The role of human papillomavirus vaccines in cervical neoplasia

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Cervical cancer is the second most common cause of cancer-related death in women, in some developing countries accounting for the highest cancer mortality. The evidence for the association of high-risk human papillomavirus types with the aetiology of cervical neoplasia is firmly established, human papillomavirus being detected in virtually all cervical cancers. The risk of progression of precursor cervical intra-epithelial neoplasia lesions is associated with persistence of human papillomavirus infection. One strategy for the management of cervical neoplasia worldwide could be the development of prophylactic and/or therapeutic human papillomavirus vaccines. This chapter will discuss the natural history of human papillomavirus infection, viral immunity and the clinical course of resultant disease as the background to the effective design and use of human papillomavirus vaccines for protection or therapy. The progress of ongoing phase I and II clinical trials for several different vaccine preparations and the challenges for establishing their future use will be discussed.

Key words: human papillomavirus (HPV); cervical cancer: cervical intra-epithelial neoplasia (CIN); vaccine; cytotoxic T lymphocyte (CTL); virus-like particle (VLP).
A knowledge of the natural history of HPV infection and the mechanisms responsible for the process of malignant transformation (Figure 1) determines the potential viral target antigens for immunological intervention strategies.

Human papillomaviruses (HPVs) are icosahedral non-enveloped viruses containing double-stranded circular DNA molecules of approximately 8 kb; the genome contains eight open reading frames (ORFs) and a non-coding region containing transcription regulatory sequences and the origin of replication. The early (E) ORFs encode proteins involved in DNA replication, transcription and cellular transformation, whereas two ORFs in the late region encode the capsid proteins L1 and L2.

HPVs are exclusively epitheliotropic, their infectious cycle being dependent on the life history of the epithelium, where cells migrate from the basal layer, differentiating as they progress to be exfoliated from the surface and replaced by cells from below. In cervical neoplasia, high-risk HPV infection (the most prevalent types being HPV 16 and 18) occurs predominantly in either the basal or the parabasal cells of the transformation zone epithelium. The viral particles, composed of capsids formed from the major (L1) and minor (L2) late proteins, access the target cells and facilitate the entry of the viral DNA, probably following minor local trauma. Subsequent viral protein expression correlates with the differentiation stages in the spinous layers.

Initially, the immediate early proteins E1, E2 and E5 can be detected. The E1 and E2 ORFs each encode DNA-binding proteins, their products being required to maintain a stable viral episome. The functions of E2 include the positive and negative regulation of viral gene expression through a specific interaction with the early promoter found in the upstream regulatory region. In the lower spinous layers, E6 and E7 are expressed in addition to the other early proteins; they are involved in regulating cell proliferation, interfering with the host cell cycle control mechanisms to activate cellular DNA synthesis, which seems to be essential for viral vegetative DNA replication. In the upper spinous layers, vegetative DNA amplification and virus assembly occurs with the expression of the L1 and L2 proteins. Although it is encoded within the early region, E4 protein expression is also largely restricted to the upper spinous layers. The precise role of E4 is unknown, but it interacts with the keratin intermediate filaments in cultured epithelial cells. Mature virions are released from exfoliating cells.

In the HPV types associated with malignant transformation, it is clear that E6 and E7 are the predominant transforming proteins, although E5 can also show oncogenic potential. The major mechanism by which oncogenic HPV E6 and E7 contribute to the development of cervical cancers is by a functional interaction with the cell proteins p53 and retinoblastoma (Rb) respectively; these proteins play a pivotal role in the negative regulation of growth. E6 from oncogenic HPVs binds to p53 with a high affinity, resulting in a loss of p53-dependent functions including G1 arrest and apoptosis. The consequence of the binding of E7 to Rb is to prevent Rb binding and the sequestration of E2-F transcription factors, leading to a disruption of cell cycle control.

