Accession number: txt001smc; **Accession date:** 3 July 1998; **Last update: 19 October 1998**
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Human cellular immune responses against human papillomaviruses in cervical neoplasia

Abbreviations: HPV, CaCx

Stephen Man

Human papillomaviruses (HPVs) are ubiquitous DNA viruses that infect cutaneous and mucosal epithelia. A subset of HPVs infects the female genital tract, to induce cervical lesions that can progress to malignancy in some women. DNA from HPVs can be found in >94% of cervical carcinomas (CaCx) worldwide; this strong association suggests that it might be possible to develop either prophylaxis or therapies for cervical neoplasia, based on the manipulation of human immune responses against HPVs. This review examines the current research into human immune responses against HPVs in CaCx and the potential impact of this research on human health.

Cervical cancer (CaCx) is the second most common cause of cancer-related deaths in women worldwide, with 500 000 new cases reported annually (Ref. 1). In the UK alone, CaCx accounts for ~1400 deaths per year, with five-year survival rates for patients with advanced cancer being ~45% (Ref. 2). Cervical neoplasia encompasses a spectrum of cervical epithelial cell abnormalities, ranging from pre-invasive cervical intraepithelial neoplasia (CIN) to CaCx (Fig. 1, fig001smc). It is thought that CaCx develops from pre-invasive CIN lesions rather than arising spontaneously. Cervical smear testing and treatment for pre-invasive CIN have reduced the overall incidence of CaCx in the UK and other developed countries (Ref. 3). Nevertheless, women treated for CIN still have a 20-fold higher risk of developing CaCx than the general population (Ref. 4); furthermore, adenocarcinoma of the cervix is increasing in incidence, in the UK at least (Ref. 5). Current therapies for cancer (surgery, radio- or chemotherapy) are of limited effectiveness for curing recurrent disease, and their use in developing countries is limited by economic constraints. The global burden of HPV-associated cervical neoplasia requires the development of novel approaches for prevention and treatment.

Association between papillomaviruses and cervical neoplasia

HPVs comprise a large family of double-stranded DNA (ds DNA) viruses that infect epithelial cells of cutaneous (skin) and mucosal surfaces. To date, at least 75 distinct types have been isolated from various human tissue biopsies. A subset (~25) of these HPVs is sexually transmitted and causes a spectrum of diseases in the male and female genital tract. These HPVs can be categorised according to their potential to cause malignancy as low risk, intermediate risk or high risk. HPV types 6 (HPV-6) and 11 (HPV-11), for example, are associated with low-risk disease, such as genital warts. By contrast, high-risk HPVs, such as HPV-16, HPV-18, HPV-31, HPV-33 and HPV-45, are associated with high-grade

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https://doi.org/10.1017/S1462399498000210 Published online by Cambridge University Press

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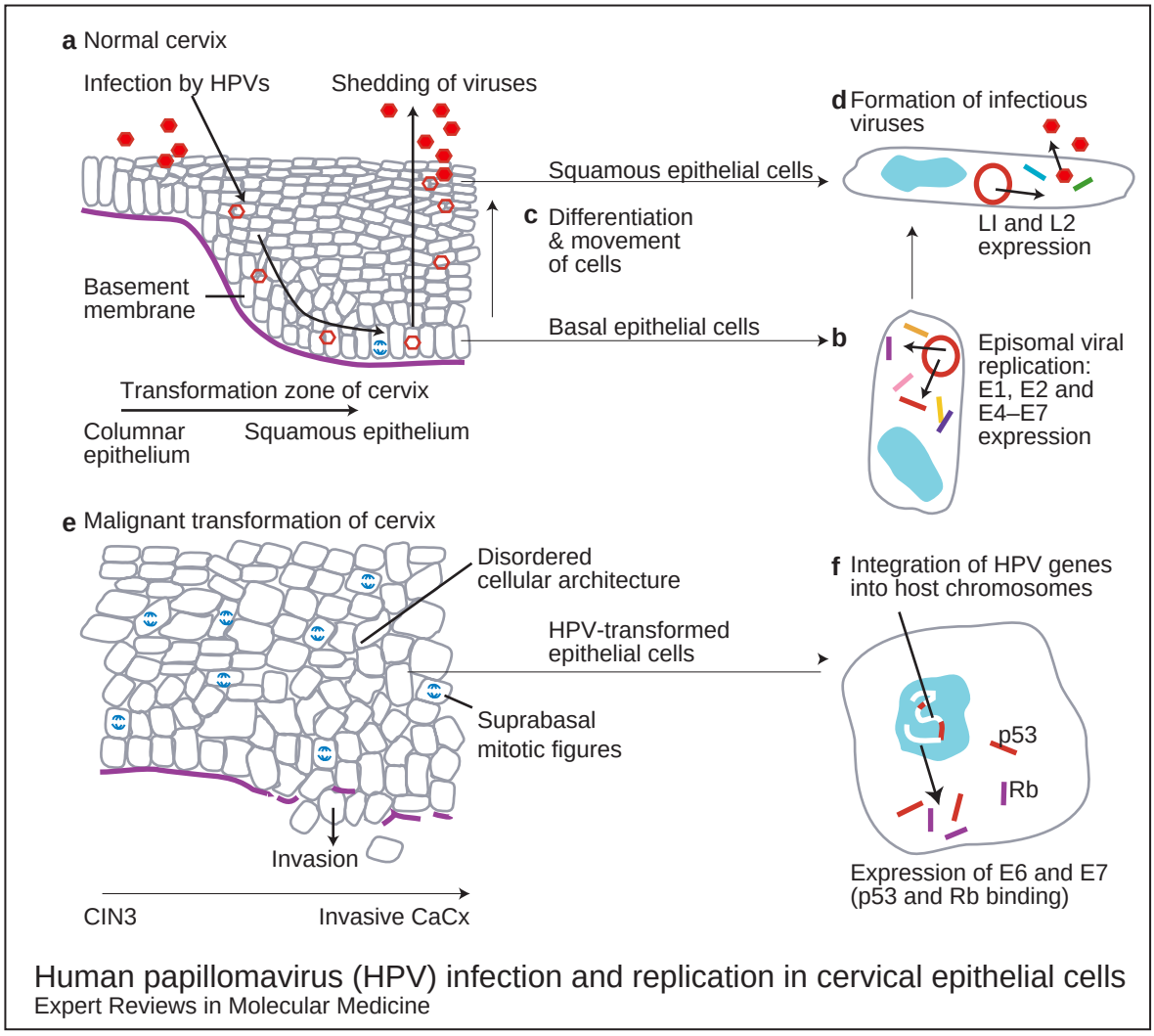

Figure 1. Human papillomavirus (HPV) infection and replication in cervical epithelial cells. (a) The normal cervix has a (narrow) transformation zone in which there is an abrupt transition from a columnar epithelium (sometimes via a metaplastic epithelium) to a squamous epithelium; HPVs are probably most infectious to cells that are close to this junction. (b) HPV viruses gain access to the basal epithelial cells of the cervix via the vagina (for example, during sexual intercourse), where they replicate episomally (outside the host chromosome in the nucleus) and express the (early) viral genes E1, E2, E4, E5, E6 and E7. (c) The infected basal cells, which show signs of cell disruption as a result of the viral infection, continue their differentiation and migration to the epithelial surface, where (d) the (now) squamous cells start to express the late HPV genes LI and L2. Infectious virus particles are formed and shed into the lumen of the vagina. (e) HPV infection (particularly with the high-risk types) can progress to: (1) HPV-induced mild dysplasia, (2) the final stages of cervical intraepithelial neoplasia (CIN3) and, eventually, (3) invasive cervical cancer (CaCx), when the basement membrane is breached by the cells, allowing local spread and also distant metastasis. (f) In transformed epithelial cells, HPV genes are integrated into the host chromosomes, with expression of (the oncogenic) E6 and E7 proteins, which bind to the tumour-suppressor proteins p53 and Rb **(fig001smc)**.

intraepithelial lesions and invasive cancer. HPV DNA has been found in >94% of CaCx cases studied, with HPV-16 and HPV-18 being the types most commonly detected. In this review, the emphasis is on the current knowledge of human immune responses against HPVs that are associated with the development of cervical neoplasia, including both premalignant disease (CIN) and invasive CaCx.

The close association of both CIN3 (the most advanced stage of CIN) and CaCx with HPV infection provides an opportunity for

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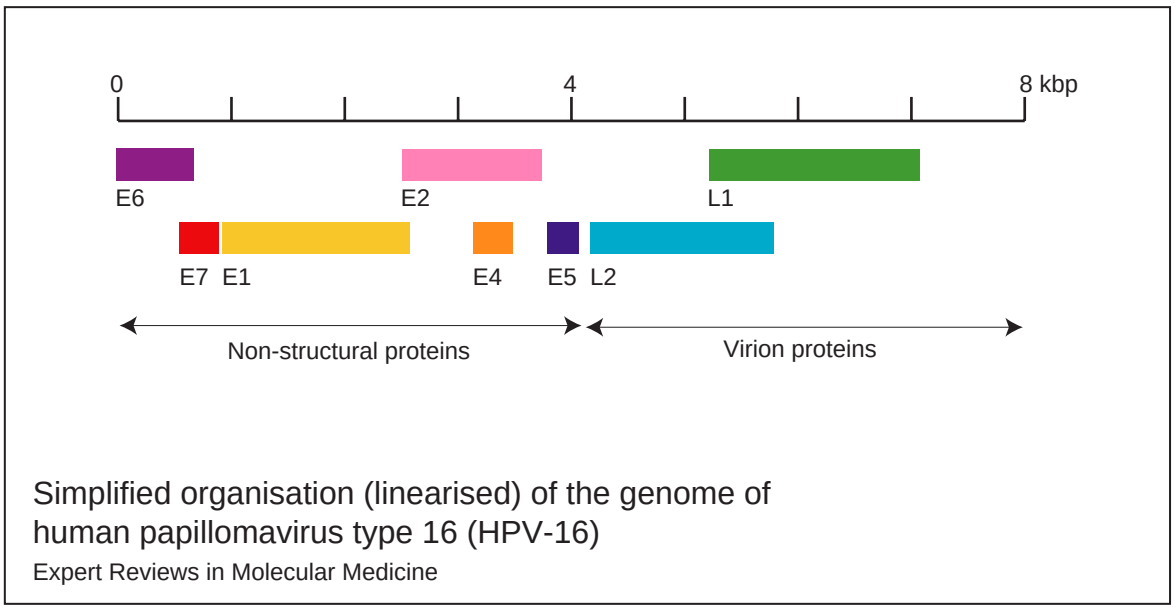

