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HPV vaccines: the beginning of the end for cervical cancer

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Vaccines prophylactic against infection with human papillomavirus (HPV) are based on alum adjuvanted virus-like particles. Two such vaccines have recently been shown to prevent persistent HPV infection and associated cervical cancer precursor lesions. The genotype-specific neutralising antibody directed at conformational epitopes of the L1 major capsid protein is likely to mediate protection. Vaccines therapeutic for persisting HPV infection can eliminate transplantable tumors in animal models, but are of limited efficacy in mice grafted with skin that expresses HPV antigens or in humans. This paradox has been partially resolved by data clarifying the immunoregulatory role of skin cytokines (e.g. transforming growth factor- β and interleukin-10) and the consequences of antigen presentation by subsets of skin-associated antigen-presenting cells.

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Introduction

An association between infection with a subset of human papillomaviruses (HPVs) and cervical cancer was first postulated in the late 1970s [1]. Formulation of this hypothesis was facilitated by the knowledge that animal papillomaviruses promote cancer, as well as identification of an epidemiological association of cervical cancer with sexual activity and recognition through cloning and sequencing that there were many different HPVs including some that infect the genital tract. Molecular mechanisms used by high-risk mucosotropic HPVs for promotion of cancer, reviewed in [2], have to a large extent been defined (Box 1). Their action has been shown to require persisting expression of two viral non-structural proteins (E6 and E7), which together inhibit differentiation and apoptotic death of epithelial cells (the only cells permissive for viral replication) and promote development of heritable somatic mutations in the infected epithelial cell.

Epidemiological evidence reviewed in [3] has confirmed that >95% of cervical cancer, and a significant proportion of other anogenital cancer, is associated with persisting infection with one of about ten high-risk HPVs. Persisting infection is unusual, occurring in <5% of healthy young women and a rather higher proportion of immunocompromised HIV-positive subjects [4[•]]. These latter data suggest that host immune responses are a major determinant of persisting infection, although other underlying viral, genetic and environmental factors are also likely to contribute. The exact nature of the immune response accountable for clearance of infection is uncertain, although direct and indirect evidence suggest that CD4 responses to viral non-structural proteins are important [5[•],6].

This review focuses on recent exciting developments in the prophylactic HPV vaccine for prevention of cervical cancer, and then examines the complexities of establishing a therapeutic vaccine with reference to recent literature on the basic biology of epithelial tumour immunity.

Prophylactic vaccines

Vaccines to protect against HPV infection and the subsequent risk of cervical cancer (Box 2) have recently become available, based on virus-like particle (VLP) technology [7,8]. VLPs are produced by expressing the capsid proteins of the virus (L1 with or without L2; Box 1) using recombinant DNA technology. When expressed in eukaryotic cells, the L1 major capsid protein self-assembles into 360-mer particles that physically and immunologically resemble the native virion. These highly immunogenic particles can, when administered as a vaccine with alum-based adjuvants, protect not only against infection with the HPV types incorporated in the vaccine [9^{••},10,11^{••}] and some cross-reactive HPV types but also against the consequent premalignant disease [12^{••}]. Protection is durable over at least five years [13], and the vaccine appears to protect nearly 100% of immunised subjects.

The VLP-based vaccines are not therapeutic, although they can safely be given to women already infected without altering the natural history of the infection. The basis of protection following vaccination is uncertain, because the 100% efficacy rates observed to date do not allow conclusions about possible surrogate markers of protection. However, animal studies have shown that protection correlates with production of virus-neutralising antibody, and passive transfer experiments in the beagle dog demonstrate that protection can be conveyed by serum raised against VLPs but not by serum raised against denatured capsid protein [14]. Thus, it is highly likely

Box 1 Human papillomaviruses.

Non-lytic species-specific dsDNA viruses that infect skin.

Require differentiating epithelium for vegetative reproduction.

Simple 7 kB genome (2 structural proteins, L1 and L2; 5–7 non-structural proteins, E1–7).

Promote epithelial cell proliferation, and inhibit cell differentiation and apoptosis.