In the initial infection, HPV is present as an episome, but in the majority of more advanced lesions and invasive tumours, HPV is integrated into the host genome. E6–E7 transcripts from integrated HPV genomes have increased stability compared with episomally derived viral mRNA, and there is an increased expression in more severe lesions and cancers. This may result from a disruption of the E2 gene during integration. Following integration, viral particles can no longer be produced, but continued E6 and E7 activity prolongs the cell cycle, leading to the loss of effective DNA repair mechanisms. This provides the opportunity for the accumulation of genetic changes in a
Infection
Metastasis
Invasive Cancer
High Grade CIN
Low Grade CIN
Subclinical Regression By Cell-Mediated Immune Functions
Viral Persistence
Modulation of cellular genes regulating viral gene expression
Viral/Genome Modification
Integration/Mutation
Mutation of genes affecting differentiation/angiogenesis
Hormones
Mutagenic Co-Factors
Genomic Instability

Figure 1. Natural history of human papillomavirus-associated cervical neoplasia.
multistep process that can result in the development of cancer. Thus, numerical and structural chromosomal changes in cervical cancer are common, and allelic losses have been observed in many different chromosomal regions. 

NATURAL IMMUNITY

Basic immune mechanisms

There are two main arms to the immune response that may play a role in the natural clearance of HPV infection: innate and adaptive immunity. Innate immunity consists of a rapidly induced, non-specific response that does not result in immune memory. It is localized at the epithelial borders and triggered by basic ‘danger’ signals associated with bacterial or viral infection. It is delivered via several immunomodulatory cytokines and cellular effectors, including monocytes, macrophages and natural killer and antigen-presenting cells (APCs).

Innate immunity-induced key effector cytokines, including interleukin (IL) 1, Interferon (IFN) α/β and tumour necrosis factor (TNF) activate APCs (e.g. dendritic cells), which act as sentinels for the host and initiate adaptive immunity. This results in the generation of antigen-specific effector cells (CD4 T-helper, CD8 T-killer and B-cells) and their products (cytokines and antibodies), which target the pathogen or pathogen-infected cell, as well as in the development of memory cells that will prevent or limit subsequent infection with the same organism. Antigen-specific T-cells are the fulcrum of anti-viral responses controlling the activation of specific B-cells to make antibodies or the differentiation of particular T-effectors (e.g. cytotoxic T-cells, or CTLs).

The critical steps in the induction of adaptive immunity likely to be involved in control of HPV infections in the cervix are summarized in Figures 2 and 3.

HPV-specific immunity in cervical neoplasia

A knowledge of the key viral targets and immune mechanisms that occur in natural viral clearance would obviously be helpful to optimize vaccine design. There are, however many limitations to our actual knowledge of the natural control of HPV infections in the cervix because of the difficulty of studying the precise local immune events in temporal relation to infection. Nevertheless, circumstantial evidence suggests a role for both the serological (e.g antibodies to capsid proteins) and the cellular (e.g. CTLs versus oncogenes) immune system in the clearance of HPV infections.

Studies with animal papillomaviruses have shown a protection against infection associated with antibodies recognizing conformational epitopes on the virus (or virus-like particles – VLPs – made of the major capsid protein); these antibodies are also able to neutralize the animal viruses. Such a serological response to HPV capsid proteins is undoubtedly a consequence of exposure to the pathogen, but the absence of such antibodies does not necessarily mean a lack of infection. Depression of the humoral immune system does not result in an increase in HPV lesions, so it is unlikely that antibodies alone are capable of viral clearance. There is, however, an increase in the frequency of HPV lesions in individuals with depressed cell-mediated immunity, such as HIV infection and allograft transplant patients.

Serological assays using VLPs indicate that a high proportion of individuals exposed to HPV with and without cervical lesions develop systemic antibodies to L1. In addition, IgA antibodies against capsid proteins are found in cervical secretions, but these may not correlate with lesion clearance. Perhaps unsurprisingly, HPV
type-specific L1 antibodies are found less frequently in patients with invasive cancer, in which the HPV is usually integrated and the ability to make viral particles is lost. Neutralizing antibodies may be an effective way of preventing viral infection and spread, but the cell-mediated surveillance of virally infected cells may also be important.
in the ultimate resolution of infection and disease. Interestingly, the cytokine patterns in cervical intra-epithelial neoplasia (CIN) lesions, which are subsequently either cleared or progress, are associated with T-helper type 1 and 2 responses respectively. It might be expected that effective T-helper responses to L1 would emerge at the early stages of infection, when late protein synthesis occurs as part of the production of infectious virions. T-helper responses to L1 presented as peptides or as VLPs have been detected in vitro by proliferation and IL-2 release assays respectively but are not found more frequently in patients with low-grade lesions compared with controls.

