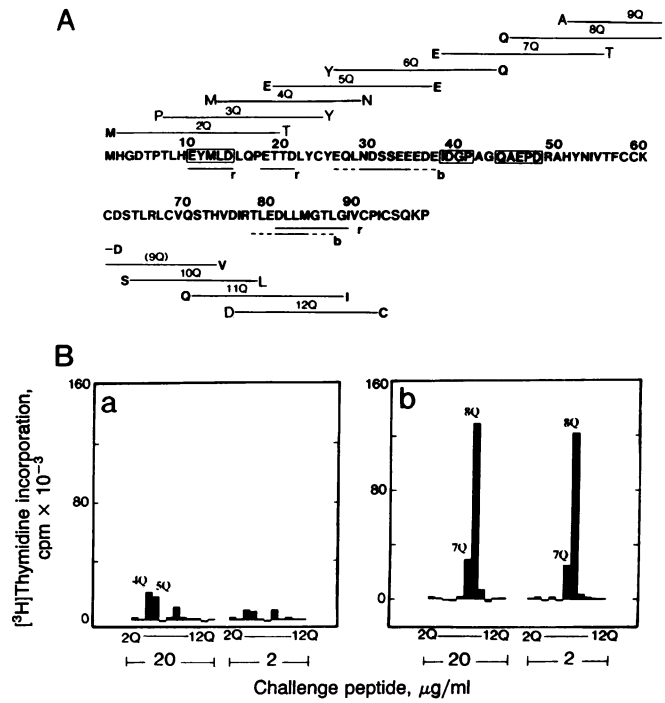

FIG. 1. (*A*) The set of overlapping peptides (termed 2Q–12Q) spanning the putative HPV-16 E7 protein, used to locate position of T-cell-proliferative epitopes (see text). Linear B-cell epitopes defined by monoclonal antibodies 8F, 4F, and 10F (10) are boxed. Underlining denotes the positions of putative T epitopes as predicted by DeLisi and Berzofsky (b) (18) and Rothbard (r) (19) algorithms. (*B*) LNC proliferation assay. LNCs from C57BL/6 mice immunized with equimolar mixes in CFA of peptides 2Q–5Q (*a*) and peptides 6Q–9Q (*b*) (three mice per group, cells pooled) were challenged *in vitro* with individual peptides 2Q–12Q at 20 μg/ml or 2 μg/ml. Counts with no added antigen (1104 ± 320) were subtracted from the results with peptide.

Challenge of LNCs from peptide 8Q-immunized mice with HPV-16 E7 fusion protein or with 8Q elicited proliferation of the same order of magnitude, provided challenges were adjusted to be approximately equimolar for 8Q (Fig. 2*B*).

Table 1. Mapping the minimal T-cell-proliferative epitope in the 8Q peptide of HPV-16 E7

Challenge peptide			
Designation	Position	Sequence	SI*
B6	44–50	QAEPDRA	1.3
B16	44–51	QAEPDRAH	1.0
B17	44–52	QAEPDRAHY	1.1
B7	44–54	QAEPDRAHYNI	**18.1**
B10	44–56	QAEPDRAHYNIVT	**23.8**
B8	44–57	QAEPDRAHYNIVTF	**27.4**
B9	44–60	QAEPDRAHYNIVTFCCK	**37.2**
B1	54–62	IVTFCCKCD	1.7
B2	51–62	HYNIVTFCCKCD	2.6
B14	50–62	AHYNIVTFCCKCD	0.9
B15	49–62	RAHYNIVTFCCKCD	0.7
B3	48–62	DRAHYNIVTFCCKCD	**29.8**
B4	45–62	AEPDRAHYNIVTFCCKCD	**25.2**
8Q	44–62	QAEPDRAHYNIVTFCCKCD	**31.7**

*LNCs from B10.A(2R) mice immunized with peptide 8Q were challenged *in vitro* with peptides. Stimulation index (SI) is defined as ratio of mean cpm of incorporated [^{3}H]thymidine for cells with added antigen to mean cpm for cells without any added antigen. Background cpm (no added antigen) was between 3807 and 5423.

