Published in final edited form as:
Lancet Oncol. 2009 October ; 10(10): 975–980. doi:10.1016/S1470-2045(09)70227-X.

Development of therapeutic HPV vaccines

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Abstract

At least 15% of human malignant diseases are attributable to the consequences of persistent viral or bacterial infection. Chronic infection with oncogenic human papillomavirus (HPV) types is a necessary, but insufficient, cause in the development of more cancers than any other virus. Currently available prophylactic vaccines have no therapeutic effect for established infection or for disease. Early disease is characterised by tissue sequestration. However, because a proportion of intraepithelial HPV-associated disease undergoes immune-mediated regression, the development of immunotherapeutic strategies is an opportunity to determine proof-of-principle for therapeutic vaccines. In this Review, we discuss recent progress in this field and priorities for future clinical investigations.

Introduction

Persistent infection with human papillomavirus (HPV), most commonly type 16, is the proximate cause of 10% of malignant diseases in women, and 5% of the total global cancer burden,[1] including cancers of the cervix, vagina, vulva, anus, and oropharynx.[2] Of these cancers, cervical cancer is the most common. Current screening strategies for preinvasive disease of the cervix (ie, high-grade dysplasia or cervical intraepithelial neoplasia [CIN] 2/3), including cytology, HPV testing, and direct visualisation and immediate triage (see-and-treat), all need infrastructure and funding that are well beyond the resources available in much of the world. Consequently, on a global scale, cervical cancer remains the second leading cause of cancer death in women.[3] Although recently available prophylactic vaccines represent a public-health milestone, they provide no therapeutic effect for prevalent infection, or for already established HPV-associated disease. Furthermore, because implementation of these prophylactic vaccines presents many of the same challenges posed by screening and treatment, the global burden of HPV disease is unlikely to decrease in the near future. The figure summarises the challenges that need to be addressed in the development of immune-based therapies for HPV disease.

HPV-associated neoplasia represents an excellent opportunity to test antigen-specific immunotherapies, because expression of two viral antigens, E6 and E7, are needed to initiate and maintain high-grade squamous intraepithelial lesions, the immediate precursors to invasive cervical cancer, and overtly invasive disease.[4] Integration of viral DNA into the host genome is strongly associated with persistent HPV infection and disease progression,

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Conflicts of interest

IHF receives royalties from sales of HPV prophylactic vaccines, and is a consultant for Merck, GlaxoSmithKine, and Commonweath Serum Laboratories on papillomavirus vaccine development. CLT declared no conflicts of interests.

although both episomal and integrated viral DNA can be present in the same lesion.[5–7] In most immunocompetent individuals, HPV infection will eventually clear without immune intervention. Immuno-suppression by drugs or HIV infection significantly reduces clearance of infection[8] and clearance correlates with the development of specific CD4-T-cell immunity to the papillomavirus E2 and E6 proteins.[9] Moreover, a subset of persistent HPV disease is susceptible to immune manipulation; topical application of an inducer of innate immune responses, imiquimod, a toll-like receptor (TLR) 7 agonist, is approved by the US Food and Drug Administration (FDA) as first-line therapy for external genital warts, most of which are caused by HPV-6 and 11. HPV-associated disease is an ideal test of antigen-specific immunotherapy, because disease is common and expression of viral oncoproteins is obligate for persisting tumour growth, providing tumour-specific non-self antigenic targets. Furthermore, pre invasive intraepithelial precursor lesions are identifiable and clinically indolent, and a proportion of dysplastic lesions undergo spontaneous immune-mediated regression. Thus, the development of immunotherapeutic strategies for patients with HPV-associated intraepithelial neoplasia represents an ideal opportunity to determine proof-of-principle of immune-based therapeutic interventions for epithelial cancer. Because the lower genital tract is relatively accessible, the effect of immune interventions on both the systemic circulation and the target tissue can be studied. Furthermore, the opportunity to study the lesion microenvironment over time after therapeutic inter ventions can provide extra insights into how immunotherapy might work for persisting viral infection.