There is no clear relationship between E7 responses and disease severity or outcome in CIN patients. In patients treated for CIN, for example, T-cell proliferation to HPV 16 E6 and E7 peptides was found to be predictive of the subsequent clearance of HPV DNA. It is, however, possible that the HPV-specific responses in these patients were generated as a consequence of taking the biopsy, creating an adjuvant effect on maintaining viral clearance. This would be compatible with the association of T-helper responses (as measured by IL-2 release), with viral persistence and high-grade premalignant lesions, whereas in patients who cleared their infections, high responses detected around the time of viral clearance subsequently declined. The observed cellular immunity in patients with resolving or persistent lesions may be reconciled by

**Figure 3.** Factors influencing the types of immune mechanism controlling human papillomaviruses. Infection by human papillomavirus and the co-ordination of the viral and epithelial life cycles determines the expression of viral antigens. The activation of dendritic cells at the site of infection by innate danger signals allows the co-ordination of the uptake and processing of the viral antigens to peptides for association with HLA molecules and the trafficking of the antigen-presenting cells to the draining lymph node. Dendritic cells concomitantly acquire optimal antigen-presentation capacity and co-stimulatory potential. Polarization of the T-helper (Th) type response: naı­ve T<sub>0</sub> cells under the influence of either an interleukin (IL)-12-yielding Th1 cytokine profile (tumour necrosis factor, interferon [IFN]γ, IL-2) leading to the generation of cytotoxic T-lymphocytes (CTLs) and activated natural killer cells, or IL-10, which drives a Th2 (IL-4, IL-5 cytokines) response that favours B-cell activation, isotype switching, etc. T-cell subset generations are biased by the first experiences of the dendritic cell that reflects the nature of the danger.
the common factor of the antigen load reaching a threshold, as part of either the natural resolution of the disease or its progression. T-helper type 1 responses to HPV 16 E2 have also been detected in HPV 16-positive patients. Longitudinal studies show that T-helper responses to the carboxy-terminal of E2 occurred at the time of viral clearance, although it was not possible to determine the actual role of the anti-E2 response in clearance.23

The constitutive expression of HPV oncogenes in cervical tumours has stimulated the search for CTLs in cervical neoplasia patients. Candidate peptide epitopes from HPV 16 E6 and E7 that could be presented by the most common HLA-A alleles have been identified and have provided a methodology for the detection of specific CTLs.24,25 These data also show that CTLs against HPV 16 E7 in CIN patients are rare in the peripheral blood. A comparison of peptide-specific responses in peripheral blood, local lymph node and tumour-infiltrating lymphocytes from cancer patients indicated that HPV-specific CTLs were more abundant at the site of antigen exposure.26 HLA type and peptide limit this CTL detection methodology, whereas in vitro re-stimulation with adenovirus recombinants expressing HPV E6 and E7 allows responses to reflect the presentation of multiple naturally processed peptides in the context of the host HLA genotype.

Using this approach, HPV 16 E6- or E7-specific CTL responses have been detected in a proportion of patients with persistent HPV infection, but only E7 CTLs were seen in the cancer patients.27 A longitudinal study of CTL responses in women with HPV 16 infection and squamous intra-epithelial lesions also implicates differential E6 responses in viral persistence.28 The demonstration of CD4+ tumour-infiltrating lymphocytes in cervical cancer recognizing an HLA-DR-restricted peptide of E7 broadens the search for relevant HPV immunity to MHC class II-restricted responses.29

In summary, there is evidence that HPV infection and associated malignancy can induce humoral and cellular immunity to capsid and oncogene viral proteins, but whether such responses are a consequence of the disease rather than the resolving factor(s) is not clear.

