Recent Advances in Human Papillomavirus Vaccines

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The human papillomavirus (HPV) is one of the most common sexually transmitted infections and the etiologic agent of cervical dysplasia and cancer. Recent research on the safety and efficacy of prophylactic vaccines against HPV has shown promising results with nearly 100% efficacy in preventing persistent infections and cervical dysplasia. Several approaches are being tested in therapeutic vaccine clinical trials whereby E6, E7, or both agents are administered in live viral vectors, as proteins, or in nucleic acid form. Cell-based therapeutic vaccines are also being tested. HPV vaccines have the potential to eradicate a major cancer and source of morbidity around the world.

Introduction

Cervical cancer is a leading cause of cancer mortality among women in developing countries. In the United States, there are more than 9000 cases of cervical cancer and more than 3000 deaths from the disease annually [1]. Over 99% of cervical cancers are linked to genital infection with human papillomavirus (HPV), which is the most common viral infection of the reproductive tract worldwide and infects an estimated 660 million people annually [2]. Vaccines against HPV infections have the potential to be a practical and cost-effective way to prevent or treat cervical cancer. This article reviews the current status of HPV vaccine development and highlights outstanding research questions. We also review available data on the epidemiology, pathogenesis, and immune responses to HPV infection.

Human Papillomavirus

More than 40 different HPV types have been identified that infect the anogenital epithelia and other mucosal membranes. Some 13 to 18 of these types are recognized as high–oncogenic risk HPV types (Fig. 1). HPV-16 accounts for approximately 60% of cervical cancers, with HPV-18 adding another 10% to 20%. Other high-risk types include types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 73 [2].

The HPV genome regulates synthesis of eight proteins. The late *L1* and *L2* genes code for the viral capsid proteins, the early proteins E1 and E2 are responsible for viral replication and transcription, and E4 seems to aid virus release from infected cells. The early genes of the high-risk HPV types (*E6* and *E7*) encode the main transforming proteins. These genes are capable of immortalization of epithelial cells and are thought to play a role in the initiation of the oncogenic process. The protein products of these early genes interfere with the normal function of tumor suppressor genes. HPV E6 is able to interact with p53, leading to its dysfunction, thereby impairing its ability to block the cell cycle when DNA errors occur. E6 also keeps the telomerase length above its critical point, protecting the cell from apoptosis [3]. HPV E7 binds to retinoblastoma protein (pRb) and activates genes that start the cell cycle, leading to tissue proliferation. To a lesser extent, E5 has also been implicated in cellular transformation [3].

It is now widely accepted that high–oncologic risk HPV infection is a necessary but not sufficient cause of virtually all cases of cervical cancer worldwide (Fig. 2) and a likely cause of a substantial proportion of other anogenital neoplasms and oral squamous cell carcinomas. An estimated 85% of anal cancers; 50% of the cancers of the vulva, vagina, and penis; 20% of oropharyngeal cancers; and 10% of laryngeal and esophageal cancers are attributable to HPV [4].

Epidemiology and Pathogenesis

An estimated 6.2 million new cases of high–oncologic risk HPV infection occur in the United States each year, and approximately 20 million Americans are infected with HPV at any one time [5].

Risk determinants for HPV infection that have been identified in various cross-sectional and prospective cohort studies include number of sexual partners (lifetime and recent), age at first intercourse, smok-