Four broad families that contain more than 200 human genotypes:

- Skin-specific, non-oncogenic: produce warts (e.g. HPV 1, 2).
- Skin-specific, oncogenic: produce flat lesions and squamous cancer (e.g. HPV 5, 8).
- Mucosotropic, non-oncogenic: produce genital warts (e.g. HPV 6, 11).
- Mucosotropic, oncogenic (~20 genotypes): produce flat lesions and squamous/adenosquamous cancers (e.g. HPV 16, 18, 33, 45).

Infections are common (>50% lifetime risk of infection) and are slow to resolve, although persistence of clinical lesions after 5 years is rare (<5%) in immunocompetent subjects.

Genotypes are generally immunologically distinct.

Box 2 Human papillomaviruses and cancer.

Between 5% and 10% of all human cancer is a consequence of HPV infection.

Cancer is associated with persistent expression of viral non-structural proteins.

100% of cervical cancer is caused by HPV infection.

Other cancers caused by HPV include squamous cancers of the anogenital skin, some head and neck cancers, and some rare cancers at mucosal/squamous epithelial junctions.

The period between infection and cancer can be from 2 years to a lifetime (commonly 10–20 years).

Human papillomavirus-associated cancers commonly have an integrated viral genome and overexpress two viral proteins (E6 and E7).

E6 and E7 together can immortalise epithelial cells by defined mechanisms.

Animal papillomaviruses can produce cancer by a 'hit and run' mechanism.

Latent HPV infection in skin and around skin cancers is common, but the role for HPV infection in most skin cancers remains controversial.

Immunosuppression is associated with significantly increased incidence of HPV-associated cancer, including squamous cancer at non-mucosal surfaces.

that virus-neutralising antibody is the means of protection in humans also.

The vaccine trial data show that there is an anamnestic response to a single dose of vaccine in previously infected subjects [15], suggesting that VLPs and natural infection raise similar immune responses, although the immune response to viral capsid in natural infection is much weaker and many infected subjects do not respond at all. The immune response to the vaccine is much better in younger subjects, before the onset of puberty [16], confirming that the involution of the immune response at puberty impacts the response to vaccines against infections previously not encountered.

Therapeutic HPV vaccines

The prophylactic vaccines for HPV infection based on HPV L1 VLPs are not therapeutic for existing HPV-associated lesions [12••]. This is not unexpected as HPV L1 protein expression is limited to superficial epithelial cells (Figure 1) and is downregulated in clones of cells that are progressing to cervical cancer, whereas these generally upregulate expression of the E6 and E7 viral non-structural proteins.

Attempts to establish therapeutic immunity in human clinical trials have therefore focussed on the HPV E6 and E7 oncoproteins as target antigens. These proteins have been delivered as vaccines using recombinant viruses, as naked polynucleotides, as proteins or as peptides in adjuvant, and as fusion constructs with potentially adjuvanting TLR agonists or with proteins that enhance antigen delivery [8]. Because chronic infection with