WHY DO SOME HPV INFECTIONS PERSIST?

The majority of apparently immunocompetent individuals infected with HPV are able to clear the infection with no further consequences, but persistent infection is correlated with the progression of cervical disease.30 Such persistent infections may result from viral stealth and immune interference strategies, leading to an escape from the normally effective immunity. Any interference with local APCs in the cervix may protect the infected tissue from the attentions of the host immunity.

A recent analysis showed that the number of Langerhans cells (immature epidermal dendritic cells (DCs)) was significantly reduced in dysplasia or HPV infection, consistent with an early failure of endogenous activation events influenced by viral gene expression.31 Others have also shown a lack of activation of Langerhans cells in cervical lesions, together with a downregulation of the cytokine TNFα produced by basal keratinocytes, and an upregulation of the suppressive cytokine IL-10 in CIN compared with normal cervical epithelium.32 In addition, high-risk HPV E6 and E7 products modulate the IFNα response pathways of the infected cells and thereby compromise any protection from interferons induced by innate immunity.33,34

All these influences in the HPV-infected tissue may serve to bias any adaptive immunity towards a T-helper type 2 rather than a T-helper type 1 response. Is it possible that the evolution of HPV has produced a pathogen that survives by a
combination of stealth and specific interference with innate immunity, with knock-on
effects on adaptive immunity that in themselves advantage the infection?

Thus, in some progressive cervical lesions, the immune system may not be triggered
until after HPV integration and other cellular events contributing to malignant
transformation have occurred, compromising the relevance of such immunity in
resolving the malignant lesion. CTLs might, for example, drive the selection of immune-
resistant tumour cells. The high frequency of HLA class I downregulation seen in cervical
neoplasia and association with progression is consistent with such immune escape
mechanisms acting at the target cell level. Recent detailed molecular analyses of cervical
carcinomas have shown that these HLA class I dysregulations result from multiple
 genetic mechanisms and occur in around 90% of tumours. In addition, the CTL effector
function may also be compromised in patients with cervical neoplasia.

**IMMUNE INTERVENTION STRATEGIES**

The goal of both prophylactic and therapeutic immunization is a stimulation of the
adaptive immune response (with memory) to produce antigen-specific effector
molecules and/or cells either to prevent infection or to eliminate infected or
transformed cells. Although the targets responsible for the immune regression of
HPV-induced lesions have not been clearly identified, the available data suggest that
HPV viral proteins can be immunogenic. Taking into account the HPV life cycle, the
most suitable candidates for prophylactic and therapeutic vaccines are likely to be HPV
L1/L2 or E6/E7 genes and their products respectively. Relatively little is known about
the immunogenicity of other viral products in humans, but E2 could provide a suitable
target for therapeutic intervention in low-grade dysplasias.

Both prophylactic and therapeutic vaccines could have a role in combating HPV-
associated disease. A prophylactic vaccine would aim to prevent HPV infection by
generating an effective immune response at the site and time of infection, thereby
inhibiting the establishment of long-term infection and re-infection. Such a vaccine
would most benefit individuals at greatest risk of exposure to HPV. For the HPV types
associated with cervical neoplasia, a suitable population could be young women at the
onset of sexual activity, since it is in this group that the highest prevalence of HPV
infection has been measured. Therapeutic vaccination would on the other hand be
directed at the elimination of established infection, in both benign and malignant
disease.

**Prophylactic vaccines**

Many viral vaccines work by inducing anti-virion antibodies that prevent virus infection.
There are currently 17 viral vaccines licensed for use in humans. These are mostly based
on live or killed virus, although that for hepatitis B is a subunit vaccine derived from a
recombinant surface antigen. The use of whole virus in a vaccine depends on an ability to
propagate the virus in vitro, which is not practical for HPV. Simple subunit vaccines
based on denatured L1 proteins were not found to be effective in animal studies.