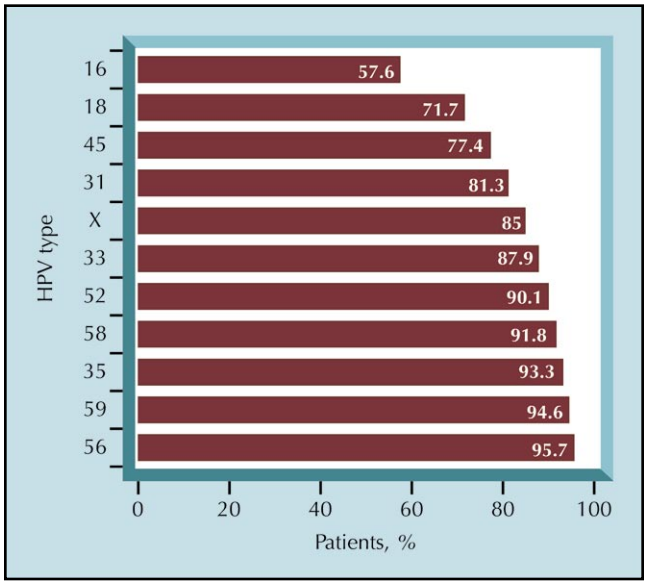

Figure 1. HPV types in cervical cancer. HPV—human papillomavirus.

ing, oral contraceptive use, other sexually transmitted infections (eg, chlamydia and herpes simplex virus), chronic inflammation, immunosuppressive conditions including HIV infection, and parity. Nevertheless, the most consistent determinant of HPV infection is age, with most studies indicating a sharp decrease in risk after the age of 30 years [6]. The decrease in risk of HPV infection with increasing age seems to be independent of changes in sexual behavior, suggesting a role for immune response.

Most infections seem to clear spontaneously; cohort studies have consistently found that only a small proportion of women positive for a given HPV type have the same type in subsequent specimens [7]. Whether infections clear completely or the virus remains latent in basal cells at undetectable levels is a matter of debate and cannot be verified empirically. What is clear, however, is that risk of subsequent cervical intraepithelial neoplasia (CIN) is proportional to the number of specimens testing positive for HPV [8]. This suggests that carcinogenesis results from HPV infections that persist productively (ie, with sustained viral replication within the squamous epithelium) for prolonged periods of time.

Immune Responses to HPV

Several studies have demonstrated that virus-neutralizing antibodies mediate protection of animals from experimental papillomavirus infection. For example, passive transfer of sera from virus-like particle (VLP)–vaccinated rabbits to naïve rabbits is sufficient for protection [9]. Similarly, vaccination with L2 peptides protects rabbits from papillomas resulting from viral but not from viral DNA challenge, consistent with the protection mediated by neutralizing antibodies [10].

Most of those who develop benign HPV lesions eventually mount an effective cell-mediated immune response that results in lesion regression. Regression of anogenital warts is accompanied histologically by a CD4+ T-cell–dominated Th1 response, and data from animal models suggest that the response is modulated by CD4+ T-cell–dependent mechanisms. Failure to develop effective cell-mediated immunity to clear or control infection results in persistent infection and, in the case of the oncogenic-HPVs, an increased probability of progression to high-grade squamous intraepithelial lesions or invasive carcinoma. The increased prevalence of HPV infection and high-grade lesions in immunosuppressed individuals as a consequence of HIV infection demonstrates the importance of CD4+ T cells in the control of HPV infection. The prolonged duration of infection associated with HPV seems to be associated with effective evasion of innate immunity as reflected in the absence of inflammation during virus replication, assembly and release, and downregulation of interferon secretion and response, thus delaying the activation of adaptive immunity [11•].

The well-characterized foreign (viral) antigens and the well-defined virologic, genetic, and pathologic progression of HPV have provided a unique opportunity for development of vaccines to prevent HPV infection and the associated pathology.

Prophylactic Vaccines

In general, prophylactic vaccines induce the generation of neutralizing antibody to the pathogen and thus prevent disease on subsequent exposure. A vaccine generating such responses must contain L1 protein in the correctly folded, tertiary, or "native" form. Technically, this is very difficult, but a major experimental breakthrough showed that the L1 protein, when expressed by vectors such as recombinant baculovirus or yeast, self-assembled into VLPs [12]. The L1 VLP is a conformationally correct, empty capsid (ie, it contains no DNA) that appears morphologically identical to, and contains the major neutralizing epitopes of, the native virion.