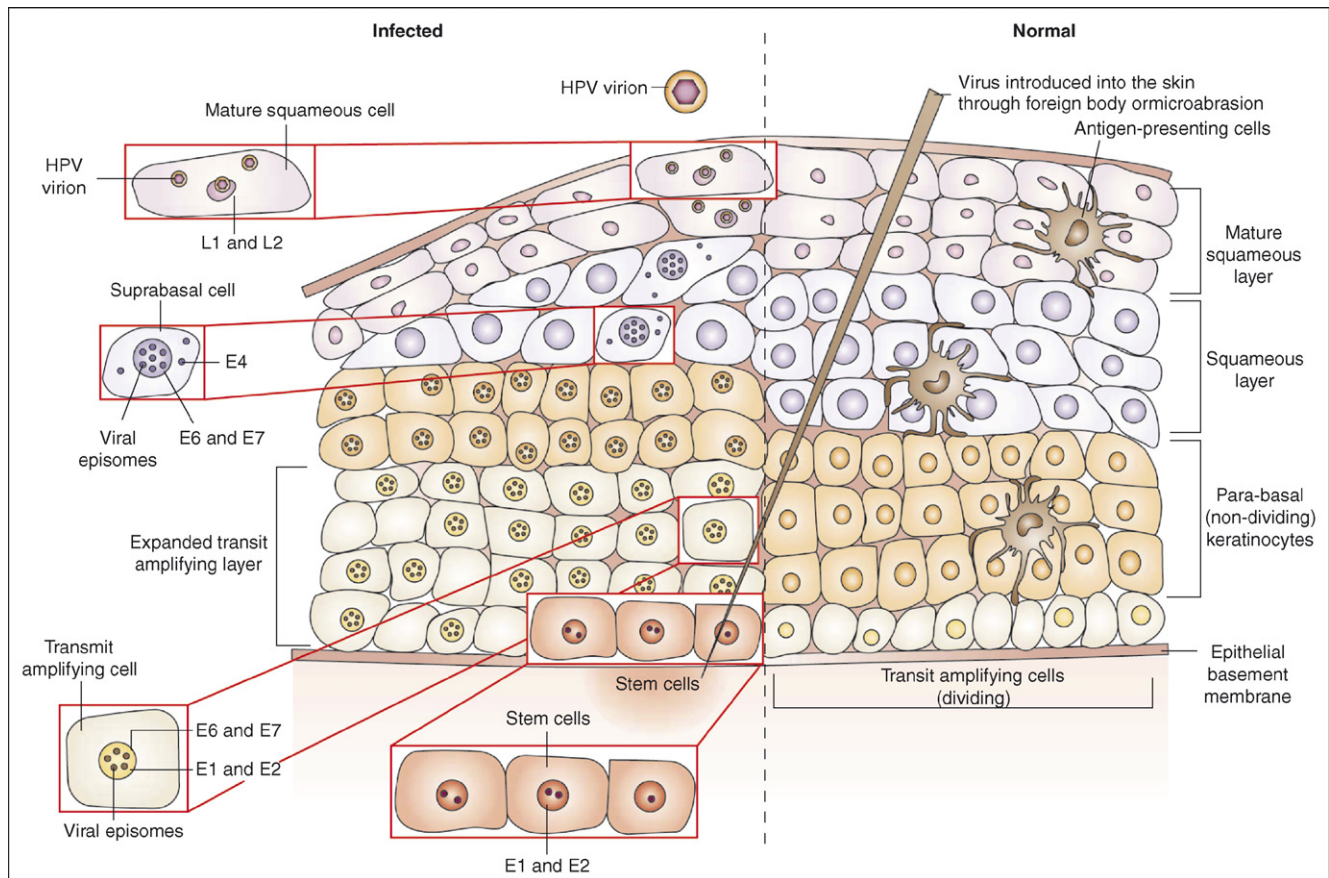
HPV is associated with an ineffective immune response to at least one viral non-structural protein — E7 [17] — IL-10-secreting antigen-specific regulatory cells might need to be silenced [18]. Trials establishing safety and immunogenicity of potential therapeutic vaccines have more recently given rise to trials seeking therapeutic efficacy [19–21]. Measurable T and B cell immunity to vaccine antigens in blood is a common finding, but objective examples of increased tumour clearance have been rare. This suggests that our underlying knowledge of induction of epithelial immunity, trafficking of lymphocytes to epithelium or effector function within the local tumour environment needs improvement.

Epithelial immunity and cervical cancer

Understanding of why the natural immune response to HPV-associated antigens E6 and E7 is apparently poor, as judged by measurable antibody responses in blood and long duration of infection, might facilitate the development of improved therapeutic vaccines (Box 3).