Figure 2. Simplified organisation (linearised) of the genome of human papillomavirus type 16 (HPV-16). Simplified organisation of the HPV-16 genome in its linearised state (scale bar is in kilobase pairs). The rectangles represent the positions of various open reading frames (ORFs). The E genes encode proteins that are produced early in the infectious cycle (the non-structural proteins), whereas the L genes encode proteins that are produced late in infection (virion proteins that are necessary for virus assembly). The actual protein products of the genes are complex owing to the production of multiple messenger RNA (mRNA) transcripts (for further details see http://hpv-web.lanl.gov/) **(fig002smc)**.

immunologists to develop strategies based on harnessing the human immune response to prevent or treat cervical neoplasia. This review focuses on cell-mediated immune (CMI) responses, but it is worth noting that the first evidence that HPV proteins were immunogenic in humans came from the detection of HPV-specific antibody responses. However, natural infection of humans by HPVs does not appear to provoke strong antibody responses; antibody responses against E6 and E7 proteins from HPV-16 are most prevalent in patients with advanced CaCx and appear to have little prognostic value (reviewed in Ref. 6). Prophylaxis against HPV infection using a vaccine will require the induction of a strong neutralising antibody response against HPV structural proteins (see Fig. 2, fig002smc). This will need to take place at the point of infection, namely at the mucosal surfaces of the female genital tract. Recently, the development of synthetic virus-like particles (VLPs) based on HPV structural proteins L1 and L2 has greatly advanced knowledge of immune responses to HPV infection (reviewed in Ref. 6). VLPs have been used as vaccines in animal papillomavirus models with encouraging results; for example, dogs were protected against papillomavirus infection by vaccination with VLPs (Ref. 7). To date, however, VLPs based on HPV L1 and L2 proteins have not been tested on humans and evaluation of clinical effectiveness in preventing the development of CaCx will require studies that are both large scale and long term.

The association between HPV infection and development of CaCx is not absolute, because the majority of women infected with HPVs do not develop CaCx. A recent study of college-age women in the USA demonstrated that ~40% of this population had evidence of HPV infection but the majority were able to eliminate HPV infection within 6 months (Ref. 8). The role of the immune system in this viral clearance is not clear, but there is circumstantial evidence that CMI is important in the control of persistent HPV infection. First, HPV-associated cervical lesions are more prevalent in immunosuppressed individuals, such as the recipients of kidney transplants who are undergoing immunosuppressive therapy (Ref. 9) or women who are infected with human immunodeficiency virus (HIV; Ref. 10). Second, in humans, so-called 'spontaneous' regression of HPV-induced genital warts is associated with an

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infiltration of lymphocytes into the lesions (Ref. 11); similar observations have also been made in animal models (Refs 12, 13, 14). Third, the presence of lymphocytes in the lesion correlates with improved prognosis in squamous CaCx (Ref. 15). These observations have fuelled an intense worldwide study of CMI responses against HPVs, to understand the roles of these responses in disease, ultimately with the aim of developing immunotherapies against CaCx.

HPV infection and gene expression

The study of human CMI against HPVs is limited by the technical inability to propagate virion particles on a large enough scale in vitro to generate HPV antigens and HPV-infected target cells. Furthermore, the HPVs that are associated with the development of cervical neoplasia have both species- and tissue-specific tropism, infecting only human keratinocytes (or cells with the potential for squamous maturation). Fortunately, advances in the molecular genetics of HPVs have allowed immunologists to express HPV-gene products in non-HPV infected cells of many species, using DNA transfection or recombinant viral vectors.

The organisation of the HPV-16 genome, consisting of 8000 DNA base pairs (bps), has been extensively studied and is shown in a simplified form in Figure 2 (fig002smc; reviewed in detail in Ref. 16). Two types of genes are expressed: the early (E) genes are involved in DNA replication, protein transcription and cellular transformation; and the late (L) genes code for viral capsid proteins. The target cell for HPV infection is the keratinocyte (epithelial cell) and expression of HPV genes in vivo is exquisitely dependent on the differentiation programme of keratinocytes. During a productive viral infection there is a cascade of HPV-gene expression that follows the differentiation programme of keratinocytes; it starts with the suprabasal epithelial stem cells and culminates in release of HPV virions from terminally differentiated keratinocytes that are being exfoliated at the skin or mucosal surface (Fig. 1, fig001smc). The genome of HPVs is maintained extrachromosomally as an episome (a self-replicating genetic element that can move into or out of chromosomes) in keratinocytes, but is frequently found to be integrated into a human chromosome in HPV-associated tumours. The molecular and cellular events leading to the change from a benign, persistently infected basal epithelial cell to a malignant transformed keratinocyte that is no longer capable of supporting HPV replication are still poorly understood. It is important to stress that this transformation process, which can take two decades, is dependent not only on HPV infection (most of the individuals who are infected with HPV do not develop cancer), but also on host genes and other co-factors. However, cellular immunologists have (unashamedly) focused their attention on the HPV-gene products that are expressed in transformed cervical keratinocytes. The HPV proteins that are invariably expressed in HPV-associated tumours are E6 and E7. These bind to the tumour-suppressor proteins, Rb and p53, respectively, to induce the transformed cell phenotype (Refs 17, 18). The absolute requirement for the continued expression of E6 and E7 to maintain the transformed phenotype makes these proteins extremely attractive tumour-specific targets for the immunotherapy of cervical neoplasia.

Processing of HPV antigens for recognition by immune cells

Specific CMI responses have been defined in humans into two broad categories: those mediated by cytotoxic T lymphocytes (CTLs) and those mediated by T-helper lymphocytes (THLs).

Cytotoxic T-lymphocyte responses

CTLs are usually $CD8^+$ (express the CD8 receptor on their cell surface) and play a vital role in the clearance of virally infected cells. Foreign viral antigens are recognised on the surface of virally infected cells in the form of short peptides bound to major histocompatability complex (MHC) class I molecules [in humans these are called human leucocyte antigens (HLAs)]. These foreign peptides are 8–10 amino acids long and are generated by the proteolysis of viral proteins in the cytoplasm of an infected cell. These peptides are then transported from the cytosol into the endoplasmic reticulum where they bind to newly synthesised MHC class I molecules. A complex of MHC class I and antigenic peptide plus β_2 microglobulin (β_2m) then migrates to the cell surface where recognition by a CTL bearing a T-cell receptor (TCR) of the appropriate specificity can take place (Fig. 3, fig003smc). MHC class I molecules are expressed on the surface of all nucleated cells, therefore anti-viral CTLs are a powerful effector mechanism for the clearance of

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virally infected cells. Activation of CTLs requires recognition of foreign antigens on so-called 'professional' antigen-presenting cells (APCs), such as macrophages, activated B lymphocytes (B cells) and dendritic cells. All of these cells (1) express high levels of both MHC class I and MHC class II molecules (see below), (2) are able to process exogenous antigens, and (3) are rich in the co-stimulation molecules (such as B7-1) that are important for T-cell activation. For CTL recognition of HPVs, it is envisaged that expression of the early HPV genes (E1–E7) within the cytoplasm of infected cells will generate HPV-derived peptides that are able to bind to HLA class I molecules (Fig. 3, fig003smc). In HPV-transformed cervical keratinocytes, it is assumed that the constitutive expression of E6 and E7 proteins should result in E6- or E7-derived peptides presented by HLA class I molecules at the keratinocyte surface. It is not clear, however, whether HPV-infected or HPV-transformed keratinocytes are able to activate HPV-specific CTLs directly, because keratinocytes have been shown to be poor APCs for $CD4^+$ T cells (Ref. 19).

T-helper lymphocyte responses

THLs are usually $CD4^+$ and can have both 'positive' and 'negative' roles in immunity. They can provide cytokines to activate B cells for the production of antibodies, and cytokines to activate and sustain $CD8^+$ CTL responses. They can also promote inflammatory responses, which in the context of a response to pathogens can be beneficial but their role is detrimental in other circumstances such as in asthma and autoimmune diseases. In contrast to $CD8^+$ T cells, $CD4^+$ T cells recognise so-called 'exogenous' antigens, which are sampled from the surroundings of the APCs by endocytosis or by phagosomes. Once inside the cell, the antigens are encapsulated into vacuoles called endosomes, which degrade the proteins into peptide fragments (generally 11–13 amino acids in length). These low-pH endosomes fuse with other vesicles that contain MHC class II molecules, allowing binding of the peptide to MHC class II molecules (Fig. 4, fig004smc). Unlike MHC class I molecules, which are expressed on all nucleated cells, MHC class II expression is largely restricted to professional APCs such as macrophages, activated B cells and dendritic cells. All of these cells have high levels of MHC class II expression, are able to process exogenous antigens efficiently, and are rich in the co-stimulation molecules (such as B7-1) that are important for T-cell activation.

The majority of peptide epitopes that have been defined for $CD4^+$ T cells have been derived from membrane-bound or exogenous proteins, presumably because these peptides have access to the MHC class II pathway (Ref. 20). Processing and presentation of HPV antigens via HLA class II molecules could occur in a number of ways. There could theoretically be 'direct' presentation of HPV antigens (i.e. peptides) because most cervical carcinomas express HLA class II molecules (Ref. 21). This is unlikely, however, because HPV early gene proteins are mostly expressed in the cytoplasm and nucleus, suggesting that these proteins would be preferentially degraded in the cytoplasm and presented by HLA class I molecules. There is also the possibility that HPV proteins might be released into the extracellular space as a result of keratinocyte death (or cell and/or tissue damage arising through trauma or surgery); these antigens could then be taken up by resident APCs and presented to local $CD4^+$ T cells. This is possible in cervical carcinoma and high-grade CIN, where infiltrates of lymphocytes (including potential APCs) have been observed (Refs 15, 22, 23). However, low-grade CIN (and HPV infection in general) are characterised by a lack of local inflammation and an absence of cell death (Ref. 24). These observations suggest that the MHC class II pathway would be less effective at presenting antigens from keratinocytes that had been freshly infected with HPV than from keratinocytes that had been transformed by HPV (Fig. 1, fig001smc).