A major breakthrough in HPV vaccine technology was the demonstration that L1 has
the intrinsic capacity to self-assemble VLPs. These are morphologically indistinguishable
from infectious virions although they lack viral DNA. They present conformational
epitopes that are highly immunogenic. The co-expression of L2 can increase the in vitro
production of VLPs, but there is no evidence that it increases immunogenicity.
HPV L1 encoded in recombinant vectors for the production of VLPs in yeast, insect cells and *Escherichia coli* has been developed, and several types have been tested in animal models\textsuperscript{42–44} and early clinical trials. The animal studies used parenteral immunization with microgram doses of purified VLPs, followed by booster injections and then challenge with high-dose purified virus to an abraded epithelium. The results show that VLPs induce antibodies to conformational capsid epitopes that can neutralize virus particles. Low doses of either L1 or L1/L2 VLPs even without adjuvant were effective in protecting against experimental challenge, as evidenced by a reduced incidence of papilloma formation. No protection was, however, detected after vaccination with denatured VLPs or if a heterologous VLP type was used. Protection could be passively transferred in immune sera or with purified IgG. The principle that VLP vaccination can induce neutralizing immunity against papillomavirus challenge is clearly established, but the lack of a genital transmission model makes it difficult to predict the efficacy of an analogous human vaccine in preventing sexually transmitted HPV infection.\textsuperscript{8}

Early-phase trials in humans are in progress using clinical-grade VLP vaccines produced in different vectors, with the aim of establishing safety and immunogenicity. The latter may be facilitated by formulation with an adjuvant (e.g. alum). HPV 16 L1 VLPs produced by the recombinant baculovirus infection of insect cells have been tested in a phase one trial in healthy volunteers at Johns Hopkins University.\textsuperscript{45} Seventy-two men and women were entered into a blinded, placebo-controlled, dose-escalation, three-inoculation regimen, preliminary analysis indicating that all subjects receiving VLP seroconverted by 1 month after the second vaccination. The neutralizing ability of these antibodies has been confirmed. There were no substantial systemic side-effects of the vaccine. Successful immunization does not appear to require the use of an adjuvant.

The National Cancer Institute is running a proof of principle trial of this HPV 16 VLP vaccine in Costa Rica. Young women will be enrolled in a population-based, randomized (3000 in each arm), placebo-controlled trial in order to control for risk factors associated with HPV infection and the progression of HPV-induced lesions. The central question to be answered is whether a simple, parenteral inoculation of VLPs will result in sufficiently high and long-lasting virion antibody concentrations in the female genital tract to prevent infection with sexually transmitted virions.

Other groups have tested similar baculovirally derived VLP products for HPV 16, HPV 18 and a combination in healthy subjects and have shown immunogenicity and safety. A yeast-produced HPV 16 VLP tested in normal females was found to be highly immunogenic and well tolerated. The high production costs in these eukaryotic systems might be reduced if *E. coli* derived VLPs could be used.\textsuperscript{46} A trial of an *E. coli*-derived HPV 16 L1 VLP preparation with alum has been initiated in Manchester. This is a placebo-controlled, double-blind, randomized, parallel group, phase I study in women with mild dyskaryosis. Patients will receive three immunizations at three dose levels and will be followed up for at least 6 months. This will establish safety, and measure and compare the specificity, magnitude and kinetics of serological and cell-mediated responses to the vaccine. A secondary objective is to evaluate the colposcopic and cytological changes in cervical lesions.

**Therapeutic vaccines**

Most therapeutic approaches have concentrated on eliciting CTL responses against the constitutively expressed viral oncogene antigens of the most prevalent high-risk HPV 16 virus. A number of animal studies have indicated that therapeutic HPV vaccines may
be effective in promoting disease regression.\textsuperscript{53} Such pre-clinical mouse models are useful in establishing the proof of concept but are of limited value for predicting immunogenicity in man with HPV-associated disease. Thus, several vaccines are currently being tested for safety, immunogenicity and clinical efficacy in phase I and II clinical trials.