Langerhans cells (LCs) — specialized dendritic cells (DCs) of the epidermis — play a major role in initiating epithelial immune responses by capturing antigen and delivering it to the T-cell areas of the draining lymph node. Given that LC function or numbers are diminished in early stage HPV-associated cervical lesions [22], recent advances in tracking the function and/or trafficking of these cells [23,24,25••] in mouse models will provide insight into the fate of LCs in persistent HPV infection. Myeloid DCs that express activation markers in the blood

Figure 1



Infection with human papillomavirus alters the biology of maturing squamous epithelium. The right side of the figure depicts normal maturing squamous epithelium. The left side demonstrates the sites of expression of human papillomavirus structural (L1 and L2) and nonstructural (E1, E2, E4, E6 and E7) proteins and the structural alterations to the epithelium produced by HPV infection. Reproduced with permission from [17].

are also present at reduced levels in patients who have cervical squamous intraepithelial lesions [26]. Equally, plasmacytoid DCs capable of secreting the anti-viral cytokine IFN- α are also found in cervical lesions [27], although their role in cervical cancer development is unclear. In other skin diseases, plasmacytoid DCs are capable of enhancing melanoma regression after treatment with topical imiquimod [28] — a TLR7 and TLR8 agonist inducing potent local inflammation — or of

suppressing the development of anti-tumour responses in the draining lymph node through indoleamine 2,3-dioxygenase (IDO) production [29]. Dissecting which of these DCs will induce effective immunity will probably require a greater knowledge of their regulation as antigen-presenting cells.

Migratory LCs are acknowledged to present epithelial antigen directly to T cells in the lymph node. This view has been challenged in recent times, with one study proposing that LCs carry viral proteins to the skin-draining lymph node but then ‘hand off’ antigen to resident CD8 α^+ DCs, which then present directly to T cells [30]. This model is supported by a second study showing that lymph node resident DCs are responsible for inducing *Leishmania*-specific T cells [31]. By contrast, skin-derived DCs induced potent CD8 T-cell immunity to genes delivered via a lentiviral vector [32], LCs can present skin antigens [33], and circulating DCs recruited to the epithelium via CCR6–CCL20 interactions are effective at cross-priming [34]. In non-inflamed skin, epithelial DCs

Box 3 Requirements for a therapeutic vaccine against cervical cancer.

- Vaccine targeting of appropriate DC subsets.
- Induction of large numbers of high avidity, effector T cells (both CD4 and CD8 T cells).
- Ensure efficient T-cell trafficking to the tumour site.
- Overcome local immunosuppression at the tumour site.
- Generation of acute, local inflammation at the tumour site.

might be expected to maintain tolerance, but one study now suggests that LCs might be constitutively active in priming anti-self T-cell responses at the lymph node [35]. Consequently, the role of different subsets of DCs in presenting HPV antigens to the immune system needs to be resolved as one strategy to improve antigen targeting for HPV therapeutic vaccines.

Natural anti-cancer immunity might also fail because of an immunosuppressive tumour environment. Cervical cancers have been shown in the past to interfere with the activity of type I interferons, cytokines such as IL-18, and antigen processing and presentation [36]. A recent study has now shown that cervical carcinoma extracts, particularly the protease component, can suppress lymphoproliferative responses [37], whereas expression of FasL on cervical tumours can cause apoptosis of infiltrating lymphocytes [38]. Evidence also exists that the production of regulatory T cells might be an important suppressive mechanism in cervical cancer, with higher numbers of functional regulatory T cells found in lymph nodes that drain cervical tumours relative to numbers in endometrial cancer [39]. Regulatory T cells that express TGF- β have also been identified within cervical intraepithelial neoplasia stage 2/3 lesions [40]. Other cell types might also promote tumour formation. A recent study suggests that regulatory T cells can recruit mast cells, which are immunosuppressive in a model of skin allograft tolerance [41^{••}]. NK cell activation was associated with an increased susceptibility to cervical neoplasia in a study of HLA/KIR combinations in patients [42]. By contrast, downregulation of the NK cell activating receptor NKG2D impaired tumour immunosurveillance in the skin [43^{••}]. Consequently, immunotherapeutic approaches to cervical cancer must circumvent the activity of these cell types and other local immunosuppressive mechanisms.