In a recent randomized, double-blind, controlled trial, Harper et al. [13••] assessed the efficacy, safety, and immunogenicity of a bivalent HPV-16/18 L1 VLP vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium) for the prevention of incident and persistent infection with these two virus types, associated cervical cytologic abnormalities, and precancerous lesions. They randomly assigned 1113 women aged between 15 and 25 years to receive three doses of either the vaccine formulated with AS04 adjuvant (aluminum salt and 3-deacylated monophosphoryl lipid A) or placebo on day 1, 1 month, and 6 months after registration in North America and Brazil (Table 1). Women were assessed for HPV infection by cervical cytology and self-obtained cervico-vaginal

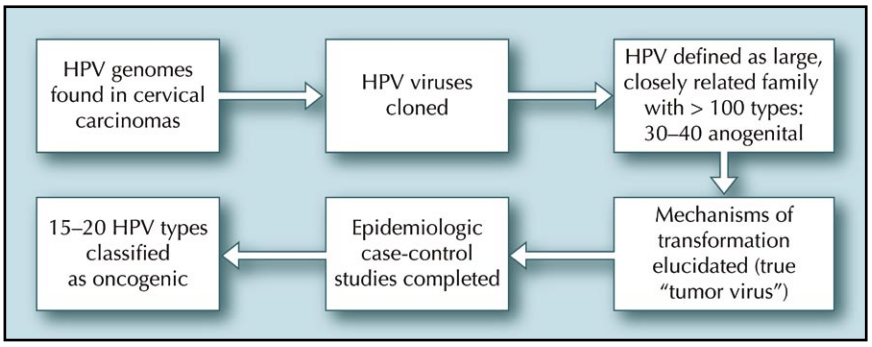

Figure 2. Discovery of the link between HPV and cervical cancer. HPV—human papillomavirus.

samples for up to 27 months and for vaccine safety and immunogenicity. In the according-to-protocol analyses, vaccine efficacy was 91.6% (95% CI, 64.5%–98.0%) against incident infection and 100% against persistent infection with HPV-16/18. In the intention-to-treat analyses, vaccine efficacy was 95.1% against persistent cervical infection with HPV-16/18 and 92.9% against cytologic abnormalities associated with HPV-16/18 infection. The vaccine was generally safe and well tolerated except for rare mild elevations in temperature and local injection site reactions, and it was highly immunogenic.

Recently, the authors performed a follow-up study of these women [14•]. More than 98% seropositivity was maintained for HPV-16/18 antibodies during the extended follow-up of 4.5 years. In a combined analysis of the initial efficacy and extended follow-up studies, vaccine efficacy of 100% against CIN lesions was associated with vaccine types. The authors also noted broad protection against cytohistologic outcomes beyond that anticipated for HPV-16/18 and protection against incident infection with HPV-45 and HPV 31. The vaccine has a good long-term safety profile.

Dubin et al. [15] compared immunogenicity and safety of the HPV-16/18 L1 VLP vaccine formulated with AS04 adjuvant (Cervarix; GlaxoSmithKline Biologicals) in 158 preteen/adolescent (10–14 years) and 458 young women (15–25 years). The vaccine was well tolerated, and adverse events were rare in both groups; however, higher antibody titers were observed in the preteen/adolescent group. The investigators concluded that higher antibody titers in the younger group might result in longer antibody persistence and be particularly advantageous when an HPV vaccine is administered at a young age well before sexual activity.