Individual viral antigens can be exogenously or endogenously processed in human APCs, and this can predict whether they are presented by HLA class I or HLA class II molecules. However, there are some notable exceptions; for example, $CD4^+$ T cells can recognise endogenous viral antigens via HLA class II molecules (Refs 25, 26). In a reciprocal manner, $CD8^+$ T cells have been shown to recognise exogenous antigens presented by HLA class I molecules (Refs 27, 28). Thus, it is conceivable that HPV antigens could be presented by these so-called 'alternative' pathways. For example, (1) exogenous HPV antigens could be presented exogenously to HPV-specific $CD8^+$ CTLs by local APCs, or (2) endogenous HPV antigens could be

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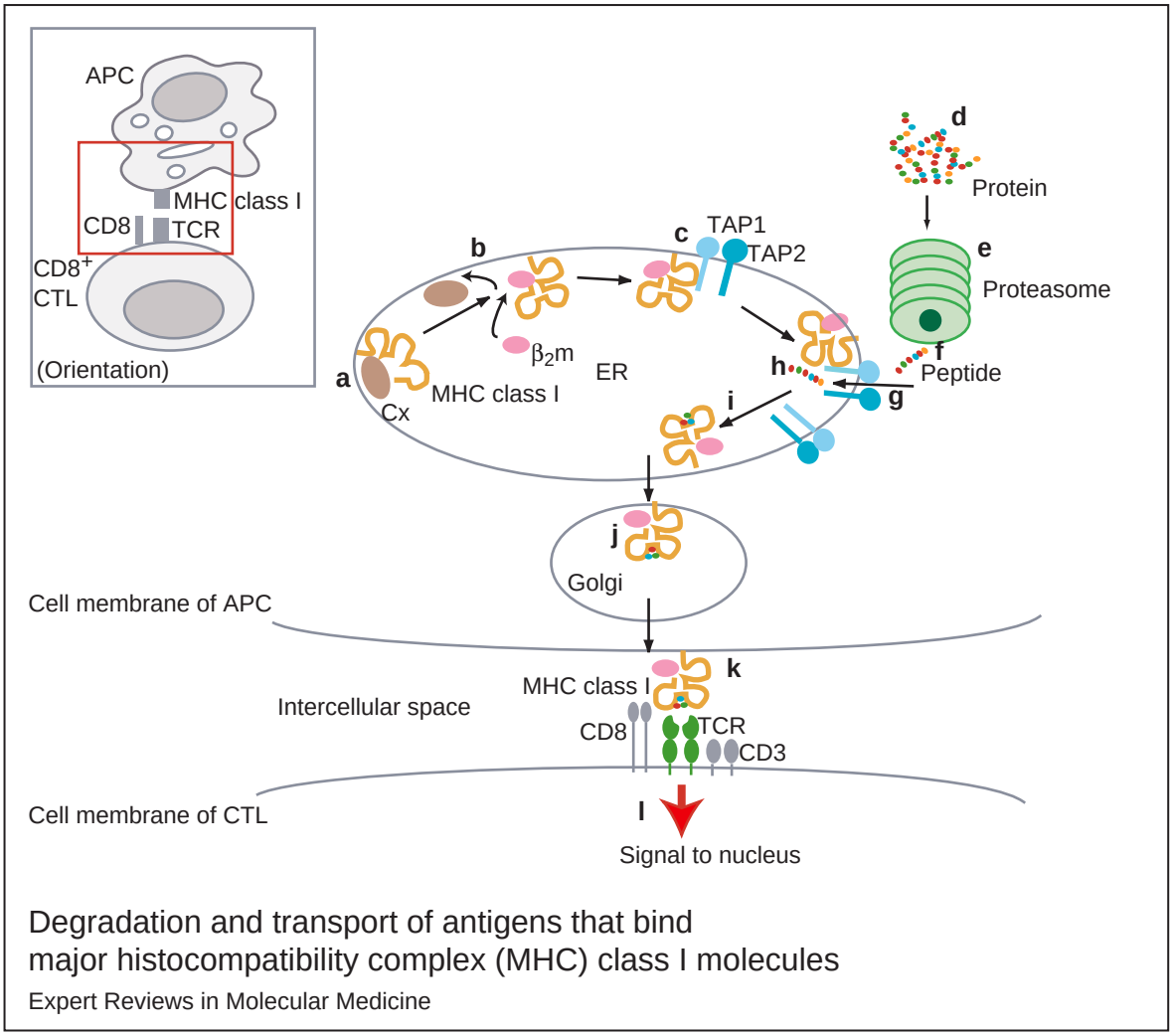

Figure 3. Degradation and transport of antigens that bind major histocompatibility complex (MHC) class I molecules. (a) In an antigen-presenting cell (APC), newly synthesised MHC class I molecules bind to calnexin (Cx), which retains them in a partially folded state in the endoplasmic reticulum (ER). (b) Binding of MHC class I molecules to β_2 microglobulin (β_2m) displaces Cx and allows binding of chaperonin proteins (calreticulin and tapasin; not shown). (c) The MHC class I–β_2m complex binds to the TAP complex (TAP1–TAP2), which awaits the delivery of peptides. (d) Peptides (e.g. from antigens) are formed from the degradation of cytosolic proteins ('self'-, pathogen- and tumour-derived proteins in the cytoplasm). (e) These are degraded by proteasomes into (f) short peptides. (g) Peptides are transported into the ER by the TAPs, where they meet the MHC class I–β_2m complex (h). This peptide binding in the antigenic groove of the MHC stablises the structure of the MHC class I molecule and (i) releases the TAP complex. (j) The fully folded MHC class I molecule with its peptide is transported to the cell surface via the Golgi apparatus. (k) Recognition of the MHC class I–peptide complex by the T-cell receptor (TCR) of an antigen-specific ($CD8^+$, $CD3^+$) cytotoxic T lymphocyte (CTL) takes place and (l) a signal transduction event activates effector functions in the MHC-class-I-restricted T cell; this requires co-stimulation to occur (not shown) **(fig003smc)**.

presented by keratinocytes to $CD4^+$ T cells. Further studies of the antigen-processing function of both HPV-infected and HPV-transformed keratinocytes are required to understand which HPV antigens might be recognised by T cells in vivo.

Methods for measuring human cell-mediated immunity

In common with human immune responses against the majority of viruses (with the exception of HIV), CMI responses to HPVs cannot be

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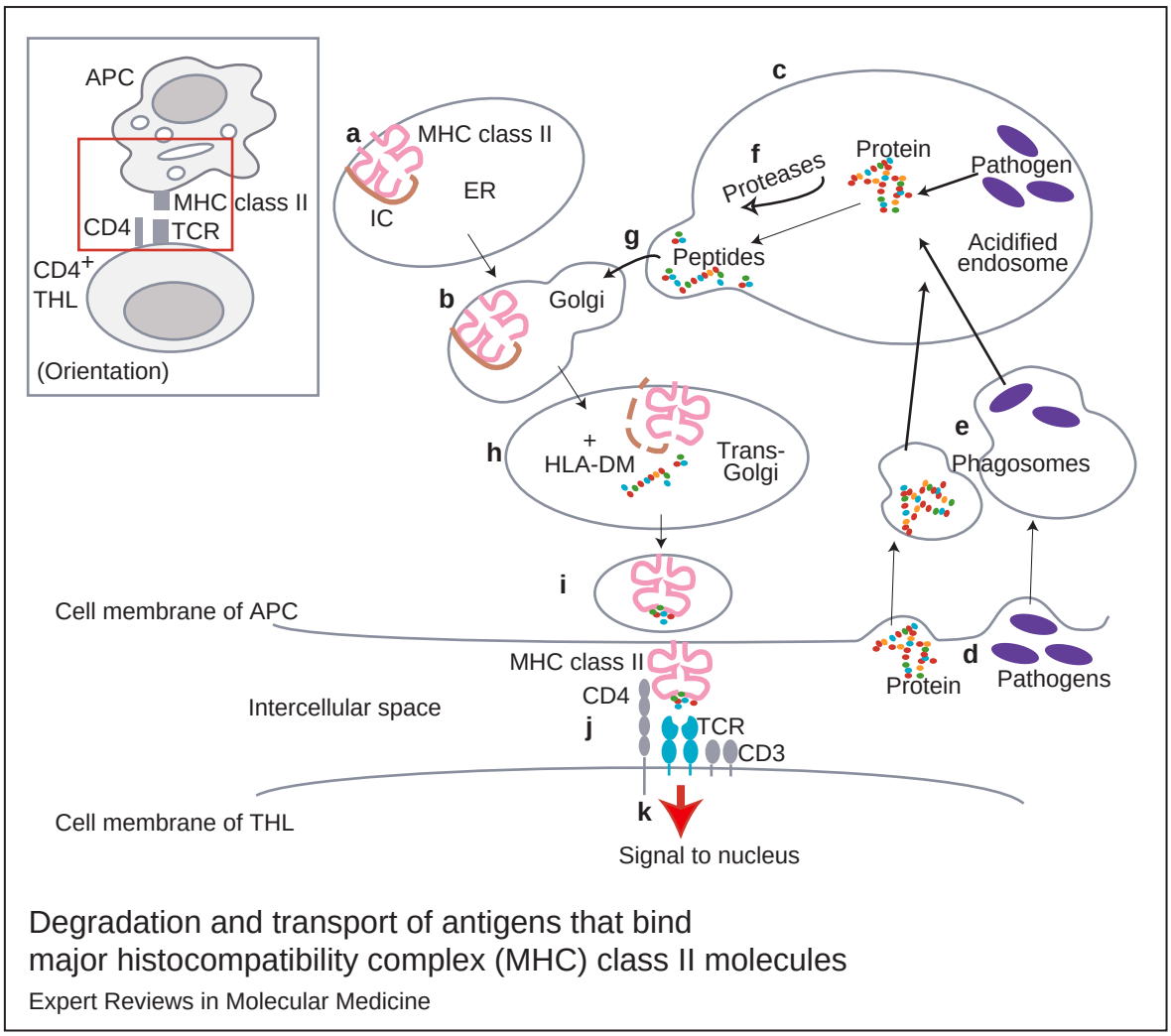

Figure 4. Degradation and transport of antigens that bind major histocompatibility complex (MHC) class II molecules. (a) In an antigen-presenting cell (APC), newly synthesised MHC class II molecules bind the invariant chain (IC), which prevents binding of peptides that are present in the endoplasmic reticulum (ER). (b) The IC allows transport of MHC class II molecules from the ER into the Golgi apparatus to acidified endosomes. (c) Endosomes contain peptides that are derived from either resident pathogens (e.g. bacteria) or (d) engulfed extracellular proteins (or pathogens) (e) in the phagosomes. (f) Proteases within the endosome degrade proteins into peptides. (g) The endosome fuses with the Golgi to form the trans-Golgi. (h) Here, the IC is cleaved and released from the MHC class II molecule. This allows the binding of peptides within the endosome to the peptide-binding cleft of the MHC molecules. An MHC-class-II-like molecule (HLA-DM) binds to MHC class II molecules to facilitate the release of the IC (not shown). (i) The MHC class II–peptide complex is then transported to the cell surface of the APC for (j) recognition by the T-cell receptor (TCR) of ($CD4^+$, $CD3^+$) T-helper lymphocytes (THLs) and (k) intracellular signalling for activation. Recycling of MHC molecules and co-stimulation are not shown **(fig004smc)**.

demonstrated directly from peripheral blood; activation of lymphocytes in vitro is required. The main methods for activating CMI are summarised in Table 1 (tab001smc). In general, $CD8^+$ T-cell responses are studied by the measurement of cytotoxicity against ^{51}Cr-labelled target cells, whereas $CD4^+$ T-cell responses rely on the measurement of specific T-cell proliferation and/or cytokine release from T cells. The advantages and disadvantages of the different methodologies are discussed below.

