Proliferative T cell responses to the human papillomavirus type 16 E7 protein in women with cervical dysplasia and cervical carcinoma and in healthy individuals

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The levels of proliferative T cell responses to peptides representing the human papillomavirus type 16 (HPV-16) E7 protein have been measured using short-term T cell lines derived from peripheral blood of healthy women and those with cervical dysplasias and carcinoma of the cervix. In healthy individuals 47% (7/15) responded predominantly to the N- and C-terminal regions of the protein and 6/7 responders were to a single peptide between amino acids 80–94. In comparison 29% (9/31) of women with cervical dysplasia responded to HPV-16 E7, with a significantly reduced response to both the N- and C-terminal regions (P = 0.03 and 0.038, respectively). A higher proportion of responders was found in patients with high grade lesions (56%, 5/9) versus those with atypical or low grade histology (20%, 4/20) and the response to a single peptide between amino acids 75–94 was also increased in this patient group (P = 0.044). This may be a reflection of higher levels of current or previous exposure to HPV-16 in patients with high grade lesions. Correlation of T cell responses with HPV DNA type (detected by PCR of cervical biopsy tissue) showed that 3/9 (33%) HPV-16 DNA-positive individuals responded. This suggests that E7 may not be the dominant target of the immune response or that the response to E7 is down-regulated in these patients. In addition 4/18 (22%) HPV-16 DNA-negative individuals responded, suggesting that their T cells may have been primed by previous exposure to HPV-16 or that a cross-reactive response was detected. Proliferative T cell responses to both HPV-16 E7 and L1 were reduced in women with cervical carcinoma in comparison to those with cervical dysplasia and healthy controls. The observed down-regulation of responses to HPV-16 E7 in women with cervical dysplasia and cervical carcinoma may reflect an altered functional balance between subsets of T helper cells in HPV-16 infections.

Introduction

The importance of cellular immunity in the control of genital human papillomavirus (HPV) infections is most evident from studies of immunosuppressed individuals, who demonstrate a dramatic increase in the incidence and severity of HPV-related lesions (Porreco et al., 1975; Melbye et al., 1990; Johnson et al., 1992). However, proliferative T cell responses to HPV infections of the genital epithelium are not well defined. Attempts to define cellular immune responses to HPV have mainly focused on T cell responses to HPV-16, the HPV type most predominantly associated with high grade cervical intraepithelial neoplasia (CIN) and carcinoma of the cervix and the E7 oncoprotein, which is highly expressed in virus-infected neoplastic cervical epithelial cells and may therefore be a suitable target antigen for vaccine therapy. A number of studies of both human and murine responses have concentrated on the identification of cytotoxic T cell epitopes within this protein (Kast et al., 1993, 1994; Sadovnikova et al., 1994) and immunization of mice with peptides derived from the E6 and E7 proteins has been demonstrated to protect against challenge with HPV-16-transformed tumour cells (Feltkamp et al., 1993). Studies of proliferative T cell responses to HPV-16 E7 in mice...
have identified epitopes spanning almost the entire molecule (Shepherd et al., 1992; Comerford et al., 1991; Tindle et al., 1991). A ‘promiscuous’ T helper cell epitope between amino acid residues 48–54 (DRAHYNI) was identified in mice which was capable of providing help for the production of several HPV-16 E7-specific antibodies simultaneously (Tindle et al., 1991). Mice immunized with hepatitis B virus core antigen particles expressing the DRAHYNI epitope in addition to major B cell epitopes of HPV-16 E7 have since been used to elicit strong E7-specific B and T helper cell responses (Tindle et al., 1994). There is no evidence that this epitope has the same functions in humans. Recent studies in mice suggest that T cells characteristic of the Th1 subset may have a role in protection against HPV-16 infection; McLean et al. (1993) and Chambers et al. (1994a, b) have demonstrated that syngeneic mice primed by epithelial grafting of HPV-16 E6- or E7-expressing keratinocyte cell lines mount a delayed-type hypersensitivity reaction on subsequent challenge with recombinant E6/E7 protein.