Naturally occurring immune responses to HPV antigens

The lifetime risk of genital infection on at least one occasion with an oncogenic strain of human papilloma-virus is thought to be greater than 80%.[10] In immunocompetent hosts, more than 90% of genital HPV infections become undetectable without intervention.[11] Because HPV infections are asymptomatic, and the timeframe for clearance is in the order of months to years, the herd burden of HPV is essentially endemic. Dysplasia develops from a chronic, mucosally-sequestered infection with HPV, and is associated with ineffective immune responses to viral non-structural proteins. Naturally occurring systemic humoral and adaptive responses to HPV antigens, even in cohorts with documented type-specific mucosal infections that have become undetectable, are hard to detect in peripheral blood.[12–14] Type-specific serum antibodies to capsid proteins are detectable in less than half of women in whom cervical HPV infections of known serotype have cleared. Nonetheless, data from cohorts undergoing prophylactic vaccination show an anamnestic response to a single dose of virus-like particle (VLP)-vaccine in previously infected individuals.[15] The antibody to the E7 protein can be measured in people with invasive cancer, but not in those with early stage disease.[16]

Women with intraepithelial HPV lesions rarely have systemic T-cell responses to HPV E6 or E7 that can be detected directly ex vivo, which probably reflects the low antigen load and tissue-compartmentalisation of early disease. By contrast to immune responses to other viral infections, the frequency of systemic memory CD8+ T cells in individuals with a known previous cervical HPV infection that has subsequently become undetectable is vanishingly low. For example, by use of direct ex-vivo assays, the frequency of systemic virus-specific CD8+ T cells after primary infection with cytomegalovirus or hepatitis C virus can be up to 5%.[17,18] By contrast, in patients with CIN, the frequency of HPV-specific T cells is two to three orders of magnitude lower, in the range of 0·1–0·01%.[19] Detection of systemic, HPV-specific T-cell responses in patients with intraepithelial neoplasia requires in-vitro sensitisation.[20–24] Various methods have been developed to identify responses to E6 and E7, including: assessment of proliferation of blood lymphocytes after incubation with HPV E6 or E7 peptides and interleukin (IL)-2 for 21 days[21] or with E7 20-mers for 7 days;[25] use of recombinant adenoviruses encoding HPV oncoproteins for secondary in-vitro restimulation

for 21 days to identify cytotoxic-T-lymphocyte responses;[26] interferon-γ enzyme-linked immunosorbent spot (ELISPOT) assays after 4 days of in-vitro sensitisation with long E6 and E7 overlapping peptides;[24] and major histocompatibility complex class I tetramer analysis done directly ex vivo by use of a fluorogenic human leucoctye antigen (HLA)-A*0201-HPV16-E7$^{11–20}$ construct.[19] After in-vitro stimulation with HPV antigens, peptide-specific T-cell frequencies increase in people with concurrent disease at the time of blood sampling, compared with individuals with no evidence of disease.[19,26] However, although amplification can identify qualitative responses to HPV antigens, this method is likely to have limited use in accurately distinguishing quantitative differences between individuals, either in the course of a natural infection, or in those with intraepithelial disease. Responses identified only after in-vitro restimulation would represent expansion of previously induced memory immune responses, rather than an ongoing response at the time of sampling. From a practical standpoint, two conclusions can be drawn concerning T-cell responses in women with intraepithelial neoplasia; first, natural infection with HPV fails to elicit a potent systemic immune response; and second, the size of natural HPV-specific T-cell responses measured in the peripheral blood of individual patients does not reliably predict lesion regression.

Natural history of HSIL

High-grade dysplasia is associated with integration of the viral genome into the host genome.[27] Nonetheless, a proportion of established cervical high-grade lesions do undergo regression over a relatively short timeframe. Both retrospective and prospective studies suggest that across all HPV types, the rate of regression of cervical high-grade squamous intraepithelial lesions (HSIL) in 4–6 months is around 35%.[28,29] Cervical HSIL associated with HPV-16 is less likely to regress than CIN2/3 associated with HPV types other than 16.[29,30] However, because it is not possible to distinguish lesions that are likely to regress from those that are not, the standard of care for a biopsy-proven high-grade dysplasia is surgical excision. Over the same timeframe, 4–6 months, high-grade cervical dysplasia is unlikely to progress, even in women who are immuno-compromised.[31] Thus, the clinical indolence of intra-epithelial lesions in combination with the fact that they can be directly visualised represents an opportunity to monitor lesions long after the active treatment window of any given immunotherapeutic intervention.

Vulvar and vaginal dysplasias are clinically more recalcitrant than cervical HSIL. Although progression to invasive carcinoma is low, in the range of 9% over a timeframe of years, spontaneous regression is rare, in the region of 1%.[32] Finally, despite the fact that high-grade anal dysplasia is less common than intraepithelial HPV lesions elsewhere in the genital tract, the incidence of this disease is increasing, both in men and women.[33]

Identification of viral epitopes recognised in natural infection, preinvasive disease, and invasive cancer can inform the monitoring of immune therapies in these patient cohorts. However, useful antigenic targets for induced immune responses are unlikely to be identified by study of HPV-antigen-specific cells in the systemic circulation. Development of improved methods to identify HPV-specific T-cell responses could enable monitoring of the functional polarisation of immune T cells in HPV-associated clinical lesions, and of the correlation of tissue and systemic immune responses to HPV proteins.