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Table 1. Currently available in vitro methods for stimulating T cells that are specific for human papillomaviruses (HPVs) (tab001smc)

Method 1
Stimulus/source of HPV antigen: HPV-transformed tumour cell.
Phenotype of responding cells: Mostly $CD8^+$ T cells.
Advantages: T cells that can recognise naturally processed tumour antigens are generated. If tumour cells and PBMCs are from the same donor, it is possible to obtain T cells recognising tumour antigens that are unique to that individual. It is possible to generate tumour-specific CTLs, reactive against non-HPV antigens.
Disadvantages: It is difficult to grow keratinocytes in vitro, and tumour cells change their properties in culture. If a non-autologous tumour line is used, an allogeneic response might occur.

Method 2
Stimulus/source of HPV antigen: Cells transfected with HPV DNA, by DNA transfection or use of recombinant viral vectors (such as vaccinia and adenovirus).
Phenotype of responding cells: Mostly $CD8^+$ T cells.
Advantages: Can identify T cells that are specific for individual HPV-gene products. Can infect HLA-typed cells, for example with the same HLA type as the donor.
Disadvantages: The peptides processed from these HPV antigens might not be the same as those found on HPV-transformed tumour cells.

Method 3
Stimulus/source of HPV antigen: Recombinant, soluble HPV proteins.
Phenotype of responding cells: Mostly $CD4^+$ T cells.
Advantages: Can detect responses to individual HPV-gene products. Can infect HPV-typed cells, for example with the same HLA type as the donor.
Disadvantages: It is difficult to control for specificity. Proteins can be impure or contaminated with proteins from the vector/purification method. The peptides processed from these HPV antigens might not be the same as those found on HPV-transformed tumour cells.

Method 4
Stimulus/source of HPV antigen: Synthetic peptides derived from HPV protein sequences.
Phenotype of responding cells: $CD8^+$ or $CD4^+$ T cells.
Advantages: Can detect T-cell responses to individual HPV-gene products. Can detect T-cell responses to single peptide–MHC combinations. Can map T-cell epitopes within a known protein sequence for sub-unit vaccine design.
Disadvantages: To study T-cell responses against a single HPV-gene product, a large number of peptides needs to be made; this can be prohibitively expensive if the protein is large. If the number of peptides that are to be synthesised is reduced by using predictive 'motifs', epitopes might be missed. Peptides that are immunogenic to T cells in vitro might not be the same as the peptides processed and presented on HPV-transformed tumour cells.

Abbreviations used: CTLs = cytotoxic T lymphocytes, a type of T cell that typically has the CD8 cell-surface receptor ($CD8^+$); CD4 = a cell-surface receptor that is typically found on T-helper lymphocytes; PBMCs = peripheral blood mononuclear cells, including T cells and some circulating antigen-presenting cells; HLA = human leucocyte antigen, the human form of the major histocompatibility complex (MHC) antigen.

$CD4^+$ T-cell-mediated responses: proliferative responses

The most commonly used method to assess specific CMI in vitro has been to test the ability of peripheral blood mononuclear cells (PBMCs) derived from patients to proliferate in response to HPV antigens. It has been assumed that these assays largely measure $CD4^+$ T-cell responses; however, $CD8^+$ T cells or natural killer (NK) cells can also contribute to the response. Early studies using relatively crude antigen preparations from warts that contained HPVs demonstrated that PBMCs from patients with a history of skin warts were able to proliferate in vitro (Ref. 29). Interestingly, PBMCs from some individuals who had no clinical history of warts also responded specifically to wart-antigen preparations. This high frequency of responders in the control group (of asymptomatic individuals) has been seen in many studies, and some of the possible reasons for this are discussed below.

As the molecular knowledge of HPVs and the mechanisms of antigen processing have advanced, it has become possible to measure

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T-cell responses against individual HPV proteins, and even to map T-cell epitopes within these proteins (for example, using synthetic peptides). However, interpretation of these assays has certain provisos; one problem associated with the use of soluble proteins to stimulate T cells in vitro is the provision of the most appropriate controls. Positive controls are needed to check that the PBMCs that are isolated are functional and can react to other antigens, whereas negative controls are needed to show that the in vitro conditions that are being used are supporting only HPV-specific T-cell proliferation. It is necessary to be wary if recombinant proteins have been produced in bacterial vectors because it is possible that insufficient purification will result in the carry over of bacterial products (such as endotoxins), which might affect immune responses in vitro. A further consideration is the use of recombinant proteins that have been 'tagged' with glutathionine S-transferase (GST) to facilitate their purification on chromatography columns. GST itself can be immunogenic and responses against GST can mask weak, HPV-specific T-cell responses. Most of the studies that have used soluble HPV proteins to assay for immune reactivity have not taken into account (and added controls) the possibility of a response against the protein tag itself; for example, by using GST tags on non-HPV-derived proteins as a control (Table 2, tab002smc).

One of the first studies to address whether HPV-specific memory $CD4^+$ T-cell responses existed in patients with CIN used soluble E6 proteins from HPV-16 and HPV-18. Weak proliferative responses were observed both in CIN patients and in normal controls, but there was no statistically significant difference between the two groups (Ref. 30). The most clear-cut results so far have been obtained using panels of synthetic peptides that overlap in sequence to stimulate T cells in vitro. Use of E7 peptides from HPV-16 (Ref. 31) and E6 and L1 peptides from HPV-16 (Ref. 32) defined eight different epitopes that were recognised by $CD4^+$ T cells from normal donors. However, the prior exposure status of the donors to HPVs was not known, so it was not clear whether these T-cell proliferative responses represented 'fortuitous' primary responses or indicated the presence of memory T cells that were specific for HPVs. Later studies addressed the clinical relevance of $CD4^+$ T cells that are specific for HPV-derived peptides, by comparing responses between patients with well-characterised HPV-associated disease and normal controls. Responses against peptides derived from HPV-16 E7 (Ref. 33) and HPV-16 L1 (Refs 34, 35) were not significantly associated with HPV disease because T-cell responses were also seen in normal controls. However, decreased numbers of patients with CaCx were able to make T-cell responses against HPV-16 E7 and HPV-16 L1 peptides, compared with either patients with CIN3 or normal controls. However, other studies using HPV-16 E7 peptides have shown more clear-cut clinical correlations. In these studies, the T-cell responses correlated with both the stage of disease and the presence of HPV-16 DNA (Refs 36, 37, 38). A limitation of all of these studies, however, is that there is no indication that the peptide epitopes used are actually produced as the result of processing of the soluble proteins by APCs, or that they are representative of those found on either an HPV-infected keratinocyte or a transformed keratinocyte.

T-cell responses from control groups

A common feature of $CD4^+$ T-cell responses against HPVs is the high frequency of proliferative T-cell responses from normal (apparently asymptomatic) control groups of volunteers or patients. There are two possible reasons for this. First, the T-cell responses to HPV antigens in the patients might not bear any relationship to HPV-associated disease (and, as such, are an artefact of the in vitro system used); owing to the lack of appropriate controls used, this cannot be excluded in many of the studies reported so far (summarised in Tables 2 and 3, tab002smc, tab003smc). Second, some researchers might argue that HPVs are ubiquitous viruses in humans and that most individuals will be able to make a T-cell memory response as a result of their prior exposure to HPV. Thus, any relevance to disease must be inferred either from a statistically significant increase in the number of people who respond (qualitatively) in a patient group (Ref. 36), or conversely from a significant decrease in the number of patients who respond (Ref. 33). This argument suggests that the T-cell proliferative responses that are often reported in asymptomatic individuals must result from long-lasting T-cell memory against HPV infection; however, there is little evidence to support this. Studying human volunteers after a natural primary infection with HPVs could address this important question: to establish both the longevity of HPV-specific

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Accession number: txt001smc; **Accession date:** 3 July 1998; **Last update: 19 October 1998**
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Table 2. Reported studies of human CD4⁺ T cells that are specific for human papillomavirus types 16 and 18 (HPV-16, HPV-18) (tab002smc)