In humans, two studies involving healthy asymptomatic individuals have identified proliferative T cell epitopes in the HPV-16 L1, E6 and E7 proteins (Strang et al., 1990; Altmann et al., 1992) and one study has examined proliferative responses of patients with cervical dysplasia by direct addition of HPV-16 E7 peptides to T cell proliferation assays (Kadish et al., 1994). In our previous studies (Shepherd et al., 1994, 1996) we utilized the short-term T cell line (STL) technique to demonstrate proliferative T cell responses in women with cervical dysplasia and healthy asymptomatic controls to the HPV-16 L1 protein and correlated these responses with the patients’ cervical biopsy histology and HPV DNA status. A role for T helper cells of the Th1 subtype in the control of genital HPV infections has been shown by Coleman et al. (1994), who demonstrated an infiltrate of activated CD4-positive cells characteristic of a delayed-type hypersensitivity response in regressing genital warts. Also, it was recently reported that Th1 responses may be impaired in patients with high grade cervical dysplasia or cervical carcinoma (Hildesheim et al., 1995).

The aim of this study was to define HPV-16 E7-specific T helper cell responses in healthy asymptomatic individuals, women with differing degrees of cervical dysplasia and in a group of women with cervical carcinoma. This was done by generating STLs from the peripheral blood using an HPV-16 glutathione S-transferase (GST)–E7 fusion protein, then identifying the epitopes recognized by these cell lines in standard 3 day proliferation assays using synthetic HPV-16 E7 peptides. We also compared T cell responses in the different study groups in terms of the magnitude of the responses detected and the epitopes recognized, and determined whether these T cell responses are associated with the presence of HPV-16 in cervical lesions by type-specific PCR of biopsy tissue. As T helper cells are central to the control of both the cell-mediated and humoral arms of the immune response, a better understanding of the HPV-16-specific proliferative T cell response is clearly necessary for the future development of successful vaccine strategies.

Methods

<table>
<thead>
<tr>
<th>Tissue culture medium.</th>
<th>The medium used was RPMI-1640 with l-glutamine (Life Technologies), supplemented with 1 mm-sodium pyruvate (Flow Laboratories), 2 mm-l-glutamine (Life Technologies), 10 mm-HEPES (BDH), 100 U/ml penicillin, 25 mg/ml gentamicin (Flow Laboratories), 50 mm-2-mercaptoethanol (Sigma) and 0·25 mg/ml Fungizone (Flow Laboratories). This medium was supplemented with autologous human sera in all tissue culture procedures.</th>
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<tr>
<td>HPV antigens.</td>
<td>The HPV-16 GST–E7 fusion protein was induced in Escherichia coli transformed with the plasmid pGEX-2T-HPV-16E7 (Smith &amp; Johnson, 1988; Comerford, 1991) and a crude extract of insoluble inclusion bodies prepared according to the method of Marston (1986). The protein was then isolated essentially as previously described (Shepherd et al., 1992). Briefly, the inclusion bodies were solubilized in 8 m-urea then diluted in nine volumes of phosphate buffer (50 mm-KH2PO4, 100 mm-EDTA, 1 m-NaCl pH 10·7). The solution was adjusted to pH 8 and then spun briefly to remove any precipitates. One-tenth volume of 10 × PBS was added to give a final protein solution in 1 × PBS (16 mm-NaH2PO4, 4 mm-Na2HPO4, and 150 mm-NaCl pH 7·3). This was then purified by affinity chromatography using a gel consisting of reduced glutathione attached to agarose beads (Sigma). After thorough washing with PBS plus 1% Triton X-100, GST–E7 was eluted by stepwise addition of elution buffer (50 mm-Tris–HCl, 5 mm reduced glutathione in PBS pH 7·5). Fractions were analysed for the presence of the protein by standard Western blotting procedures as previously described (Shepherd et al., 1992).</td>
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<tr>
<td>Synthetic peptides.</td>
<td>In the initial part of the study (eight patients), a set of 20-mer peptides were used which overlapped by 15 amino acids and spanned the entire E7 molecule. These were a gift from S. Comerford (Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, USA) and were synthesized by the method of Houghten (1985) by R. Campbell (formally Department of Virology, Wellcome Research Laboratories, Beckenham, Kent, UK). For the majority of the study (23 patients) ten 15-mers were selected from the set shown in Fig. 1 such that they represented the whole E7 molecule and overlapped by five amino acids. These were synthesized commercially by standard Fmoc chemistry, followed by HPLC purification and amino acid sequence verification (Peptide and Protein Research Consultants).</td>
</tr>
</tbody>
</table>
| Patient selection, collection of peripheral blood and biopsy material. | Patient selection was on a voluntary and random basis from women referred to the colposcopy clinic at Guy’s Hospital Trust for investigation of abnormal cervical smears. PBMCs were obtained from each patient by taking 50 ml of venous blood which was then delipidated by rotating in a sterile centrifuge tube containing glass beads for 10 min at room temperature. Serum was collected after centrifugation (780 g for 10 min) and the cells separated by layering onto 2 × 15 ml Lymphoprep (Nycomed) and spinning at 942 g for 20 min. After washing, each patient’s cells were resuspended in autologous serum containing 10% (v/v) DMSO (BDA) and stored as three 1·5 ml aliquots in liquid nitrogen until required. Patient details were recorded by the investigating physician who was also responsible for clinical investigation and performing cervical biopsies. Clinical diagnosis was made on histological evidence from cervical biopsy material. Patients were then grouped into one of three categories; atypical (i.e. patients referred for colposcopy with atypical changes on cytology only and no evidence of CIN on
MHGDTPTLHEYMLDL (1)
TPTLHEYMLDLQPET (2)
EYMLDLQPETTDLYC (3)
LQPETTDLYCYEQLN (4)
TDLYCYEQLMDSSSE (5)
YEQLNSSEEDDIE (6)
DSSEEDDIEGPAGQ (7)
EDEIDGPAGQAEPOP (8)
GPAGQAEPOPHAHNI (9)
AEPDRAHNYTFFCC (10)
AHYNIVFCCKCDST (11)
VFCCKCDSTRLCV (12)
KCDSLRLCVQSTHV (13)
LRlacQSTHVDIRTL (14)
QSTHVDIRTLEDLM (15)
DIRTLEDLMOTGI (16)
EDLMOTGI1CIPC (17)
MGTLI1CIPCQSQKP (18)