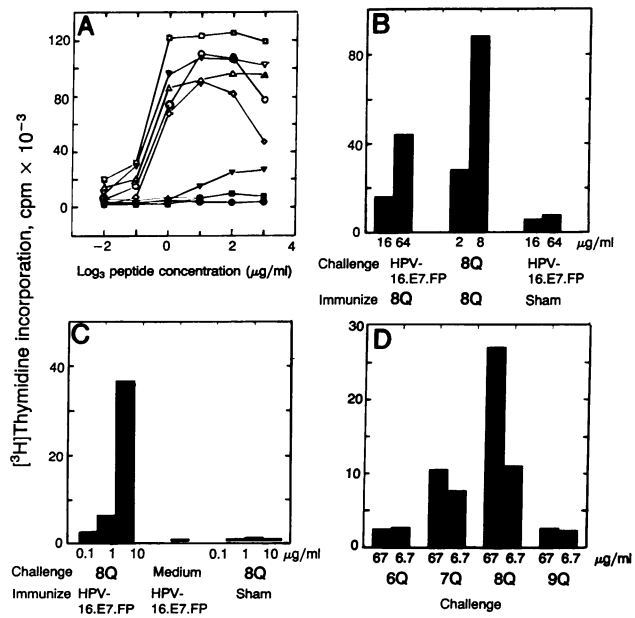

FIG. 2. The *in vitro* proliferative response (*A–C*) and lymphokine production (*D*) of LNCs from mice immunized 8 days previously with HPV-16 E7 or E7 peptides and challenged with HPV-16 E7 or various E7 peptides. FP, fusion protein. (*A*) LNCs from congenic mice (three mice per group, cells pooled) immunized with an equimolar mix of peptides 8Q and 6Q were challenged with various concentrations of 8Q (open symbols) or 6Q (closed symbols). ■ and □, B10.D2 (I-A^dI-E^d); ▲ and △, B10.A (I-A^aI-E^a); ◆ and ◇, B10.BR (I-A^kI-E^k); ● and ○, B10.A (2R) (I-A^kI-E^d); ▼ and ▽, B10.A(4R) (I-A^kI-E^b). Mean background cpm (no added antigen) was 3302, and mean purified protein derivative response was 87,753. (*B*) LNCs from 8Q or "sham"-immunized B10.A(2R) mice (five per group) were challenged with HPV-16 E7 fusion protein at 16 or 64 μg/ml or 8Q at 2 or 8 μg/ml. The HPV-16 E7 and 8Q challenge doses were approximately equimolar for the 8Q sequence. Background controls (no added antigen) and purified protein derivative controls were 1,269 cpm and 98,775 cpm, respectively. (*C*) B10.A(2R) mice were immunized with 100 μg of HPV-16 E7 fusion protein or RPMI (sham) (five mice per group), and LNCs were challenged with 8Q at 0.1, 1.0, and 10 μg/ml. Background controls (no added antigen) and purified protein derivative controls were 361 cpm and 68,872 cpm, respectively, for HPV-16 E7 fusion protein-immunized mice. (*D*) B10.A(4R) mice were immunized with 50 μg of equimolar mixtures of peptides 6Q–9Q. LNCs were pooled and challenged with each of the peptides at 67 and 6.7 μg/ml individually in separate wells (three wells per concentration per peptide). Culture supernatants were harvested 3 days later and added at 1:2 dilution to HT-2 cells. The HT-2 cells were pulsed 42–44 hr later for 6 hr with [^{3}H]thymidine, harvested, and counted. Background (RPMI medium added to HT-2 cells in place of supernatants) was 1870 ± 720 cpm.

LNCs from mice immunized with HPV-16 E7, but not "sham"-immunized mice, proliferated when challenged *in vitro* with 8Q (Fig. 2*C*). Supernatant fluid from LNC from mice previously immunized with a mixture of 6Q–9Q peptides induced proliferation of the interleukin-2/interleukin-4-dependent cell-line HT-2 (Fig. 2*D*) when the LNCs were challenged with 8Q or 7Q but not when they were challenged with 6Q or 9Q. Supernatants of LNCs from mice immunized and challenged *in vitro* with the other Q-series peptides failed to induce significant proliferation of HT-2 cells.