The results of a randomized, double-blind, placebo-controlled multicenter phase II trial of a quadrivalent VLP vaccine were published recently [16••]. The vaccine included four recombinant HPV type-specific VLPs consisting of the L1 major capsid proteins of HPV-6, -11, -16, and -18 adsorbed onto amorphous aluminum hydroxyphosphate sulfate adjuvant (Gardasil; Merck Research Laboratories, Whitehouse Station, NJ). Two hundred seventy-seven young women (mean age, 20.2 years) were randomly assigned to quadrivalent HPV (20-μg type 6, 40-μg type 11, 40-μg type 16, and 20-μg type 18) L1 VLP vaccine and 275 (mean age, 20.0 years) to one of two placebo preparations at day 1, month 2, and month 6. In the according-to-protocol cohort, the incidence of persistent HPV-6, -11, -16, or -18 infection or associated disease decreased by 90% (95% CI, 71%–97%) in women who received the vaccine compared with those who received placebo. The results were similar in an intention-to-treat analysis. All women who received vaccine developed HPV antibody to the four HPV types after the series was completed, and antibody titers were substantially higher than in placebo recipients who had had a previous HPV infection. Mean antibody titers at month 36 remained at or above the titers in women who had a natural HPV infection and cleared the virus. Pain was the most common injection-site adverse event and headache the most common systemic adverse event. No vaccine-related serious adverse events were reported (Table 1). A phase III trial of the quadrivalent vaccine, involving 17,800 women aged 16 to 23 years, has recently been completed. Data from this clinical trial, the Females United to Universally Reduce Endo-ectocervical disease (FUTURE II) study, were presented recently [17]. In a subsample of 12,167 women who were randomized to receive quadrivalent HPV-6/11/16/18 recombinant vaccine (Gardasil; Merck, Inc.) or placebo and who followed the protocol closely, the vaccine was 100% effective in preventing incident HPV-16/18–related CIN 2/3, adenocarcinoma in situ, and cervical cancer during 2 years of follow-up. The vaccine was well tolerated, and no vaccine-related serious adverse events were reported. On June 8, 2006, the US Food and Drug Administration announced the approval of Gardasil, the first vaccine developed to prevent cervical cancer, precancerous genital lesions, and genital warts due to HPV types 6, 11, 16, and 18 [18].

From a technical perspective, vaccination with VLPs appears promising. Nevertheless, several practical issues must be addressed before these vaccines can be deployed in clinical practice and public health programs.

How will HPV vaccines affect recommendations for cervical screening? In the short to medium term, there should be little impact on frequency of screening. Because the vaccines may initially cover only types 16 and 18, one must continue to screen for the other 30% of HPV disease caused by the types not in the first versions of

Table 1. Comparison of quadrivalent and bivalent L1 VLP prophylactic vaccines

Study	Harper et al. [13••]	Villa et al. [16••]
Design	Randomized, double-blind controlled trial	Randomized, double-blind, controlled trial
Vaccine type	Bivalent HPV-16/18 VLP; L1 capsid component	Quadrivalent HPV-6/11/16/18 VLP, L1 capsid component
Age, *y*	15–25	16–23
Trial size	56 vaccinees, 553 placebo	277 vaccinees, 275 placebo
Site	US, Canada, Brazil	US, Brazil, Europe
Antigen	20 μg HPV-16	20 μg HPV-6,
	20 μg HPV-18	40 μg HPV-16, 20 μg HPV-11
	500 μg aluminum hydroxide	
Adjuvant	50 μg 3-deacylated monophosphoryl lipid (ASO4)	225 μg aluminum hydroxyphosphate sulfate
Dose and administration	0.5 mL intramuscular	0.5 mL intramuscular
Schedule, *mo*	0, 1, 6	0, 2, 6
Follow-up, *mo*	Up to 27	Up to 35
Clinical outcome	100% efficacy in preventing persistent HPV-16/18 infection	90% efficacy in preventing HPV-6/11/16/18 infection
	93% efficacy in preventing cytologic abnormalities	100% efficacy in preventing cytologic abnormalities
Major adverse effects	None	None

HPV—human papillomavirus; VLP—virus-like particle.

these vaccines. Screening programs may evolve from a cytopathologic basis to a DNA testing base over time. In the longer term, screening recommendations might be modified based on the field data and cost-effectiveness considerations, but some level of screening is likely to be required for decades [19].