Peptides

The natural processing of viral proteins in vivo results in the production of many different peptides that are presented in conjunction with MHC molecules on the surface of the cell. The HLA molecules expressed by an individual restrict the repertoire of peptides presented. It is possible to predict immunodominant or subdominant peptides of viral antigens that would associate with particular HLA allelic products and then show that they can indeed be recognized by human T-cells. Unfortunately, suitable patients need to be of an appropriate HLA type. Forty per cent of Caucasians carry the HLA-A2 allele, and two-phase I/II, peptide-based clinical trials of HLA\textsuperscript{*}0201 binding HPV 16 E7 peptides have been reported.

In the first study, 12 HLA-A2-positive women with refractory cervical or vaginal cancer were vaccinated with four E7 lipopeptide inoculations at 3-weekly intervals. Although there were no clinical responses, E7 peptide-specific CTLs were detected in four out of 10 evaluable patients before vaccination, five out of seven evaluable patients after two vaccinations, and two out of three evaluable patients after all four vaccinations.\textsuperscript{54} Another phase I trial vaccinated 18 women with CIN or vulvar intra-epithelial neoplasia (VIN) II/III with E7 peptides four times at 3-weekly intervals. Six patients showed a partial regression of their CIN lesions, three patients had a complete disease resolution, and 10 patients demonstrated increased HPV-specific immunological responses as assessed by cytokine release and CTL assays.\textsuperscript{55}

Although the vaccines in these studies have included just one or two E7 peptides, vaccines consisting of multiple CTL epitopes strung together in a linear polypeptide can be envisaged. These ‘polytope’ vaccines could be individualized according to a particular patient’s HLA genotype, or generalized to include CTL epitopes that are applicable for several common HLA types.\textsuperscript{56} The advantages of the peptide approach are cost-effectiveness, as peptide vaccines are cheap to produce, and the fact that immunological responses following vaccination are relatively straightforward to measure.

Recombinant proteins

Recombinant proteins have the advantage over peptide approaches in the delivery of all potential epitopes to the antigen-processing cells of the immune system. Such vaccines can be administered regardless of the individual’s tissue type, as the APCs of the immune system process and present one or more peptide epitopes in association with host HLA molecules. Challenges involve the production and purification of the viral proteins and their formulation with appropriate adjuvants in order to elicit the necessary processing and subsequent activation of T-cells.

Exogenous proteins are usually naturally processed and presented on the surface of APCs via the MHC class II restricted pathway, which results in the induction and priming of CD4\textsuperscript{+} T-helper cells rather than CD8\textsuperscript{+} cytotoxic T-cells.\textsuperscript{57} Several animal studies have, however, demonstrated that CTL responses can be induced in vivo if the recombinant protein is constituted in adjuvant. A clinical trial in five late-stage cancer
of the cervix patients immunized with a glutathione-S-transferase HPV 16 E7 fusion protein in Algammulin adjuvant showed evidence of HPV 16 E7 antibody responses in all patients. Two out of three evaluable patients made proliferative T-cell responses to the E7 protein and peptide.58

**Viral vectors**

Live viral expression vectors such as recombinant vaccinia viruses have also been used to generate HPV vaccines. The advantage of these vaccines is that HPV proteins are endogenously synthesized from viral DNA by host cells, with the result that an array of HPV peptides is produced, processed and presented on the cell surface in conjunction with MHC class I molecules. Such a system poses no restriction on patient HLA genotypes or CTL repertoire and allows several HPV types or antigens to be included in the vaccine.

Vaccinia vaccines were used in the immunization schedule that resulted in the worldwide eradication of smallpox, and the vaccinia virus is very amenable to genetic manipulation. Although generally safe to use, vaccinia viruses are, however, known occasionally to cause host skin and CNS complications, especially in immunocompromised individuals.59 Alternatives to vaccinia vectors include the modified vaccinia virus Ankara, a highly attenuated strain of vaccinia that is significantly less likely to cause safety complications, and recombinant avipox, a virus with an excellent safety profile in human subjects.60

A number of early clinical trials using recombinant vaccinia vaccines have been performed. Borysiewicz et al61 administered a live recombinant vaccinia virus encoding HPV 16 and 18 E6 and E7 (TA-HPV) to eight patients with advanced cervical carcinoma. Three patients developed an HPV-specific antibody response, and one out of three evaluable patients developed transient HPV-specific CTLs at 9 weeks following vaccination. This patient showed disease remission and was tumour-free 15 months post-vaccination. In a multicentric EORTC phase II trial, TA-HPV was administered to early-stage cervical cancer patients, the immune response being measured before and after immunization. TA-HPV has also been used in the treatment of CIN3 and VIN3, and the results of these trials are awaited.