To determine whether primed LNCs would "help" B cells produce specific antibody to B-cell epitopes of HPV-16 E7, we exploited the fact that peptide 8Q, in addition to the T-cell epitope DRAHYNI defined above, also contained an immunodominant B epitope at positions 44–48, QAEPD (10). The sera of mice immunized with 8Q and infected 3½ weeks later with VAC–E7, but not with WR-VAC, contained antibodies reactive with 8Q (Fig. 3*B*) and with HPV-16 E7 (Fig. 3*A*),

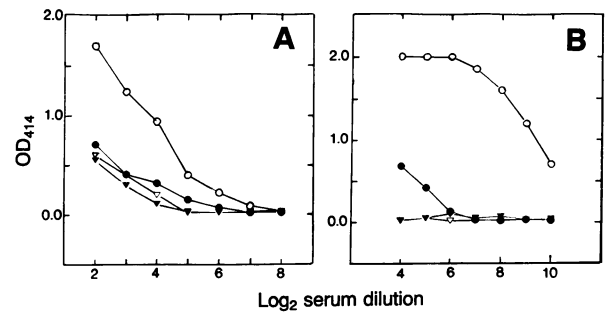

FIG. 3. Immunization with peptide 8Q-primed mice for *in vivo* challenge with HPV-16 E7 produced from a recombinant vaccinia virus. ELISA results on pooled 8-day sera from 8Q-primed mice (○, ●) and sham-primed mice (▽, ▼) challenged with VAC–E7 (○, ▽) or WR-VAC (●, ▼) on HPV-16 E7 fusion protein (*A*) or peptide 8Q (*B*) are shown.

both of which contain the QAEPD B epitope. Sera from mice immunized with peptides 2Q–5Q or 10Q–12Q and infected with VAC–E7 failed to react with HPV-16 E7 or with any peptide containing an E7 B epitope. Results on 13-day sera were similar to those shown for 8-day sera.

Sera from mice immunized with peptide 8Q reacted in ELISA assay with 8Q and 7Q (Fig. 4 *A* and *B*) and HPV-16 E7 fusion protein (Fig. 4*D*) but not control peptide (Fig. 4*C*). Reactivity with HPV-16 E7 could be absorbed out by preincubation of the sera of 8Q-immunized mice with peptide 8Q (containing QAEPD) but not with irrelevant peptide 2Q. These data suggested that the serum antibodies may have recognized the B-cell epitope QAEPD contained within 8Q, 7Q, and HPV-16 E7. Sera from three of nine mice immunized with peptides B7 or B8 reacted with 8Q and 7Q, whereas sera from mice immunized with B16, B17, B19, or B3 did not so react (Table 2). Thus, to elicit an antibody response to peptides and HPV-16 E7 fusion protein containing the B-cell epitope QAEPD, the immunogen was required to contain the T-cell epitope DRAHYNI, in addition to QAEPD.

Mice immunized with peptide B11 contained antibodies to peptides containing B-cell epitopes EYMLD and QAEPD and reacted with HPV-16 E7 (Table 2). The serum of one of

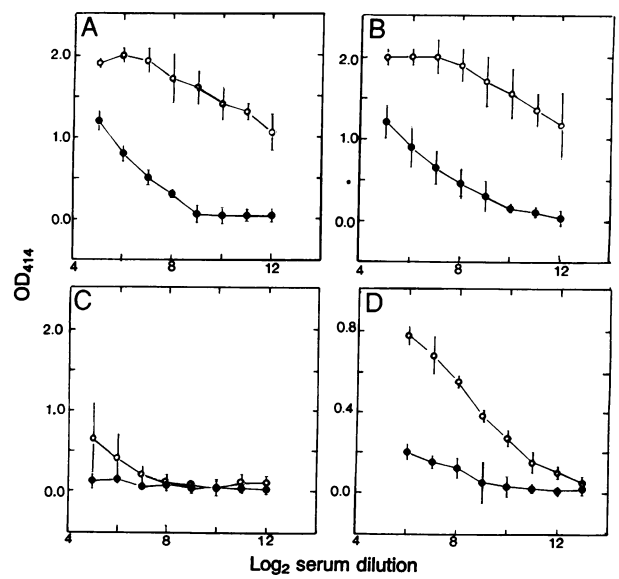

FIG. 4. Sera from B10.A(2R) mice immunized with peptide 8Q (○) or control peptide 3Q (●) in CFA were assayed by ELISA for antibody to peptide 8Q (*A*), peptide 7Q (*B*), peptide 6Q (*C*), or HPV-16 E7 fusion protein (*D*). Data points are arithmetic means (± SEM) of sera collected individually from three mice.