Summary of therapeutic vaccine approaches

Despite the fact that the epithelial compartment of cervical cancers and also preinvasive anogenital lesions can express up to nine papillomavirus-encoded proteins (L1, L2, and E1–E7), the E6 and E7 proteins are of specific interest for vaccine development, not only

because of their functional role in the neoplastic process, but also because natural immune responses to these antigens, although limited, have been identified in relation to disease. Up to now, delivery systems tested clinically have included fusion proteins used alone and with adjuvant, encapsulated polynucleotides, protein with adjuvant, recombinant viruses, DNA constructs, dendritic cells, and chimeric VLP constructs (table). These vaccines have been tested in a spectrum of patient cohorts, from patients with end-stage cervical cancer to those with intraepithelial neoplasia of the cervix, vulva, or perianal area who are otherwise healthy. Overall, these investigations have established the safety, feasibility, tolerability, and limited immunogenicity of these vaccine constructs. However, despite optimistic preclinical data, evidence of therapeutic benefit from induced T-cell responses in humans has been limited. Early clinical trials were done in patients with late-stage disease, who were immunocompromised both by disease and by previous treatment for disease. Moreover, late-stage disease is probably a poor target for antigen-specific therapies as a stand-alone modality, because these tumours commonly have mutations in addition to deletions of genes involved in antigen processing and presentation.[52,53]

More recently, the design of trials testing HPV immunotherapies has undergone a paradigm shift towards testing immunisation strategies in patients with preinvasive disease. An emerging body of evidence from clinical studies testing a non-HPV-specific immune modulator, imiquimod, on intraepithelial HPV disease suggests several potentially crucial insights for the design of subsequent clinical trials. [54,55]

The use of topical imiquimod on high-grade vulvar intraepithelial neoplasia (VIN) has been reported with a complete response in nine (34·6%) of 26 patients over a 12-month period.[54] The expected rate of spontaneous regression in this timeframe is less than 1%. The use of sequential imiquimod and photodynamic therapy has also been reported in this patient cohort, with six (30%) of 20 patients showing a complete response in a 12-month period.[55] Notably, in both of these studies, patients with detectable systemic HPV-specific T-cell responses before study intervention were more likely to have a clinical response to manipulation of the lesion microenvironment than patients who did not have a detectable HPV-specific response at study entry. This finding suggests that combinatorial regimens should incorporate both induction of virus-specific T-cell responses and subsequent manipulation of the target lesion microenvironment. Both studies also reported an increase in the ratio of CD8:Foxp3 cells in lesional lymphocytes, in patients who responded to imiquimod. It is also worth noting for future clinical trial design that clinical responses were noted a long time after the completion of the intervention phase of the trials. These data suggest that clinical trials testing immunotherapies in this patient cohort should include monitoring of the lesion compartment, in addition to the more conventional systemic measures of immunological parameters. Although eliciting an HPV-specific T-cell response is a rational proxy measure of vaccine efficacy, the development of immunotherapeutic strategies for HPV disease should also include immunological monitoring of the lesion site.

Animal models of therapy for HPV disease

Preclinical data to support clinical trials of HPV-specific immunotherapy have emerged from three different models: naturally occurring animal papillomavirus infections in cows, dogs, and rabbits; murine transplantable tumours expressing HPV antigens; and mice transgenic for HPV genes. Natural animal papillomavirus infections have limited use, because most disease regresses spontaneously. Bovine papillomavirus (BPV)-associated oesophageal tumours develop in cattle fed bracken fern, but do not express papillomavirus-encoded antigens,[56] whereas BPV-associated sarcoids in horses, which express some papillomavirus antigens,[57] are not widely available for preclinical research. Studies of canine oral papillomavirus infections, which are generally benign and self-limiting, have