Fig. 1. Overlapping HPV-16 E7 synthetic peptides. T cell proliferation assays used peptides (15-mer) of E7 that overlapped by 5 amino acids and spanned the entire molecule. In preliminary studies the peptides were 20-mers with sequences the same as each of the above but extended by 5 residues at their C termini.

histology), CIN I/II or CIN III. The age distribution of these groups was as follows: atypical group, age range 22–41 (n = 5; mean ± SD = 28.6 ± 7.3); CIN I/II group, age range 23–50 (n = 15; mean ± SD = 33.3 ± 7.7); CIN III group, age range 19–33 (n = 9; mean ± SD = 27.4 ± 4.8). For all patients, sections were taken from selected biopsy blocks with evidence of abnormal histology and the material used to prepare DNA extracts for PCR analysis as described below. A control group of women (n = 15) was selected from the same population area of London who had normal routine cervical smears, no previous history of genital warts and a similar age distribution to the patients (age range 22–67; mean ± SD = 33.8 ± 11.7). For ethical reasons it was not possible to obtain cervical biopsy material from this control group. Peripheral blood cells from women with cervical carcinoma and information regarding HPV DNA type were generously provided by M. Duggan-Keen and P. Stern (Paterson Institute for Cancer Research, Christie Hospital, Manchester, UK).

■ HPV typing by PCR. DNA was obtained from paraffin-embedded biopsy material as described by Wright & Mans (1990). HPV typing was performed essentially as described by van den Brule et al. (1992). Consensus primers from a homologous region of L1 were used to detect HPV DNA from a wide range of HPV types; HPV DNA-positive samples were then subjected to type-specific PCR analysis using a mixture of type-specific primers. In our experiments the primers used for consensus PCR were GP6 (Snijders et al., 1990) and MY11 (Resnick et al., 1990) which amplified a 184 bp fragment of L1, and the type-specific primers for detection of HPV-6, -11, -16, -18, -31 and -33 were as described by van den Brule et al. (1992). Consensus PCR-negative samples were checked for amplifiable DNA using primers (0.1 μM) which amplify a 355 bp segment of the β-globin gene (British Biotechnology).