**Other vaccines**

A number of candidate HPV therapeutic vaccines have been studied in animal models but have yet to be tested in a clinical trial. These include:

1. tumour antigen (e.g. E7)-pulsed autologous dendritic cells62;
2. the administration of CTLs engineered ex vivo to secrete IL-263;
3. vaccination with autologous tumour cells engineered to secrete cytokines such as granulocyte–macrophage colony-stimulating factor64 and IL-1265;
4. salmonella engineered to express HPV 16 E7 and/or E666;
5. re-routing the antigen by inserting a signal sequence in the construct (e.g. LAMP-1), which addresses the MHC class II processing pathway67;
6. plasmid DNA-based immunizations.68

A particularly attractive strategy utilizes chimeric (E7 and L1/L2) VLPs that are effective in inducing antibodies, and T-helper and cytotoxic T-cell responses, which can elicit both prophylactic and therapeutic responses.69
Prime-boost strategies

Prime-boost strategies utilizing a priming immunization with DNA (plasmid or viral vector) followed by a heterologous boost with a different viral vector encoding the immunogen are very effective in several models of viral, parasite and tumour antigen vaccination. Several features of the priming vectors used may contribute to the enhanced immune responses. These include:

1. a stimulation of IL-12 production that may favour cell-mediated immunity;
2. greatly enhanced secondary responses resulting from a low but persistent expression of the immunogen;
3. the affinity maturation of antibodies after multiple antigen exposure;
4. the selection of high-affinity T-cell receptors from limited antigen availability;
5. a focus of specificity on the target antigen by the use of non-replicating viral vectors for boosting.

Prime-boost protocols using recombinant vectors and boosting with protein have unfortunately tended to generate T-helper 2-driven humoral immunity, which can militate against the generation of CTL activity. In a situation in which both humoral and cell-mediated immunity may provide for prophylaxis and therapy, it is certainly worth investigating various combinations of immunization protocol.

SUMMARY

HPV depends absolutely upon the differentiation programme of the keratinocyte for the completion of its life cycle. It infects cells in the basal layer of the epithelium but produces virus only in the superficial, terminally differentiated cells, ‘hijacking’ a maturation process which takes weeks to complete. This makes the evasion of host immunity extremely important, HPV having evolved a number of strategies that enable it to frustrate the natural response of the host.

Although genital HPV infection is extremely common, cervical carcinoma develops in a relatively small proportion of patients, specifically in those with persistent HPV infection. In addition, epidemiological studies show a greatly increased risk of HPV-associated disease in immunocompromised women, such as those with HIV. Taken together, this evidence suggests a role for natural immunity in the control of HPV infection, and many studies have provided evidence of serological and cell-mediated immune responses to HPV in patients with HPV-associated disease.

The precise role for these responses in the natural history of papillomavirus infection is still unclear, but the identification of HPV-specific immunity has provided the impetus for the development of vaccines both to prevent and to treat HPV-associated disease. The ultimate aim of these vaccine strategies is to effect a worldwide eradication of high-risk HPV types and thus prevent the hundreds of thousands of deaths that occur annually from cancer of the cervix.

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Research agenda

Prophylaxis

- does systemic immunization induce appropriate local immunity in the genital tract of humans? The vaccination route might be important in inducing mucosal immunity. The intranasal instillation of VLPs in mice can, for example, induce both systemic IgG and genital IgA. Alternatively, salmonella-based vaccines containing HPV 16 L1 expressing plasmids can be administered intranasally to induce IgA production in mice.

- are neutralizing antibodies to capsids sufficient for long-term protection, or is there a role for cell-mediated immunity in the prevention of an HPV infection? Methods for determining local T-cell and cytokine influences need to be developed.