confirmed that immune responses to two viral early proteins (E2 and E6) correlate with lesion regression.[58] Rabbit oral papillomavirus infections and cottontail rabbit papillomavirus infections are self-limiting in most strains; chronic infection leads to localised skin cancers, for which regression can be induced by immunotherapy.[59] However, the mechanism of regression remains unclear, due to the low availability of reagents and of genetically homogenous rabbits. Several transplantable murine tumours expressing the early open reading frames of HPV have been established, and are generally susceptible to a wide range of antigen-specific immunotherapy.[60,61] However, immunotherapeutics effective in these animal models, have, when tested in humans, shown limited or no clinical efficacy. These data suggest that transplantable tumour models are not representative of persistent papillomavirus infection in humans, perhaps because the 2% of humans that develop persistent infection do so because of specific genetic predisposition. In transgenic mice, HPV early proteins expressed in the epithelium from a keratin promoter can be induced with oestrogen to develop cervical carcinoma.[62] These mice develop partial tolerance to the papillomavirus-encoded transgenic proteins, and are unable to generate papillomavirus antigen-specific cytotoxic T cells after immunisation.[63] However, such mice might model the requirements for induction of papillomavirus protein-specific immunity in humans already tolerant of papillomavirus proteins from extended exposure during infection. Skin from papillomavirus E6 and E7 transgenic mice is not spontaneously rejected when grafted to immunocompetent recipients,[64,65] by contrast to the skin from animals expressing other transgenes from the same promoter,[65] and such grafts provide a further model for assessing papillomavirus protein-specific immunotherapy. E7-expressing grafts are not rejected after immunisation with E7, although such immunisation induces a specific immune response sufficient to reject an E7-expressing transplantable tumour.[66] This finding suggests that papillomavirus E6 and E7 proteins are either poorly immunogenic or interfere with their own immunogenicity. Local immuno-regulation in skin, attributable in part to properties of the E7 protein, and in part to the immuno-regulatory properties of hyperproliferative epithelium, also contributes to the failure of immunotherapy in resulting in E7 graft rejection. In this model, possible mechanisms allowing persistent HPV infection can be identified (figure), which might be relevant to persisting HPV infection in some apparently immunocompetent patients. These mechanisms include inhibition of interferon-γ mediated upregulation of antigen presentation, a requirement for local inflammation, inducible by topical imiquimod therapy, for optimum effector T-cell function, and cellular inhibitors of antigen-specific CD8 T-cell induction and effector function, including regulatory CD4 T cells, and natural killer T cells.

Conclusions

Clinical trials that have been done up to now have been moderately successful in eliciting cell-mediated immune responses to HPV E6 and E7 in patients with a spectrum of HPV-associated disease. However, clinical responses have not been consistent. Advances in immunotherapies for HPV-associated disease will be predicated on the development of instruments for measuring tissue-localised mucosal immune parameters. Mechanisms of immune-cell traffcking to the genital mucosa, and, more generally to epithelial surfaces in the absence of local inflammation, are incompletely understood, and this knowledge would provide valuable direction for optimising vaccination strategies. Clinical assessment of nasal, oral, rectal, intramuscular, and intravaginal immunisation suggest that, although intravaginal priming and boosting is the most effective schema for eliciting detectable antigen-specific genital immune responses, nasal immunisation also generates genital immune responses.[67] Nasal immunisation has certain practical advantages over genital immunisation, including more straightforward logistics, and a greater likelihood of cultural acceptability.

The ability to identify mechanisms of immune dysfunction in the lesion milieu could allow temporary systemic immune modulation in conjunction with strategies to elicit E6-specific or E7-specific T-cell responses. The development of methods to quantitate immunological parameters in the mucosal micro environment could also allow the identification of predictors of lesions that are likely to respond either to vaccination or to manipulation of the local immune environment.

Acknowledgments

CLT has received grants from the National Cancer Institute, Bethesda, MD, USA, from the Maryland Cigarette Restitution Fund, and from the Dana Foundation. IHF has received grants from the National Health and Medical Research Council of Australia, the Cancer Research Institute New York, Wellcome foundation, the Australian Cancer Research Foundation, and the Cancer Council Australia.

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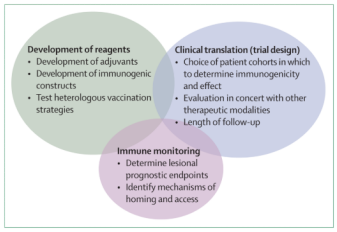

Figure.
Identifying barriers to therapeutic vaccination for human papillomavirus disease