Study and assay details[a]	Comments
(Ref. 30) Cubie et al. (1989) **Antigen:** HPV-16 E6 protein; HPV-18 E6 protein; HPV-16 E4 protein. **T-cell responses:** Women with CIN: 8/29. Controls: 3/15.	Low responses in T cells. No significant difference in T-cell responses between patients and controls. HPV types of patients not known. No specificity controls. No HPV typing.
(Ref. 31) Altmann et al. (1992) **Antigen:** HPV-16 E7 protein. HPV-16 E7 peptides. **T-cell responses:** HPV-16$^+$ controls: 2/3. HPV-16$^-$ controls: 0/2.	Normal donors only, who were either seropositive or negative for HPV-16 E4 and E7. CD4 T-cell clones isolated. Peptide epitopes were defined using pooled peptides. A fusion protein control (for GST) was used.
(Ref. 32) Strang et al. (1990) **Antigen:** HPV-16 E6 peptides. HPV-16 L1 peptides. **T-cell responses:** Several controls tested.	Normal donors only. T cells were HLA-DR restricted and HPV-16 specific.
(Ref. 36) Kadish et al. (1994) **Antigen:** HPV-16 E7 peptides. **T-cell responses:** Women with CIN: 12/42. Controls: 3/13.	Correlation between ongoing HPV infection and T-cell responses. 9/25 women who had CIN and were infected with HPV made a T-cell response compared with 3/17 women who had CIN and were HPV$^-$.
(Ref. 33) Luxton et al. (1996) **Antigen:** HPV-16 E7 protein. HPV-16 E7 peptides. **T-cell responses:** Women with CIN: 9/31. Women with CaCx: 0/5. Controls: 7/15.	No significant difference between T-cell responses in patients and controls. No significant association between HPV-16 infection and T-cell responses in CIN patients. Decreased T-cell responses in cancer patients.
(Ref. 38) de Gruijl et al. (1996) **Antigen:** HPV-16 E7 protein. HPV-16 E7 peptides. **T-cell responses:** Women with CIN and HPV-16$^+$: 15/26. Women with CIN and HPV-16$^-$: 0/15.	T-cell responses were most frequent in women with CIN3 who had persistent HPV-16 infection. Responses were also found in women with CIN3 who had cleared HPV-16 or had a fluctuating HPV-16 infection.
(Ref. 34) Shepherd et al. (1996) **Antigen:** HPV-16 L1 peptides. **T-cell responses:** Women with CIN: 26/41. Controls: 5/11.	T-cell responses were most prevalent in the CIN3 group who had HPV-16 infection. Responding T cells were CD4$^+$.
(Ref. 37) Kadish et al. (1997) **Antigen:** HPV-16 E6 peptides. HPV-16 E7 peptides. **T-cell responses:** Response to E6 peptides of women with CIN2: 32/49. Response to E7 peptides of women with CIN2: 21/48. Controls: not tested.	T-cell responses were associated with clearance and regression of HPV-associated CIN2 lesions. Controls for PHA and ConA were present. No controls for normal donors.
(Ref. 40) de Gruijl et al. (1998) **Antigen:** HPV-16 E7 peptides. **T-cell responses:** Women with CIN3 and HPV-16$^+$: 14/15. Women with CaCx and HPV-16$^+$: 7/15.	T-cell responses were measured only by IL-2 release. The highest frequency of responders were among women with CIN3 who had persistent HPV-16 infection. Responses were also found in women with CIN3 who had cleared HPV-16 (8/13). Decreased responses were found in women who had CaCx.

[a] Antigen = the antigen source used in in vitro assays to test for HPV-specific T-cell responses; T-cell responses = the numbers of people who had T cells that responded in vitro to HPV antigens and the types of people (including HPV status) who the T cells were derived from.

Abbreviations used: CaCx = cervical cancer; CD4 = a cell-surface receptor that is typically found on T-helper lymphocytes; CIN = cervical intraepithelial neoplasia, including all stages (1–3); CIN2 = CIN stage 2; CIN3 = CIN stage 3; ConA = concanavalin A, a lectin used to stimulate polyclonal T cells (T lymphocytes); E4, E6, E7, L1 = HPV proteins (E = early, L = late); GST = glutathione S-transferase; HLA-DR = a type of human leucocyte antigen, which is one of the human forms of the major histocompatibility complex (MHC) class II; IL-2 = interleukin 2; PHA = phytohaemagglutinin, a lectin used to stimulate polyclonal T cells.

https://doi.org/10.1017/S1462399498000210 Published online by Cambridge University Press

Accession number: txt001smc; **Accession date:** 3 July 1998; **Last update: 19 October 1998**
Archived previous version: no; **Replaced by revised version**: no

Table 3. Reported studies of human CD8+ cytotoxic T-cell responses that are specific for human papillomaviruses (HPVs) (tab003smc)

Study and assay details[a]	Comments
(Ref. 68) Ressing et al. (1995) **Re-stimulation:** HPV-16 E7 peptides bound to HLA-A*0201 on activated B cells or T2 cells. **Detection:** Peptide on B-LCL, or CaSki cells (HPV-16-transformed cervical keratinocytes). **T-cell specificity:** HPV-16 E7.	Controls: 6/9 responded. CTLs were $CD8^+$. HPV-16-peptide-specific CTLs recognised HPV-16 E7 expressed on CaSki cells. CTL responses were likely to be primary responses (they were obtained from healthy donors who had no HPV disease).
(Ref. 50) Ressing et al. (1996) **Re-stimulation:** HPV-16 E7 peptides bound to HLA-A*0201 on PBMCs. **Detection:** Peptide on B-LCL, or CaSki cells. **T-cell specificity:** HPV-16 E7.	Women with CIN3 and who were $HPV\text{-}16^+$: 2/11 responded. Women with CaCx and who were $HPV\text{-}16^+$: 2/11 responded. Controls: 0/10 responded. CTL responses in patients were likely to be memory-CTL responses because the donors were $HPV\text{-}16^+$. CTL responses against influenza M1 peptide decreased in CaCx patients compared with women with CIN3 and controls.
(Ref. 53) Evans et al. (1996) **Re-stimulation:** CaSki cells ($HLA\text{-}A*0201^-$). **Detection:** HPV-16 E6 or E7 genes transfected into C33A cells or CaSki cells. **T-cell specificity:** HPV-16 E7 and E6.	Women with CIN2 and who were $HPV\text{-}16^+$: 1/11 responded. No normal controls tested.
(Ref. 51) Evans et al. (1997) **Re-stimulation:** HPV-16 E7 peptides [or mitogen (PHA)] bound to HLA-A*0201 on PBMCs. **Detection:** Peptide on B-LCL, or B-LCL cells infected with r vaccinia-HPV (expressing early proteins), or Caski cells. **T-cell specificity:** HPV-16 E7. HPV-16 E6/E7. HPV-18 E6/E7.	Women with CaCx and who were $HPV\text{-}16^+$ and $HPV\text{-}31^+$: 4/5 responded. Controls: 0/4 responded. After using peptides to stimulate CTLs in vitro, T-cell responses were detected against HPV-16 E7 peptides. 3/4 CaCx patients were tested for HPVs; 2 were $HPV\text{-}16^+$, 1 was $HPV\text{-}31^+$. HPV-16-peptide-specific CTLs recognise B-LCL infected with r vaccinia-HPV. CTLs recognising full-length HPV-16 E6/E7 or HPV-18 E6/E7 could be detected among tumour-infiltrating lymphocytes.
(Ref. 55) Nimako et al. (1997) **Re-stimulation:** HPV-16 E6/E7 or HPV-18 E6/E7 protein (expressed in recombinant adenoviruses) on multiple HLA types on PHA blasts. **Detection:** B-LCL infected by r vaccinia-HPV (expressing early proteins), or Caski cells. **T-cell specificity:** HPV-16 E6/E7. HPV-18 E6/E7.	Women with CIN3 and who were $HPV\text{-}16^+$: 6/10 responded. Controls: 0/10 responded. The HPV-specific CTLs obtained recognised both r vaccinia-HPV and CaSki. Three of the women with CIN3 had evidence of infection with HPV-16. Multiple HLA class I restriction patterns were seen.
(Ref. 52) Jochmus et al. (1997) **Re-stimulation:** HPV-16 E7 peptides bound to HLA-A*0201 on dendritic cells (as APCs). **Detection:** HPV-16 E7 peptides or SIHA cells and CaSki cells. **T-cell specificity:** HPV-16 E7.	Controls: 1/1 responded. Woman with CIN3 and who were $HPV\text{-}16^+$: 1/1 responded. CTLs specific for HPV-16 E7 peptide could be derived using only dendritic cells as APCs. Likely to be a primary CTL response. CTLs were unable to recognise full-length HPV-16 E7 protein.
(Ref. 54) Nakagawa et al. (1997) **Re-stimulation:** HPV-16 E6 and E7 proteins on multiple HLAs on PBMCs. **Detection:** B-LCL infected with r vaccinia-HPV (expressing early proteins). **T-cell specificity:** HPV-16 E7. HPV-16 E6.	Women who were $HPV\text{-}16^+$ but did not have CIN: 6/8 responded. Women with CIN and who were $HPV\text{-}16^+$: 2/7 responded. HPV-specific CTL responses occurred more frequently in HPV-16-infected patients without CIN.

(continued)

https://doi.org/10.1017/S1462399498000210 Published online by Cambridge University Press

Accession number: txt001smc; **Accession date:** 3 July 1998; **Last update: 19 October 1998**
Archived previous version: no; **Replaced by revised version**: no

Table 3. Reported studies of human CD8⁺ cytotoxic T-cell responses that are specific for human papillomaviruses (HPVs) (tab003smc) (continued)

Study and assay details[a]	Comments
(Ref. 69) Konya et al. (1997) **Re-stimulation:** HPV-16 E2 peptides binding to HLA-A*0201 on PBMCs as APCs. **Detection:** HPV-16 E2 peptides or HPV-16-transfected cell line. **T-cell specificity:** HPV-16 E2.	Controls: 2/3 responded. CTLs that were specific for HPV-16 E2 peptide could recognise full-length HPV-16 E2. CTL responses were likely to be primary responses because they were obtained from healthy donors without HPV disease.

[a] Re-stimulation = source of HPV-derived antigen (or mitogen) used to re-stimulate (or stimulate) responding T cells, and source of antigen-presenting cells and their HLA type; Detection = source of HPV-derived antigen, and source of target cells; T-cell specificity = HPV-derived antigen that the responding T cells were specific for.

Abbreviations used: APC = antigen-presenting cell; B cells = B lymphocytes; B-LCL = B-lymphoblastoid cell line (B cells transformed by Epstein–Barr virus); CaCx = cervical cancer; CaSki = HPV-16-transformed cervical keratinocyte line, which expresses HLA-A*0201; CD8 = cell-surface receptor characteristic of cytotoxic T cells (CTLs); CIN = cervical intraepithelial neoplasia; CIN3 = cervical intraepithelial neoplasia stage 3, the most advanced stage before invasion; C33A = cervical keratinocyte line, which is HPV16⁻ and HLA-A*0201⁺; E2, E6, E7 = HPV early proteins; HLA = human leukocyte antigen, which is the human form of the major histocompatibility complex (MHC); PHA blasts = PBMCs stimulated by PHA; PBMCs = peripheral blood mononuclear cells; PHA = phytohaemagglutinin; r vaccinia-HPV = recombinant vaccinia virus expressing HPV proteins; SIHA = HPV-16-transformed cervical keratinocyte cell line; T2 = human cell line that is defective for antigen processing but good at presenting peptides.

immunity (measuring antibodies, CD4⁺ and CD8⁺ T-cell responses) and its role in the development of cervical neoplasia. However, setting up such studies will be ethically and technically difficult and would require a long-term follow-up period.