For all PCR reactions, 40 cycles of amplification were performed on a Biometra Trio-thermoblock TB1. Reaction conditions for consensus PCR, type-specific PCR and β-globin PCR were all as previously described (Shepherd et al., 1996). PCR products were analysed by electrophoresis on a 1.5% agarose gel.

■ STLs. Patients’ cells were thawed rapidly at 37 °C, washed in culture medium containing 10% autologous serum and adjusted to 3 x 10^6/ml. GST-E7 fusion protein was diluted in culture medium and 100 μl (10 μg/ml) was dispensed into 25 wells of a 96-well round-bottomed microtiter plate (Greiner). A 100 μl cell suspension (3 x 10^5 cells) was added to each well and the plates incubated at 37 °C in a humidified 5% CO₂ atmosphere for 14 days. Cell cultures were fed on days 3 and 7 with 100 μl medium containing 5% autologous serum, recombinant interleukin 2 (20 U/ml; Sandoz) and recombinant interleukin 4 (4 U/ml; Glaxo).

■ Specificity assays on STLs. Each of the 20 STLs was tested on day 14 against GST-E7 fusion protein (10 μg/ml), 10 synthetic peptides representing HPV-16 E7 (10 μM) and culture medium in duplicate wells. The antigens were dispensed first at 100 μl/well. Cell counts were performed on five pooled cell lines and the mean counts used to estimate the numbers of cells per line (this number varied between 1–3 x 10^4 cells). Each cell line was diluted to 10^5/ml in medium plus 5% autologous serum and dispensed together with irradiated antigen-presenting cells at 2.5 x 10^5/ml in a further 100 μl. The latter were obtained from two vials of the patient’s cells stored in liquid nitrogen, thawed, washed and irradiated (4000 Rads). The plates were incubated for 3 days at 37 °C in 5% CO₂ and pulsed with 20 μl of [methyl-3H]thymidine (Amersham; 9.25 kBq/well) for the final 4 h of incubation. Incorporation of labelled thymidine was measured by counting the harvested wells in a β-spectrometer (Hewlett Packard). The data was plotted as the response in
Fig. 2. (a) T cell responses to HPV-16 E7 peptides in women with cervical dysplasia. The numbers of antigen-specific STLs from nine responding patients out of 31 tested are represented. Twenty STLs were established per patient and assayed against GST-E7 fusion protein, GST (§, except these patients; see Results) and 15-mer peptides representing the E7 protein (*, 20-mer peptides). Criteria required for a positive STL response are given in Methods. (b) T cell responses to HPV-16 E7 peptides in healthy asymptomatic individuals. The numbers of antigen-specific STLs from seven responding individuals out of 15 tested are represented. Twenty STLs per individual were assayed against GST-E7 fusion protein and 15-mer peptides representing E7.
positive proliferative response was defined by a stimulation index (SI) of c.p.m., for each cell line to all the antigens and medium controls. A contingency tables of responders and non-responders to peptides in the patient and control groups, and on grades of cervical biopsy histology and HPV DNA status. Fisher's exact test was applied when sample numbers were small. A P value of less than 0.05 was taken as statistically significant.

Statistical analyses. Chi squared tests were performed on contingency tables of responders and non-responders to peptides in the patient and control groups, and on grades of cervical biopsy histology and HPV DNA status. Fisher's exact test was applied when sample numbers were small. A P value of less than 0.05 was taken as statistically significant.

Results

Mapping of the proliferative T cell response to HPV-16 E7

We examined the proliferative T cell responses to the HPV-16 E7 protein in a cross-sectional study involving 31 women with cervical dysplasia and 15 healthy asymptomatic individuals. The age distribution was similar in the patient (31.8 ± 9.3 years) and control (33.8 ± 11.7 years) groups. Twenty STLs were established from the individuals' peripheral blood mononuclear cells using a GST-E7 fusion protein as the antigen and their specificity for HPV-16 E7 was determined in 3 day proliferation assays using synthetic peptides (Fig. 1). By including GST as a test antigen in our initial patient proliferation assays (patients 3, 5, 7, 8; Fig. 2a) we found that the majority of GST–E7 driven STLs included T cells that responded to GST and this antigen was therefore not included in subsequent assays.