- antibodies to capsid proteins alone cannot cure a latent infection. Do other viral antigens (e.g., oncoproteins) need to be included in a vaccine in order to evoke the necessary cell-mediated response? Chimeric VLPs containing both late and early gene products have been shown to induce neutralizing antibodies against L1 and E7 specific CTLs. Such a vaccine could prevent virus infection as well as eliminating any infected cells that might progress to malignant disease.

- what is a suitable end-point for assessment of the clinical efficacy of VLP vaccines? The long delay between infection and the development of cervical cancer makes the latter impracticable as well as unethical. CIN3 is an option, but not all infections progress to this stage, and most are treated before such a progression. If low-grade CIN is actively monitored for 3–5 years, a randomized trial of about 10 000 young women could provide an indication of the efficacy of an HPV 16 VLP vaccine.

- the potential impact of an effective prophylactic vaccination begs the question of who will pay for whom to be vaccinated. The real need is in countries that have no screening or only inadequate treatment options. In the developed world, at what point, if ever, will it be possible to abandon cervical screening programmes to reduce cost?

- is it easier to test a vaccine effect on the therapy of genital wart-associated HPV infections, with additional resulting protection? Thus, a fusion protein vaccine consisting of HPV 6 L2E7 with alum (TA-GW) was developed for the treatment of genital warts. The phase IIa safety and immunogenicity trial of TA-GW in persons with genital warts provided encouraging results. Further trials were designed to evaluate its efficacy in preventing the recurrence of genital warts in patients with severe disease in whom conventional therapies had previously failed. The latest results have, however, shown that, at 6 months, there was no significant difference in the wart recurrence rate between patients who received the vaccine and those in the control group. Why this vaccine did not work as anticipated is not clear, but this highlights the requirement for the continued analysis of multiple potential HPV encoded vaccine targets and vaccination protocols.

- as even closely related HPV types show no cross-protection from in vitro neutralization, and cervical cancer is associated with multiple types of HPV, it will be necessary to use a polyvalent vaccine to suppress the development of the majority of cervical cancers. A vaccine containing VLPs against HPV 16, 18, 31 and 35 might theoretically prevent 80% of cervical cancers. The possible emergence of a previously less virulent strain of HPV causing a significant number of replacement cancers after a longer period of infection needs to be considered.
Research agenda

Prophylaxis

Clinical trials aimed at evaluating the clinical and immunological aspects of therapeutic vaccines in cervical neoplasia face several difficulties:

- 80% of CIN I lesions regress spontaneously, so large cohorts need to be vaccinated and receive long-term follow-up in order to allow any benefits to be established.
- For high-grade CIN, the standard treatment will play the major role in disease outcome and may mask any additional benefit provided by the vaccine. The low recurrence rate dictates a need for large cohorts in long-term follow-up studies.
- Cervical cancer patients will have received conventional therapies prior to vaccination. Thus, early-stage patients treated by surgery will require a long follow-up since 76% have a 5-year survival rate. In contrast, late-stage patients have a poorer survival, providing the potential to measure any effect of immunization. The role of immune escape mediated at the target or effector cell may, however, compromise the therapy, and these variables must also be assessed in any trial design.
- VIN is characterized by HPV 16-associated lesions that are extremely difficult to eradicate. It is acceptable to treat VIN III expectantly with regular repeated biopsies and clinical review. Thus, the clinical effectiveness of a vaccine can be assessed in VIN without the confounding influence of other treatments. This may be a cost-effective way of delivering evidence of clinical efficacy in HPV-associated disease.
- There is clearly a need for simplified measures of the immune response of vaccinated patients that can ultimately be correlated with clinical efficacy. Newer techniques that quantify the number of virus-specific T-cells including γ-IFN ELISPOT or HLA tetramer assays may offer an advantage. The measurement of CTL responses in lesions rather than in the peripheral blood of patients is, however, probably more relevant, albeit logistically difficult.
- It is necessary to establish the longevity of the HPV-specific immunological memory that results from vaccination. This is likely to depend on the precise vaccination regimen, the nature, frequency and timing of which can take diverse forms.

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