Cytokine production

CMI is regulated by cytokines that are produced by CD4⁺ T-helper cells. The major cytokines associated with the development of antiviral CMI are interleukin 2 (IL-2) and interferon γ (IFN-γ), whereas cytokines such as interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 10 (IL-10) and interleukin 13 (IL-13) are thought to inhibit the development of CMI. However, few studies have addressed the production of specific cytokines by HPV-specific CD4⁺ T cells. Clerici and colleagues (Ref. 39) measured cytokine release by PBMCs after stimulation with soluble influenza antigens, allogeneic APCs or mitogens. They studied patients who had either extensive HPV infection (HPV DNA was found at multiple sites of the lower genital tract) or localised HPV infection (HPV DNA was found only in the cervix). There was a significant difference between the levels of cytokines produced by patients who had extensive HPV disease and by those who had localised HPV disease. Decreased levels of IL-2 production were found in patients who had extensive HPV disease, compared with those who had localised HPV disease or those in control groups. By contrast, elevated levels of IL-4 and IL-10 were found in the patient group that had extensive disease, compared with those with only localised HPV disease or no HPV disease (controls). This study, which was performed on a small group (30) of patients using non-HPV antigens, suggests that there is a shift towards increased T-cell production of cytokines (IL-10, IL-4) that are associated with the suppression of CMI. A more recent study (examining IL-2 production by patient-derived T cells in response to HPV-16 E7-derived peptides) suggests that the generation of T-cell responses is dependent on additional factors (Ref. 40). No significant responses were observed from T cells derived from: (1) umbilical cord blood, (2) patients with evidence of HPV-16 infection but without CIN, or (3) patients with CIN but no evidence of HPV infection. Interestingly, significant responses were observed in patients with CIN who had persistent HPV infection, and also to a significantly lesser extent in patients with CaCx. On follow-up, patients who cleared HPV-16 [measured using the polymerase chain reaction (PCR)] were still able to make a positive IL-2 response; however, this decreased with time after clearance of HPV infection. This might suggest a role for THLs in

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Accession number: txt001smc; **Accession date:** 3 July 1998; **Last update: 19 October 1998**
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the clearance of HPVs, but this interpretation must be reconciled with the increased T-helper activity found in patients with persistent HPV infections. This study illustrates the difficulties in determining whether HPV-specific T cells play any role in disease, and suggests that additional (immune and non-immune) parameters will need to be studied to gain a clearer picture.

Because immune responses are regulated by HLA molecules, it is possible that possession of certain HLA class II alleles might contribute to disease susceptibility. This has been extensively studied in patients with CIN and CaCx; however, few studies have reported significantly increased or decreased frequencies of HLA alleles in patient groups compared with controls (Ref. 41).

$CD8^+$ T-cell responses

HPV-specific CTLs can be readily induced in rodent models after injection of the animals with recombinant viruses containing HPV genes (Ref. 42) or rodent cells transfected with HPV genes (Ref. 43). Furthermore, such HPV-specific CTLs can protect against subsequent challenge with tumour cells transfected with HPV genes (Refs 43, 44). This has driven the search in humans for so-called 'naturally occurring' CTL responses. Until recently the results have been disappointing, with a consensus that HPVs are able to evade the human immune system and establish a state of immune tolerance (Refs 45, 46). This view did not, however, hinder the development of vaccines designed to elicit HPV-specific immunity. It is noteworthy that early clinical trials of such vaccines were set up in the absence of any data that CMI responses occurred naturally in human HPV infection or, indeed, were relevant to human cervical disease. Three clinical trials involving patients with advanced CaCx have been set up using: (1) HPV-derived peptides (Ref. 47), (2) HPV-derived soluble proteins (Ref. 48), or (3) recombinant vaccinia viruses expressing HPV proteins (Ref. 49). Although the clinical results or immune responses from these trials have not been extensively reported in the literature, it is clear from at least two of the trials that HPV antigens can be immunogenic in humans, inducing a T-cell response (Refs 48, 49). All of the trials were performed on groups of patients with advanced CaCx, making assessment of 'natural' T-cell immunity in the absence of the vaccine difficult. More recently, there has been significant progress with the first reports that HPV-specific human CTLs can occur naturally (Table 3, tab003smc). Ressing and co-workers (Ref. 50) demonstrated that peptides derived from HPV-16 E7, which were able to bind to HLA-A*0201 (the commonest HLA class I allele among Caucasians), were capable of inducing peptide-specific CTLs in vitro from normal donors. Some of these CTLs were able to recognise a cell line derived from human cervical carcinoma that had been transformed by HPV-16 and was expressing HLA-A*0201; this suggests that at least some of the CTLs that were stimulated by peptides in vitro also recognised naturally processed HPV epitopes. This approach was extended to the study of patients with CIN3 and CaCx, and showed that the same peptides were recognised by T cells present in PBMCs (Refs 50, 51) and also by T cells isolated from cervical biopsy specimens (Ref. 51). By contrast, another study (Ref. 52), which used the same peptides, was unable to detect CTLs among patients unless populations of APCs that were enriched for dendritic cells were used for T-cell stimulation in vitro. This study raises the possibility that the peptides that are selected for re-stimulation in vitro might not be truly representative of the HPV epitopes that are actually expressed in vivo as a consequence of HPV-associated disease. Another disadvantage of the peptide re-stimulation approach is that for technical reasons the HPV responses examined will be restricted to a single (or only a few) HLA allele(s). Evans and co-workers (Ref. 53) have used HPV-16-transformed CaSki cells (an HPV-transformed cervical keratinocyte line, which expresses HLA-A*0201) for re-stimulation of PBMCs in vitro, rather than peptides. This approach should re-stimulate CTLs with naturally processed HPV epitopes; however, the use of a tumour cell line that has not been derived from the same person as the responder cells were derived from can result in responses preferentially directed against allogeneic MHC molecules, rather than HPV antigens. This might, in part, explain the low numbers of patients (9%) who were able to mount an HPV-specific CTL response in this study. An alternative approach to re-stimulation with HPV-derived peptides (or tumour cells) is to use full-length HPV proteins, either in a soluble form (Ref. 54) or expressed by recombinant viral vectors (Ref. 55). Using the latter approach (recombinant adenoviruses expressing full-length HPV-16 or HPV-18 E6/E7), naturally occurring HPV-specific CTLs were

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found in the PBMCs of most (6/10) of the patients with CIN3 tested (Ref. 55). Some of the CTLs generated were also able to lyse the HPV-transformed CaSki target cells, and CTLs could be detected at multiple time points over an eight-month period. The CTL responses also appeared to be disease associated, because no responses were detected among normal controls. Further testing of the MHC restriction of the CTL lines from one patient revealed that HPV antigens could be recognised in the context of at least three different HLA class I alleles. These data suggest that the natural CTL response against HPVs encompasses a broad range of MHC- and HPV-antigen specificities. A high CTL response rate (6/8) was also observed using soluble HPV-16 E6 and E7 proteins (Ref. 54); however, it was not clear whether these responses represented $CD8^+$ or $CD4^+$ CTLs. The CTL responses were most frequent among individuals who were HPV-16^+ (by PCR typing) with no CIN; controls who were HPV-16^- with no CIN were not tested.

Overall, the studies of CTL function in humans with HPV-associated cervical disease suggest that naturally occurring HPV-specific CTLs do occur, although with variable detection rates (resulting from the differing methodologies employed). Encouragingly (and unlike the studies of $CD4^+$ T-cell function in human HPV-associated cervical neoplasia), responses in 'normal' asymptomatic subjects appear to be rare, suggesting the CTL responses are associated with HPV disease. However, these preliminary studies have not addressed whether these CTLs have any role in the prevention, control or treatment of disease.

Clinical implications/applications

The in vitro assays described above suggest that HPV proteins are immunogenic in humans both to $CD4^+$ T cells and to $CD8^+$ T cells. From epidemiological studies, it is clear that most individuals who encounter 'genital' HPV types are able to clear them without further complications (Ref. 8). It is not known whether CMI plays any part in this clearance, largely because immune parameters after primary HPV infection have not been studied. Interestingly, it is known that among women with pre-invasive cervical neoplasia (specifically, CIN3) spontaneous regression of lesions occurs in ~30% of cases (Ref. 56). However, we do not know whether HPV-specific CMI plays any role in this regression, or whether the absence of an HPV-specific CMI response predisposes towards the development of cancer. Carefully controlled studies of large cohorts of women who have been exposed to HPV and exhibit stages of cervical disease that are well characterised are needed; furthermore, these studies will require long-term follow-up. It is also possible that CMI is important in both the control of disease and susceptibility towards cancer without being directed against HPV itself; alternatively, other types of CMI (such as that mediated by NK cells or γδ T cells, which are a subset of T cells) might play an equally or more important role. Paradoxically, HPV-specific CMI (both $CD4^+$ and $CD8^+$ T-cell responses) has been most consistently found in patients with persistent CIN3 or cancer (Refs 36, 37, 38, 40, 50, 51, 55). It could be argued that this represents a failure of CMI to halt the progression of disease, and that these CMI responses have arisen as an indirect consequence of disease. Further studies will be required to resolve this; however, these are still likely to be limited by the need to use conventional assays of T-cell function, which require extensive in vitro culture. Recent advances in technology have increased considerably the sensitivity of detection of effector T cells in blood samples, allowing direct quantification and functional assessment with minimal in vitro handling (Ref. 57).

Another consideration of the available data on CMI against HPV is that they are largely sampled from PBMCs; T-cell responses in this cellular compartment might be different from those occurring at sites of disease. Recently, it has been shown that HPV-specific CTLs can be found among lymphocytes that are infiltrating cervical tumours (Ref. 51) and that the numbers of these CTLs are higher than those detected in PBMCs in the same individual. Histological analysis of tumour tissue has demonstrated an enrichment of activated $CD8^+$ CTLs, but it is not known whether these cells are HPV specific (Ref. 58).