In the dysplasia patient group there were nine individuals (29%) who demonstrated a proliferative T cell response to one or more of the E7 peptides tested. The specificity and number of responding cell lines from each of those who responded is illustrated in Fig. 2(a) (where a positive response was equivalent to two or more cell lines responding to the same peptide per patient). These T cell responses were distributed across the central and C-terminal regions of the E7 molecule (amino acids 25–94) with each responding individual recognizing between one and three different peptides. No peptides were recognized in the N-terminal region of the molecule (amino acids 1–34) and no single peptide stimulated a dominant T cell response within the patient group.

In the healthy asymptomatic group, seven individuals (47%) demonstrated proliferative T cell responses to one or more of the E7 peptides tested. These responses (Fig. 2b) were predominantly to peptides in the C-terminal region of the protein (amino acids 70–98). One or more of the peptides representing this region were recognized by all responding asymptomatic individuals (7/15 or 47%). However, in patients with cervical dysplasia this response was significantly reduced, with only 5/31 (16%) of women responding (P = 0.038). In 6/7 of the healthy controls who responded to the C-terminal region (40% of the group as a whole) a response was made to a single immunogenic peptide representing the region between residues 75–94. This was compared to 4/31 or 13% of patients with dysplasia who responded to the same region (P = 0.057).

The number of STLs responding per individual to region 75–94 was also higher in healthy individuals (0–14) than in patients with cervical dysplasia (0–3), indicating a higher number of circulating T cells were specific for this peptide. Three out of 15 healthy asymptomatic individuals (20%) responded to the N-terminal portion of the molecule (amino acids 1–34) whereas no responses to this region were detected in patients with cervical dysplasia (P = 0.03). The number of responders to the central portion of the protein (residues 25–74) was similar in the patient (5/31 or 16%) and control (2/15 or 13%) groups.

Thus, healthy asymptomatic individuals were significantly more likely to demonstrate proliferative T cell responses to the N- and C-terminal regions of E7 than women with cervical dysplasia. An immunogenic region between 75–94 was identified in responding healthy individuals who also demonstrated a greater number of responding cell lines than patients with dysplasia responding to the same region.

T cell responses to HPV-16 E7 in relation to cervical biopsy histology and detection of HPV DNA

Cervical biopsy tissue was obtained from 29 patients and was analysed and assigned to one of three histological categories; atypical, CIN I/II or CIN III. Hence, 5/29 patients (17%) were found to have atypical histology, 15/29 (52%) had CIN I/II lesions and 9/29 (31%) had CIN III lesions. The highest proportion of responders was found in the CIN III group (5/9 or 56%) with less responders found in the remaining patients who were atypical or CIN I/II (4/20 or 20%). This difference was not statistically significant (P = 0.067) but may reflect a higher level of previous or current exposure to HPV-16 in the CIN III group. The ability of patients to mount a response in relation to the histological grade and the HPV DNA type in their biopsies is shown in Fig. 3. Patients in the CIN III group differed significantly from patients in the atypical or CIN I/II groups in their ability to respond to the immunogenic region at amino acids 75–94 (3/9 versus 0/20 individuals respectively; P = 0.044; data not shown).

Cervical biopsy tissue was available for HPV DNA typing in 27 of the 29 patients described above. HPV-16 DNA was detected in 9/27 biopsies (33%), the majority of which (5/9) were from CIN III lesions but this was not a significant association (P = 0.072). HPV-18 DNA was detected in 3/27 biopsies (11%) all of which were CIN I/II lesions. Six (22%) contained HPV DNA which could not be typed by this method (HPV type ‘X’) and were predominantly CIN I/II lesions; nine (33%) were HPV DNA-negative and represented...
Table 1. HPV-16 L1- and E7-specific T cell responses of women with cervical cancer

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Response to β-gal-L1 L1 peptides</th>
<th>Response to GST-E7 E7 peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>+ (12)</td>
<td>NT</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>+ (5)</td>
<td>335(5)</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>+ (4)</td>
<td>+ (1)</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>+ (10)</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>54</td>
<td>-</td>
<td>NT</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td>+ (4)</td>
<td>281(2), 311(6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>316(7), 335(2)</td>
</tr>
</tbody>
</table>

* T cell lines were generated using a HPV-16 β-gal-L1 fusion protein representing amino acids 199–409 and specificity demonstrated using 15-mer peptides representing previously identified immunogenic regions of L1 between 199–346 (Shepherd et al., 1996); numbers in parentheses show responding cell lines from a total of 20 assayed.
† T cell lines generated and proliferation assays performed as described in Methods. NT, Not tested.
‡ Nine cell lines assayed.