Table

Clinical trials of human papillomavirus (HPV)-specific immunotherapy

	Delivery system	Antigen	Disease group	Immunogenicity	Clinical outcome
Double-blind placebo-controlled trial[34]	Fusion protein (TA-CIN)	HPV-16 L2–E6–E7 fusion protein (no adjuvant)	Healthy volunteers (N=40)	Antibody, T cell, interferon γ, and ELISPOT all detected	No HPV infections
Open-label uncontrolled trial (warts not HPV-16+)[35]	HSP fusion protein (HSP-E7)	HPV-16 E7 peptide	Genital warts (N=22)	ND	Regression of warts: 3/14 CRs and 10/14 PRs
Open-label uncontrolled trials (anal dysplasia and HPV-16+ cervical dysplasia)[36–38]	Encapsulated polynucleotide (ZYC101)	HPV-16 E7	Anal and cervical dysplasia; HPV-16+; HLA-A2 (anal: N=12, cervical: N=15)	Most individuals ELISPOT positive; induction of E2-specific immunity	Regression of AIN: 3/12 PRs; regression of CIN: 5/15 CRs
Multicentre, double-blind, randomised, placebo-controlled trial (CIN2/3, any HPV type)[38,39]	Encapsulated polynucleotide (ZYC101)	HPV-16 E7 peptide	CIN2/3, any HPV type (assessable N=127)	Most individuals ELISPOT positive; induction of E2-specific immunity	Lesion regression higher in patients <25 years of age; not restricted to HPV-16 or 18+ lesions
Randomised placebo-controlled trial[39,40]	Protein/Iscomatrix adjuvant (E6, E7–IMX)	HPV-16 E6–E7 fusion protein	CIN (N=31)	Antibody, DTH, CTLs	HPV-type-specific reduction in HPV infection: 7/14 CRs and 7/14 PRs/no clinical regression
Open-label phase I/II uncontrolled trial[41]	Vaccinia virus (TA-HPV)	E6–E7 fusion protein	Late-stage cervical cancer (N=8)	CTLs (1/8), antibody (3/8)	Outcome not documented
Open-label uncontrolled trial[42]	Vaccinia virus (TA-HPV)	E6–E7 fusion protein	Vulval HPV/VIN (N=18)	Antibody, CMI (13/18)	50% reduction in disease in 8/18; loss of viral load in 12/18
Open-label uncontrolled trial[43]	Vaccinia virus (TA-HPV)	E6–E7 fusion protein	VIN (N=12)	T-helper cell ELISPOT increase (6/10); vaccinia response in all patients	>50% reduction in disease in 5/12
Open-label uncontrolled trial[44]	Peptide/oil plus water adjuvant	E7 peptides	Refractory cervical cancer; HPV-16+; HLA-A201 (N=19)	No CTL response	2/19 stable disease
Open-label uncontrolled trial[45]	Protein/algammulin adjuvant	E7–GST fusion protein	Cervical cancer (N=24)	Antibody, DTH	No alteration in natural history of disease
Open-label uncontrolled, trial HPV-16+[46]	Peptide plus IFA	E7 A0201 peptide	VIN/CIN; HPV-16+; HLA-A2 (N=18)	CTLs 10/16, no DTH	3/18 CRs and 6/18 PRs
Open-label uncontrolled, trial[47]	VLPs	L1	Genital warts (N=33)	Antibody, DTH	Regression of warts: 25/33 CRs
Open-label uncontrolled, trial[48]	Dendritic cells	HPV-16 E7 and HPV-18 E7	Cervical cancer, stage IV (N=15)	Antibody, proliferation, ELISPOT (3/11)	No objective clinical response

	Delivery system	Antigen	Disease group	Immunogenicity	Clinical outcome
Randomised placebo-controlled trial[49]	Chimeric virus-like particles (CVLP)	HPV-16 1 E7 protein	CIN2/3; HPV-16 only (N=39)	Antibody, CTL	39% histological improvement in vaccinated patients *vs* 25% in placebo group; 59% of responders became HPV-16 DNA-negative
Open-label uncontrolled, trial[50]	Peptide montanide ISA-51 adjuvant	HPV-16 E6 combined or separated from HPV-16 E7 overlapping long peptides	End-stage cervical cancer (N=35)	ELISPOT	Immunity against E6 in patients vaccinated with E6 and E7 at the same site; greater response to E7 in patients vaccinated with E6 in one limb and with E7 in a different limb
Open-label uncontrolled, trial[51]	DNA vaccine	Sig-E7(detox)-heat-shock protein-70 fusion protein	CIN2/3; HPV-16+; (N=15)	ELISPOT	Complete histological regression in 33% (3/9) in the highest dose cohort; new responses to E7 at 6 months

IFN=interferon. ELISPOT=enzyme-linked immunosorbent spot. ND=not done. CR=complete response. PR=partial response. HSP=heat shock protein. HLA-A2=human leucocyte antigen serotype A2. AIN=anal intraepithelial neoplasia. CIN=cervical intraepithelial neoplasia. DTH=delayed hypersensitivity reaction. CTL=cytotoxic T lymphocyte. VIN=vulvar intraepithelial neoplasia. CMI=cell-mediated immunity. GST=glutathione-S-transferase. IFA=incomplete Freund's adjuvant. VLP=virus-like particle.