The measurement of HPV-specific CMI in situ in the cervix is important, because there are several methods by which HPV-transformed cervical keratinocytes could evade immune recognition. First, it has been shown that reduced or absent expression of MHC class I molecules occurs frequently in cervical tumour cells (Refs 59, 60). This is a possible mechanism for HPV-transformed keratinocytes to evade CTL responses; however, it is not known whether

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MHC class I downregulation is a direct consequence of immune selection (as seen for melanoma; Ref. 61), or simply the result of the genetic instability of tumour cells. What is clear is that downregulation of the MHC class I pathway does correlate with poorer prognosis for patients with CIN3 (Ref. 62) or CaCx (Ref. 63). Second, HPV-transformed keratinocytes might produce cytokines that suppress immune responses (Ref. 39) or proteins that can inactivate stimulatory cytokines (Ref. 64). Third, HPV-transformed keratinocytes might resist CTL killing, either by producing proteins that interfere with CTL-lytic mechanisms or by inducing apoptotic cell death in the CTLs themselves. For these reasons, it will be important in the future to measure CMI in tissue biopsies, although this will be technically more challenging. Such analyses will be vital to understanding the role of CMI in the natural history of HPV-associated cervical disease and for assessing the therapeutic potential of vaccines designed to induce HPV-specific CTLs.

Human antiviral CTLs have been shown to have powerful therapeutic effects both against infectious virus (Ref. 65) and against virally induced tumours (Refs 66, 67). Regardless of the natural role of HPV-specific CMI against HPVs, there remains considerable optimism that human immune responses against HPVs can also be harnessed for the therapy of CaCx. Continuing studies of the interplay between HPV-transformed keratinocytes and HPV-specific CTLs (both in vitro and at sites of disease) will allow the rational design of immunotherapies. These immunotherapeutic approaches, in combination with conventional treatments and prophylaxis, should aim to diminish the global impact of CaCx.

Research in progress and outstanding research questions

HPV-specific killing of cervical tumour cells by CTLs

For successful immunotherapy of CaCx, HPV-specific CTLs must be able to destroy cervical tumours. The studies described above have used either synthetic peptides or recombinant viral vectors to re-stimulate HPV-specific CTLs in vitro. It is not known whether these methods will mimic adequately the HPV antigens displayed on cervical tumour cells, such that the CTLs generated can kill tumour cells.

Interactions at the sites of disease between HPV-transformed cervical keratinocytes and T cells

It is known that downregulation of MHC class I molecules frequently occurs in CaCx, but it is not known whether this downregulation has a functional impact on $CD8^+$ CTL recognition of tumour cells. For example, does downregulation of HLA-A2 in cervical tumour cells effect the recognition of tumour cells by CTLs that are restricted by HLA-A2? If this is so, then CTLs that are not restricted by HLA-A2 should be selected for use in immunotherapy.

Cytokine regulation of the immune response

Activated CTLs can be found among lymphocytes infiltrating cervical tumours. Do cytokines, or other mechanisms, suppress the action of these CTLs? If the interactions between T cells and cervical keratinocytes are understood, it might be possible to circumvent the effects of MHC downregulation (or immunosuppression) in the cervix, for example by local delivery of cytokines.

HPV-specific CTLs and immunotherapy of cervical cancer

Promising results in other systems suggest that HPV-specific CTLs might be effective in the immunotherapy of CaCx. Ongoing vaccine trials (designed to elicit HPV-specific CTLs in vivo) are not yet measuring clinical responses (tumour regression). Results from immunological assays will not be known for 2–3 years. Subject to answering the questions posed above, the most direct method of testing the concept that $CD8^+$ CTLs are important in the immunotherapy of CaCx is to infuse patients who have CaCx with large numbers of (autologous) HPV-specific CTLs that have been generated in vitro. As fundamental research continues, it is increasingly likely that this approach will be attempted within the next five years.

Prophylactic vaccination, design and testing

Considerable effort (by academic groups and industry) is now being spent on the development of vaccines against HPVs, some of which are being tested in clinical trials. In brief, two main approaches are being taken: the vaccination of individuals therapeutically to control or eliminate an existing infection (and/or cancer), or the development of prophylactic vaccines to protect an individual from acquiring HPV disease (and HPV-related cancer).

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The therapeutic vaccine approaches are based on the fact that E6 and E7 proteins of the high-risk HPV types are continually expressed in the transformed cells; as such, they are potential tumour-specific target antigens for an immune response, particularly CMI responses, such as CTLs. Several strategies are being used to try to induce these responses including: (1) recombinant vaccinia vectors that express E6 and E7, (2) soluble recombinant proteins with adjuvant, or (3) synthetic peptides that represent predicted CTL epitopes (for example, delivered parenterally or coated onto dendritic cells).

Attempts to generate effective prophylactic vaccines to prevent HPV infection are focusing on mechanisms to induce long-lasting mucosal immunity directed against L1 and L2, the major structural proteins of HPVs. The strategies being tried include immunisation with DNA (DNA vaccines) or the highly immunogenic VLPs. In VLPs, L1 is expressed with or without L2 (in eukaryotic or, in some cases, prokaryotic systems); these proteins self-assemble into 'empty' virus particles (that do not contain any viral DNA) consisting of proteins in their correct conformation (as found in the virus).

Acknowledgements and funding

Funding for Stephen Man's research has been provided, in part, by the Royal Society (RS University Research Fellowship) and the Higher Education Funding Council of Wales (HEFCW). The author is grateful to Hetty Bontkes (Department of Pathology, Free University Hospital, Amsterdam, The Netherlands) and Dr Alison Fiander (Department of Obstetrics and Gynaecology, Llandough Hospital, Penarth, Wales) for critically reviewing this manuscript.

References

1 Boyle, P. (1997) Global burden of cancer. Lancet 349 (suppl. II), 23–26
2 Cannistra, S.A. and Niloff, J.M. (1996) Cancer of the uterine cervix. N. Engl. J. Med. 334, 1030–1038
3 Forsmo, S. et al. (1997) Treatment of pre-invasive conditions during opportunistic screening and its effectiveness on cervical cancer incidence in one Norwegian country. Int. J. Cancer 71, 4–8
4 Soutter, W.P. et al. (1997) Invasive cervical cancer after conservative therapy for cervical intraepithelial neoplasia [see comments]. Lancet 349, 978–980
5 Stockton, D., Cooper, P. and Lonsdale, R. (1997) Changing incidence of invasive adenocarcinoma of the uterine cervix in East Anglia. J. Med. Screen. 4, 40–43
6 Frazer, I.H. (1996) Immunology of papillomavirus infection. Curr. Opin. Immunol. 8, 484–491
7 Suzich, J.A. et al. (1995) Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. Proc. Natl. Acad. Sci. USA 92, 11553–11557
8 Ho, G.Y.F. et al. (1998) Natural history of cervicovaginal papillomavirus infection in young women. N. Engl. J. Med. 338, 423–428
9 Halpert, R. et al. (1986) Human papillomavirus and lower genital neoplasia in renal transplant patients. Obstet. Gynecol. 68, 251–258
10 Petry, K.U. et al. (1994) Cellular immunodeficiency enhances the progression of human papillomavirus-associated cervical lesions. Int. J. Cancer 57, 836–840
11 Coleman, N. et al. (1994) Immunological events in regressing genital warts. Am. J. Clin. Pathol. 102, 768–774
12 Jarrett, W.F. et al. (1991) Studies on vaccination against papillomaviruses: prophylactic and therapeutic vaccination with recombinant structural proteins. Virology 184, 33–42
13 Knowles, G., O'Neil, B.W. and Campo, M.S. (1996) Phenotypical characterization of lymphocytes infiltrating regressing papillomas. J. Virol. 70, 8451–8458
14 Selvakumar, R. et al. (1997) Regression of papillomas induced by cottontail rabbit papillomavirus is associated with infiltration of $CD8^+$ cells and persistence of viral DNA after regression. J. Virol. 71, 5540–5548
15 Tosi, P. et al. (1992) Prognostic factors in invasive cervical carcinomas associated with human papillomavirus (HPV). Path. Res. Pract. 188, 866–873
16 zur Hausen, H. and de Villiers, E.M. (1994) Human papillomaviruses. Annu. Rev. Microbiol. 48, 427–447
17 Crook, T. et al. (1989) Continued expression of HPV-16 E7 protein is required for maintenance of the transformed phenotype of cells co-transformed by HPV-16 plus EJ-ras. EMBO J. 8, 513–519
18 Vousden, K.H. (1994) Interactions between papillomavirus proteins and tumor suppressor gene products. Adv. Cancer Res. 64, 1–24
19 Bal, V. et al. (1990) Antigen presentation by keratinocytes induces tolerance in human T cells. Eur. J. Immunol. 20, 1893–1897

https://doi.org/10.1017/S1462399498000210 Published online by Cambridge University Press