Proliferative T cell responses to HPV-16 L1 and E7 in patients with cervical carcinoma

Proliferative T cell responses to the HPV-16 L1 and E7 proteins were studied in a small group of patients (n = 7) with cervical carcinoma by generating STLs using a β-galactosidase (β-gal)–HPV-16 L1 fusion protein representing amino acids 199–409 or the GST–E7 fusion protein, respectively. STL specificity was determined in 3 day proliferation assays with E7 peptides as previously described or peptides representing previously identified immunogenic regions of L1 between residues 199–346 (Shepherd et al., 1996); the data are summarized in Table 1.

In previous studies we examined the response of 41 women with cervical dysplasia to β-gal–L1 (199–409) and found that all 41 patients responded to the fusion protein (range 10–20 STLs per patient) and 26/41 (63 %) responded to one or more peptides representing this region of L1. We found that 5/7 (71%) of the cervical carcinoma patients responded to the L1 fusion protein and 2/5 (40%) to the E7 fusion protein, which represents a significantly lower response than that of cervical dysplasia patients (100% to both fusion proteins, P = 0.019 and 0.001, respectively). Similarly, the range of responses was lower in women with cervical carcinoma (0–12 STLs per patient for β-gal–L1 and 0–4 STLs per patient for GST–E7) than those with cervical dysplasia. These observations suggest that responses to either the β-gal and GST components of the fusion proteins, or to the HPV-16-specific antigens L1 and E7, or both, may be impaired in this patient group. HPV DNA typing data were available for three of these patients (2, 4 and 5) who were all HPV-16-positive.

The HPV-16 peptide-specific STL responses of our three study groups are summarized and compared in Fig. 4. We observed a reduced response to L1 peptides in women with cervical carcinoma where 2/7 (29%) responded to one or more L1 peptides compared to 26/41 (63 %) of women with cervical dysplasia and 9/15 (60%) of healthy individuals. T cell responses to E7 were reduced in women with cervical dysplasia (9/31 or 29 %) when compared with healthy individuals (7/15 or 47%). No specific T cell responses to the E7 peptides were found in five patients with cervical carcinoma. Hence, we observed a reduction in proliferative T cell responses to E7 which correlates with an increase in disease severity of the study group, although none of the observed differences proved to be statistically significant (P > 0.05).
### Discussion

T cell responses to papillomavirus infections in man are not well defined, particularly with regard to their potential role in the pathogenesis of genital disease. In this study we describe our findings relating to proliferative T cell responses in healthy individuals and women with cervical disease and cervical carcinoma to the E7 protein of HPV-16, the HPV type most closely associated with high grade cervical dysplasia and cervical carcinoma. We identified differences in the T cell responses of these subject groups which may reflect differences in the ability of these individuals to control the virus and prevent the subsequent development of HPV-16-related disease.

All patients and controls demonstrated T cell responses to the GST–E7 fusion protein. We have previously demonstrated that in most individuals a component of this response is GST-specific. HPV-16 E7-specific T cell responses were demonstrated in 7/15 (47%) of healthy individuals. All of these individuals made a response to the C-terminal end of the protein (amino acids 70–98) and the peptide 80–94 was found to be the most immunogenic within this group's response; 6/7 of responding individuals recognized this single peptide. The number of women with cervical dysplasia who responded to HPV-16 E7 peptides was lower (9/31 or 29%) although not significantly so. However, fewer women with cervical dysplasia than healthy individuals responded to the C-terminal region of E7 or to single peptides representing the region 75–94 (P = 0.038 and 0.057, respectively). Also, significantly fewer women with cervical dysplasia responded to the N-terminal region at residues 1–34 than healthy individuals (P = 0.03), although the number of responders in this group was small. The shift in recognition pattern may reflect the importance of the N- and C-terminal regions in immune responses in healthy individuals.