Table 2. Immunization with peptides containing T- and B-cell epitopes elicits specific antibody

Immunizing peptide		Mice producing serum antibody, no.						
		E7 peptide					Whole E7	
Designation	Sequence*	8Q and 7Q (QAEPD)	6Q (IDGP)	2Q and 3Q (EYMLD)	12Q (None)	GF13† (DEIDGV-NHQHL)	HPV-16‡ E7FP	HPV-18 E7FP
8Q	QAEPDRAHYNIVTFCCKCD	12/14	—	—	—	NT	12/14	NT
B7	QAEPDRAHYNI	2/6	—	—	—	NT	2/6	NT
B17	QAEPDRAHY	—	—	—	—	NT	—	NT
B16	QAEPDRA	—	—	—	—	NT	—	NT
B8	QAEPDRAHYNIVTF	1/3	—	—	—	NT	1/3	NT
B19	DRAHYNI	—	—	—	—	NT	—	NT
B3	DRAHYNIVTFCCKCD	—	—	—	—	NT	—	NT
B3 + 8Q§	DRAHYNIVTFCCKCD + QAEPDRAHYNIVTFCCKCD	3/3	—	—	—	NT	—	NT
7Q	EIDGPAGQAEPDRAHYNIVT	3/3	—	—	—	NT	2/2	NT
6Q	YEQLNDSSEEDEIDGPAGQ	—	—	—	—	NT	—	NT
B11	EYMLDAGIDGPAGQAEPDRAHYNIVTFCCKCD	3/3	1/3	3/3	—	NT	3/3	NT
B12	RAHYNIVTFCCKCDQAEPDAGIDGPAGEYMLD	1/5	—	2/5	—	NT	2/5	NT
B13	QAEPDAGIDGPAGEYMLD	—	—	—	—	NT	—	NT
GF15	QAEPDRAHYNIDEIDGVNHQHL	2/3	NT	—	NT	3/3	2/3	3/3
GF12	AHYNIDEIDGVNHQHL	—	NT	—	NT	—	—	—
GF11	DEIDGVNHQHL	—	NT	—	NT	—	—	—

Sera from individual mice (at least three per group), immunized with various peptide constructs, reacted over a range of doubling dilutions (1:64–1:4096) with microtiter plates to which were bound peptides 8Q, 7Q, 6Q, 2Q, 3Q, 12Q, or GF13, and HPV-16 E7 or HPV-18 E7, in ELISA assay. —, No antibody detected; NT, not tested. Positive and negative results were indicated by OD_{414} readings of >0.5 and <0.05, respectively, at serum dilution of 1:256 for peptides and by readings of >0.5 and <0.1, respectively, at serum dilution of 1:256 for E7 fusion protein. Sequences (in one-letter code) in parentheses indicate the B-cell epitopes contained by the peptides.

*B-cell epitopes of HPV E7 (10, 11) are indicated in peptide sequences in boldface type; T-cell epitope DRAHYNI is underlined.

†Full sequence of GF13 is RAHYNIDEIDGVNHQHL.

‡None of the sera reactive with HPV-16 E7 fusion protein reacted with HPV-16 E6 fusion protein (negative control).

§Mice were immunized two to three times i.p. with 50 μg of peptide B3 in CFA at 2-week intervals followed by a final injection of peptide 8Q. Sera were prepared 3 days later.

three mice also contained antibodies to 6Q peptide containing B-cell epitope IDGP. The sera of mice immunized with 7Q contained antibody to peptide containing QAEPD, but did not contain antibody to IDGP (Table 2). To determine whether inclusion of DRAHYNI in a synthetic peptide could drive production of heterologous antibody, mice were immunized with peptides GF11, GF12, and GF15 containing an immunodominant linear B-cell epitope DEIDGVNHQHL of HPV-18 E7 (11). Serum antibodies that recognized a peptide (GF13) containing the B-cell epitope, and whole HPV-18 E7, were produced in all three mice immunized with GF15, which contains intact DRAHYNI, but not in mice immunized with GF12 or GF11, where DRAHYNI is truncated or absent. GF15-immunized mice simultaneously produced antibody that recognized peptide containing the HPV-16 E7 B epitope QAEPD and whole HPV-16 E7 (Table 2). Mice immunized with peptide B3 (containing T-cell epitope DRAHYNI but no B-cell epitope) and later challenged with peptide 8Q (containing B-cell epitope QAEPD and T-cell epitope DRAHYNI) produced antibody, detectable at 3 days, which recognised 8Q (Table 2). Mice challenged with B3 did not produce antibody. Nor did "sham"-immunized mice challenged with 8Q produce any antibody.