Mouse models of cervical cancer

Transplantable tumours have long been used to demonstrate vaccine efficacy in preclinical trials in mice [44]. These tumours do not resemble their *in vivo* counterparts in that they are fast-growing, have been exposed to *ex vivo* growth supplements, are often highly immunogenic, and grow in non-cervical locations. Immunological therapies that are successful on E7-containing transplantable tumours are rarely successful when applied to clinical trials of cervical cancer. Immunization before tumour establishment is a particularly poor model of therapeutic cancer immunity whereas immune-mediated regression of established transplantable tumour masses at least mimics some aspects of cancer tissue. In this regard, a fusion protein of *Bordetella pertussis* adenylate cyclase and E7 showed promise in regressing established E7-expressing TC-1 tumour [45]. Occasionally, transplantable tumour models reveal an unexpected role for innate immune cells in preventing tumour growth, such as

NKT cells [46] or eosinophils [47], which can then be assessed in human cancers.

An alternative tumour model for HPV immunotherapy is the use of transgenic skin grafts that express E6 and/or E7 proteins in epithelial cells under the control of a keratin 14 (K14) promoter. E7 expression leads to epithelial hyperplasia in these mice, and grafting of this skin onto syngeneic non-transgenic mice enables both the induction of effector responses and the role of the local environment in antigen-specific effector cell function to be analysed in detail. K14E7 grafts are not rejected from immunocompetent hosts unless large numbers of activated CD8 T cells are generated by adoptive transfer of antigen-specific T cells with immunization [48]. Rejection of skin grafts also requires a local inflammatory environment because well-healed grafts, despite persisting antigen expression, do not undergo rejection from animals simultaneously rejecting a newly placed graft [48], although such rejection can be induced by local delivery of pro-inflammatory Toll-like receptor agonists (Zhong *et al.*, personal communication). Local inflammation has been shown to be important in the recruitment of effector T cells during graft-versus-host disease (GVHD) in the skin [49[•]]. A unique population of skin-resident T cells — $\gamma\delta$ T cells — are also important in regulating skin inflammation [50]. Thus, a proinflammatory environment is likely to be necessary for clearance of persistent HPV infection and associated cancer, whether by specific immunotherapy or by naturally occurring immune responses. Induction of successful effector function by acute inflammation might assist with rejection of HPV-infected skin cells, but caution might be needed in clinical trials because chronic inflammation can promote rapid HPV tumour growth in mouse models [51[•],52]. Also keratinocytes can, through up-regulation of RANKL in response to pro-inflammatory stimuli [53^{••}], increase regulatory T cell numbers. Thus, the nature of any local inflammatory immune response may determine whether it enhances or impairs local immune effector function to assist in eliminating epithelial cells expressing tumor or viral antigen.

T-cell recruitment to epithelial sites is one parameter of inflammation that is being dissected at the molecular level. During the induction of immune responses in the lymph node, DCs originating in the skin are able to imprint skin-specific homing during T-cell activation [54[•]]. T cell and skin DC (but not LC) exit from peripheral issue is not random and is controlled by the chemokine receptor CCR7 [55,56]. T cell trafficking to normal human skin appears to be controlled by the chemokine receptor CCR8 [57], and CCR4–TARC and CCR10–CTACK also participate in skin homing during inflammation [58]. However, whereas homing of effector T cells to skin will be necessary for effective immunotherapy, the absolute need for such direction is yet to be established in any skin tumor model.

Conclusions

Generation of a successful prophylactic HPV vaccine has turned attention to immunotherapy of persistent HPV infection and associated cancer, as five million cervical cancer deaths worldwide could be prevented over the next twenty years with interventional HPV-specific immunotherapy. HPV non-structural proteins (E6 and E7) are model nonself tumour antigens, necessary for maintaining the malignant state of transformed epithelium. Although empiric clinical trials have been successful in inducing cell-mediated immunity directed against E6 and E7, cure of HPV-associated epithelial disease is rare. Improved epithelial tumour immunotherapy will require better understanding of the cell types and molecular events involved in initiating and permitting immune effector function at cutaneous and mucosal sites. Overcoming local immunoregulation in the skin will be a key determinant of success.

Disclosure statement

Patents held by the University of Queensland with Ian Frazer as a named inventor have been licenced to CSL Ltd, and Ian Frazer might benefit financially from the sale of vaccines described in this article and developed using the patented technology.

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