When comparing levels of T cell response to HPV-16 E7 of patients with lesions of different histological grades (irrespective of HPV DNA status) there was a trend for those patients with high grade or CIN III lesions (5/9) to respond rather than patients with either low grade (CIN I/II) lesions or atypical histology (4/20), although this did not reach statistical significance (P = 0.067). However, the peptide-specific responses of those patients with high grade dysplasia were more often to one peptide representing the region 75–94 than those of patients with low grade or atypical histology (P = 0.023). These findings might be explained by the higher incidence of HPV-16 positivity in high grade versus low grade lesions (63% versus 21% in this study). Unlike our findings on HPV-16 L1 (Shepherd et al., 1996) the ability to respond to HPV-16 E7 was not significantly associated with the presence of the virus in the lesion as detected by PCR (P = 0.65). There are a number of possible explanations for these observations. In individuals who are HPV-16 DNA-positive but fail to respond, E7 may simply not be the dominant target antigen of their HPV-specific T helper cell responses. Alternatively, E7-specific T helper cell responses may be specifically down-regulated. In immunocompetent mice it has been shown that sustained unresponsiveness may be induced by immunization with low amounts of E7 antigen (Chambers et al., 1994b). It is therefore possible that during genital HPV-16 infection, low level expression of E7 in cervical keratinocytes lacking the appropriate secondary signalling molecules induces a similar state of immunological tolerance. Responders were also found in other HPV DNA type categories (HPV-18, X and negative). Again, responding individuals may have been previously exposed to HPV-16 or have a current HPV-16 infection at a site other than that which was biopsied, or the T cell response detected may have been a cross-reactive response to a determinant represented on another HPV type. It is also

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**Fig. 4.** Comparison of T cell responses to HPV-16 L1 and E7 in healthy individuals, women with cervical dysplasia and with cervical carcinoma. STLs were expanded with β-gal–L1 (amino acids 199–409) or GST–E7 fusion proteins and HPV-16 specificity demonstrated in 3 day assays with peptides (15-mers) representing immunogenic regions of L1 (between residues 199–346, Shepherd et al., 1996) or the whole of E7, respectively.
possible that PCR failed to detect HPV-16 DNA due to sampling procedures or destruction of virus DNA during the fixation process.

We have also studied T cell responses to HPV-16 L1 in healthy individuals and women with cervical dysplasia (Shepherd et al., 1996) and to both HPV-16 L1 and E7 proteins in a small group of women with cervical carcinoma (n = 7). On comparison of T cell responses to L1 in the three study groups we found that the number of responders to L1 was similar in the former two groups but reduced in women with cervical carcinoma. We observed a trend for patient groups of increasing disease severity to show a reduced ability to respond to E7, where no response was seen in women with cervical carcinoma. That these findings did not prove to be statistically significant is due to the small size of the latter group. However, we have demonstrated that 100% and 33% of HPV-16-positive women with cervical dysplasia respond to the L1 (Shepherd et al., 1996) and E7 proteins, respectively, compared to 29% and 0% of women with cervical cancer, the majority of whom are likely to be HPV-16-positive. We conclude that there is evidence for the down-regulation of T helper cell responses to the L1 and E7 proteins in this patient group, even though only a small number of patients were studied.

The reduction in the T helper cell responses which we observed in women with cervical dysplasia and those with cervical carcinoma could be important in allowing progression of virus-induced disease, although we have no direct experimental evidence for this. There are a number of mechanisms which might be involved in inducing a state of non-responsiveness to HPV antigens. The virus may evade immune recognition by sequence variation within T cell-immunogenic proteins, as proposed by Ellis et al. (1995) following their identification of an HLA B7-binding HPV-16 E6 variant. We did not HLA type our dysplasia patients or controls in this study but preliminary studies on responses to L1 in a similar group of patients did not reveal any HLA association of responses observed, although the numbers were small (n = 12). It is possible that the regulation or function of T helper cells of a particular subtype is impaired in patients who fail to respond. In patients with an impaired Th1 response for example, mechanisms regulated by these cells and shown to be important in controlling HPV-related disease (i.e. delayed-type hypersensitivity or cytotoxic T lymphocyte responses) would be down-regulated such that the host would have a reduced ability to control the virus infection, leading to progression of disease.

The correlation of these findings in the peripheral blood of healthy individuals and women with cervical disease with the function of HPV-specific T helper cells in cervical lesions is currently under investigation.

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References