DISCUSSION

We have defined a T-cell epitope DRAHYNI in HPV-16 E7, which stimulates proliferation and cytokine production by T cells of all tested strains of mice. The proliferative response to 8Q was always an order of magnitude greater than that to 7Q in LNCs from appropriately primed mice, suggesting that flanking sequences outside the minimal epitope DRAHYNI could influence response (17). DRAHYNI was not predicted by the DeLisi and Berzofsky (18) or Rothbard and Taylor (19) T-cell epitope algorithms. We have demonstrated that immunization of mice with DRAHYNI joined to a B-cell epitope will elicit antibody reacting specifically with peptides containing the B-cell epitope and with the whole E7 molecule. Furthermore, immunizing mice with a peptide containing DRAHYNI but no B-cell epitope elicited a secondary antibody response when mice were subsequently challenged with DRAHYNI plus B-cell epitope, suggesting that T activation in the absence of a B-cell response is sufficient to prime (13).

Unexpectedly, one major T-cell epitope was identified in the entire HPV-16 E7 molecule by using the Q-series range of peptides and the five major histocompatibility complex haplotypes we used. Minor proliferation induced by 4Q and 5Q, both of which contain algorithm-predicted T-cell sites (Fig. 1), was observed in primed B10.A(2R) and C57Bl/6 mice, although no cytokine production was detectable. The putative T-cell sequence of 4Q was unable to provide help for antibody production to the adjacent EYMLD B-cell epitope when peptide 3Q, which contains both, was used to immunize mice.

The experiments in which mice were immunized with DRAHYNI and B-cell epitopes linked in various conformations indicated that DRAHYNI could provide cognate help to more than one clone of antibody-secreting B cells to produce multiple antibodies of different specificities. The production of antibody occurred in several combinations of the position and orientation of the B-cell epitopes with respect to the T-cell epitope. Similar findings on epitope orientation have been reported by others (13). DRAHYNI joins a small number of stimulating peptides that are recognized in association with multiple major histocompatibility complex haplotypes (20–24). It has been suggested that widely reactive peptides are capable of forming a structure closer to an "ideal" T-cell epitope that can associate with the protein products of many class II alleles (25). Although introduction of strong heterologous T-cell epitopes into vaccines has been

advocated [e.g., hepatitis B virus core antigen (26)] ideally synthetic HPV vaccines would be composed of T- and B-cell sites derived from the same organism, so that latent and/or subsequent infections would elicit a response from both populations of lymphocytes, critical when T-cell immunity as well as antibody production is required for protection.

A vaccine for HPV for prophylaxis and for therapy of cervical cancer is desirable, and for reasons previously discussed (10), a peptide-based vaccine is the likely choice. Development of a peptide vaccine requires the delineation of immunogenic B- and T-cell epitopes within open reading frame peptides. Our group has recently defined immunodominant B-cell epitopes in HPV-16 E7 and HPV-18 E7 peptides (10, 11, 27). Studies with other viruses have shown convincingly that T-cell epitopes relevant to infection with native virus can be defined by synthetic peptides (28–30). We have chosen to seek T-cell epitopes recognized by mice because of the difficulties in testing large numbers of potential T-cell epitopes in patients. Experience from influenza (31), human immunodeficiency virus (32), and malaria (13) indicates a good correlation between epitopes recognized by murine T cells and those recognized by human T cells. The studies reported here describe a functional T_h-cell epitope within an open reading frame protein of anogenital HPV. DRAHYNI is a T_h-cell-stimulating epitope that can be used for eliciting cognate interaction between T and B lymphocytes for the production of antibody against whole E7 protein. By these criteria the DRAHYNI peptide may be suitable for inclusion into a synthetic subunit vaccine for anogenital HPV.

Prof. L. Gissmann provided the pPLc 24 vector containing the HPV-16 E7 open reading frame gene. Dr. L. Selvey provided HPV-18 E7 monoclonal antibodies. Initial peptide syntheses were done by Dr. G. Jones. We thank Dr. M. Good for helpful discussion. This work was funded by National Health and Medical Research Council of Australia, the Clive and Vera Ramiciotti foundation, the Mayne Bequest Fund, and the Queensland Cancer Fund. R.W.T. is supported by the Lions Kidney and Medical Research Foundation. J.C.S. was a Training Fellow of the Medical Research Council of Great Britain.

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