20 Ramensee, H. (1995) Chemistry of peptides associated with MHC class I and class II molecules. Curr. Opin. Immunol. 7, 85–96
21 Glew, S.S. et al. (1992) HLA class II antigen expression in human papillomavirus-associated cervical cancer. Cancer Res. 52, 4009–4016
22 Ferguson, A. et al. (1985) Expression of MHC products and leucocyte differentiation antigens in gynaecological neoplasms: an immunohistological analysis of the tumour cells and infiltrating leucocytes. Br. J. Cancer 52, 551–563
23 Coleman, N. and Stanley, M.A. (1994) Analysis of HLA-DR expression on keratinocytes in cervical neoplasia. Int. J. Cancer 56, 314–319
24 Tay, S.K. et al. (1987) Subpopulations of Langerhans cells in cervical neoplasia. Br. J. Obstet. Gynaecol. 94, 10–15
25 Jaraquemada, D., Marti, M. and Long, E.O. (1990) An endogenous processing pathway in vaccinia virus-infected cells for presentation of cytoplasmic antigens to class II-restricted T cells. J. Exp. Med. 172, 947–954
26 Nuchtern, J.G., Biddison, W.E. and Klausner, R.D. (1990) Class II MHC molecules can use the endogenous pathway of antigen presentation. Nature 343, 74–76
27 Carbone, F.R. and Bevan, M.J. (1990) Class I-restricted processing and presentation of exogenous cell-associated antigen in vivo. J. Exp. Med. 171, 377–387
28 Speidel, K. et al. (1997) Priming of cytotoxic T lymphocytes by five heat-aggregated antigens in vivo: conditions, efficiency, and relation to antibody responses. Eur. J. Immunol. 27, 2391–2399
29 Lee, A.K. and Eisinger, M. (1976) Cell-mediated immunity (CMI) to human wart virus and wart-associated tissue antigens. Clin. Exp. Immunol. 26, 419–424
30 Cubie, H.A. et al. (1989) Lymphoproliferative response to fusion proteins of human papillomaviruses in patients with cervical intraepithelial neoplasia. Epidemiol. Infect. 103, 625–632
31 Altmann, A. et al. (1992) Definition of immunogenic determinants of the human papillomavirus type 16 nucleoprotein E7. Eur. J. Cancer 28, 326–333
32 Strang, G. et al. (1990) Human T cell responses to human papillomavirus type 16 L1 and E6 synthetic peptides: identification of T cell determinants, HLA-DR restriction and virus type specificity. J. Gen. Virol. 71, 423–431
33 Luxton, J.C. et al. (1996) Proliferative T cell responses to the human papillomavirus type 16 E7 protein in women with cervical dysplasia and cervical carcinoma and in healthy individuals. J. Gen. Virol. 77, 1585–1593
34 Shepherd, P.S. et al. (1996) Proliferative T cell responses to human papillomavirus type 16 L1 peptides in patients with cervical dysplasia. J. Gen. Virol. 77, 593–602
35 Luxton, J.C. et al. (1997) Serological and T helper cell responses to human papillomavirus type 16 L1 in women with cervical dysplasia or cervical carcinoma and in healthy controls. J. Gen. Virol. 78, 917–923
36 Kadish, A.S. et al. (1994) Cell-mediated immune responses to E7 peptides of human papillomavirus (HPV) type 16 are dependent on the HPV type infecting the cervix whereas serological reactivity is not type-specific. J. Gen. Virol. 75, 2277–2284
37 Kadish, A.S. et al. (1997) Lymphoproliferative responses to human papillomavirus (HPV) type 16 proteins E6 and E7: outcome of HPV infection and associated neoplasia. J. Natl. Cancer Inst. 89, 1285–1293
38 de Gruijl, T.D. et al. (1996) T-cell proliferative responses against human papillomavirus type 16 E7 oncoprotein are most prominent in cervical intraepithelial neoplasia patients with a persistent viral infection. J. Gen. Virol. 77, 2183–2191
39 Clerici, M. et al. (1997) Cytokine production patterns in cervical intraepithelial neoplasia: association with human papillomavirus infection. J. Natl. Cancer Inst. 89, 245–250
40 de Gruijl, T.D. (1998) Differential T helper cell responses to human papillomavirus type 16 E7 related to viral clearance or persistence in patients with cervical neoplasia: a longitudinal study. Cancer Res. 58, 1700–1706
41 Stern, P.L. (1996) Immunity to human papillomavirus-associated cervical neoplasia. Adv. Cancer Res. 69, 175–211
42 Meneguzzi, G. et al. (1991) Immunization against human papillomavirus type 16 tumor cells with recombinant vaccinia viruses expressing E6 and E7. Virology 181, 62–69
43 Chen, L.P. et al. (1991) Human papillomavirus type 16 nucleoprotein E7 is a tumor rejection antigen. Proc. Natl. Acad. Sci. USA 88, 110–114
44 Chen, L. et al. (1992) Induction of cytotoxic T lymphocytes specific for a syngeneic tumor expressing the E6 oncoprotein of human papillomavirus type 16. J. Immunol. 148, 2617–2621

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45 Beverley, P.C. et al. (1994) Strategies for studying mouse and human immune responses to human papillomavirus type 16. In Ciba Found. Symp. 187, pp. 78–86, Wiley, Chichester, UK
46 Tindle, R.W. and Frazer, I.H. (1994) Immune response to human papillomaviruses and the prospects for human papillomavirus-specific immunisation. Curr. Top. Microbiol. Immunol. 186, 217–253
47 Melief, C.J.M. et al. (1996) Peptide based cancer vaccines. Curr. Opin. Immunol. 8, 651–657
48 Tindle, R.W. (1996) Human papillomavirus vaccines for cervical cancer. Curr. Opin. Immunol. 8, 643–650
49 Borysiewicz, L.K. et al. (1996) A recombinant vaccinia virus encoding human papillomavirus type 16 and type 18, E6 and E7 proteins as immunotherapy for cervical cancer. Lancet 347, 1523–1527
50 Ressing, M.E. et al. (1996) Occasional memory cytotoxic T-cell responses of patients with human papillomavirus type 16-positive cervical lesions against a human leukocyte antigen-A*0201-restricted E7-encoded epitope. Cancer Res. 56, 582–588
51 Evans, E.M. et al. (1997) Infiltration of cervical cancer tissue with human papillomavirus-specific cytotoxic T-lymphocytes. Cancer Res. 57, 2943–2950
52 Jochmus, I. et al. (1997) Specificity of human cytotoxic T lymphocytes induced by a human papillomavirus type 16 E7 derived peptide. J. Gen. Virol. 78, 1689–1695
53 Evans, C. et al. (1996) HLA-A2-restricted peripheral blood cytolytic T lymphocyte response to HPV type 16 proteins E6 and E7 from patients with neoplastic cervical lesions. Cancer Immunol. Immunother. 42, 151–160
54 Nakagawa, M. et al. (1997) Cytotoxic T lymphocyte responses to E6 and E7 proteins of human papillomavirus type 16: relationship to cervical intraepithelial neoplasia. J. Infect. Dis. 175, 927–931
55 Nimako, M. et al. (1997) Human papillomavirus-specific cytotoxic T lymphocytes in patients with cervical intraepithelial neoplasia grade III. Cancer Res. 57, 4855–4861
56 Ostor, A.G. (1993) Natural history of cervical intraepithelial neoplasia: a critical review. Int. J. Gynecol. Pathol. 12, 186–192
57 McMichael, A. and O'Callaghan, C. (1998) A new look at T cells. J. Exp. Med. 187, 1367–1371
58 Bontkes, H.J. et al. (1997) Assessment of cytotoxic T-lymphocyte phenotype using the specific markers granzyme B and TIA-1 in cervical neoplastic lesions. Br. J. Cancer 76, 1353–1360
59 Connor, M.E. and Stern, P.L. (1990) Loss of MHC class-I expression in cervical carcinomas. Int. J. Cancer 46, 1029–1034
60 Cromme, F.V. et al. (1994) Loss of transporter protein, encoded by the TAP-1 gene, is highly correlated with loss of HLA expression in cervical carcinomas. J. Exp. Med. 179, 335–340
61 Ikeda, H. et al. (1997) Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. Immunity 6, 199–208
62 Bontkes, H. et al. (1998) Specific HLA class I downregulation is an early event in cervical dysplasia associated with clinical progression. Lancet 351, 187–188
63 Ellis, J.R. et al. (1995) The association of an HPV16 oncogene variant with HLA-B7 has implications for vaccine design in cervical cancer. Nat. Med. 1, 464–470
64 Smith, G. (1996) Virus proteins that bind cytokines, chemokines or interferons. Curr. Opin. Immunol. 8, 467–471
65 Riddell, S.R. et al. (1992) Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. Science 257, 238–241
66 Papadopoulos, E.B. et al. (1994) Infusions of donor leukocytes to treat Epstein Barr virus associated lymphoproliferative disorders after allogeneic bone marrow transplantation. N. Engl. J. Med. 330, 1185–1191
67 Rooney, C.M. et al. (1995) Use of gene-modified virus-specific T lymphocytes to control Epstein–Barr-virus-related lymphoproliferation. Lancet 345, 9–13
68 Ressing, M.E. et al. (1995) Human CTL epitopes encoded by human papillomavirus type 16 E6 and E7 identified through in vivo and in vitro immunogenicity studies of HLA-A*0201 binding peptides. J. Immunol. 154, 5934–5943
69 Konya, J. et al. (1997) Identification of a cytotoxic T lymphocyte epitope in the human papillomavirus type 16 E2 protein. J. Gen. Virol. 78, 2615–2620

https://doi.org/10.1017/S1462399498000210 Published online by Cambridge University Press

Accession number: txt001smc; **Accession date:** 3 July 1998; **Last update: 19 October 1998**
Archived previous version: no; **Replaced by revised version**: no

Further reading, other resources and other contacts

Cancer and cervical cancer
Oncolink, for information on cancer, including cervical cancer (CaCx); this includes a 'lecture' entitled 'Neoplasia of the female lower genital tract', which contains schematic diagrams and photographs.
http://www.oncolink.upenn.edu/specialty/gyn_onc/cervical/

The US National Institutes of Health has information on CaCx.
http://text.nlm.nih.gov/nih/upload-v3/CDC_Statements/Cervical/cervical.html

Human papillomavirus and virology
All the Virology on the Web contains a large collection of information about viruses, including photomicrographs of HPVs.
http://www.tulane.edu/~dsander/garryfavweb.html
UK mirror site: http://www-micro.msb.le.ac.uk/garryfavweb/garryfavweb2.html

The Human Papillomaviruses Database at Los Alamos National Laboratory contains nucleic acid and amino acid information.
http://hpv-web.lanl.gov/

Tables
Table 1. Currently available in vitro methods for stimulating T cells that are specific for human papillomaviruses (HPVs) (tab001smc).
Table 2. Reported studies of human $CD4^+$ T cells that are specific for human papillomavirus types 16 and 18 (HPV-16, HPV-18) (tab002smc).
Table 3. Reported studies of human $CD8^+$ cytotoxic T-cell responses that are specific for human papillomaviruses (HPVs) (tab003smc).

Schematic figures
Figure 1. Human papillomavirus (HPV) infection and replication in cervical epithelial cells (fig001smc).
Figure 2. Simplified organisation (linearised) of the genome of human papillomavirus type 16 (HPV-16) (fig002smc).
Figure 3. Degradation and transport of antigens that bind major histocompatibility complex (MHC) class I molecules (fig003smc).
Figure 4. Degradation and transport of antigens that bind major histocompatibility complex (MHC) class II molecules (fig004smc).

Animations
Degradation and transport of antigens that bind major histocompatibility complex (MHC) class I molecules (swf001smc).
Degradation and transport of antigens that bind major histocompatibility complex (MHC) class II molecules (swf002smc).

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