Concerns have been raised about the impact of HPV vaccination on both sexual risk behaviors and screening behaviors. Some have expressed concern that adolescents who receive an HPV vaccine may feel less vulnerable to sexually transmitted infections (STI) and thus practice riskier sexual behaviors; however, no published data support this concern. Vaccinated women should understand that HPV vaccines will not prevent infection with other sexually transmitted diseases, nor will their introduction eliminate the need for cervical cancer screening [20••].

Therapeutic Vaccines

Despite encouraging results in preventive vaccine studies, development of therapeutic HPV vaccines for cervical cancer or precancerous lesions remains a high priority. HPV viral DNA is present in 99.7% of cervical carcinomas, and it is often integrated into the host genome. HPV therapeutic vaccines must include the early HPV proteins/peptides (eg, E7) rather than the late proteins as used in VLPs that are explored for preventive vaccinations [21]. Consensus has also been reached that therapeutic vaccines for cervical cancer need to induce a cell-mediated immune response. Two oncogenic HPV proteins, E6 and E7, are constitutively expressed in malignant lesions. These proteins elicit and maintain cervical cancers and are not deleted from HPV-transformed cells.

HPV infection of epithelial cells results in expression of immunosuppressive cytokines by these cells [22•]. In addition, low protein expression by HPV-infected cells, the absence of double-stranded RNA, inhibition of type 1 interferon production in infected cells, the lack of a danger signal, and cross presentation of antigens all serve to aid HPV in evading the immune system [23]. To overcome these obstacles, a successful HPV vaccine must be designed to enhance immunogenicity, accelerate antigen presentation, induce maturation and activation of antigen-presenting cells, rapidly expand specific CD8+ and CD4+ T cells, induce long-lasting memory cells, support a Th1 cytokine milieu, and awaken the immune system by danger signals [24].

Various forms of HPV vaccines have been described in experimental systems targeting HPV-16 E6 and E7 proteins (Table 2). Most studies have focused on E7 because it is more abundantly expressed and better characterized immunologically [25]. Furthermore, its sequence is more conserved than that of the *E6* gene.

Viral Vector Vaccines

Viral vector vaccines have the advantages of being highly immunogenic and having different immunogenic

Table 2. Characteristics of therapeutic HPV vaccines

Vaccine type	Advantages	Drawbacks
Vector-based: viral/bacterial	Highly immunogenic	Safety concerns, previous immunization
Peptide-based	Safe, easy to produce	Weakly immunogenic, requires HLA compatibility
Protein-based	Safe, no HLA restriction	Weak activator of cell-mediated immunity
DNA	Easy to produce, store, and transport; sustained antigenic expression	Weakly immunogenic
DC-based	Highly immunogenic	Difficult to produce and biodeliver

DC—dendritic cell; HPV—human papillomavirus.

properties of viruses. The drawbacks include safety concerns and preexisting viral immunity in the recipient (Table 2). Several preclinical studies have shown that immunotherapy targeting E6, E7, or both using vaccinia vectors generates strong cytotoxic T-lymphocyte (CTL) activity and antitumor responses. A live recombinant vaccinia virus encoding HPV-16 and HPV-18 E6 and E7 (TA-HPV) has been used in the treatment of high-grade vulvar intraepithelial neoplasia (VIN) [26]. Some, but not complete, correlation was shown between HPV immunity and clinical response defined by lesion shrinkage 24 weeks after vaccination. The best correlation with response was local immune infiltration with CD4+, CD8+, and CD1a+ immune cells.

Garcia-Hernandez et al. [27] recently conducted a phase II clinical trial to evaluate the potential use of the MVA E2 recombinant vaccinia virus in treatment of high-grade lesions (CIN 2 and CIN 3) associated with oncogenic papillomavirus. Fifty-four female patients with high-degree lesions were treated either with an MVA E2 therapeutic vaccine or with conization. Thirty-four women received the therapeutic vaccine, injected directly into the cervix once every week over a 6-week period. Twenty control patients were treated with conization. By colposcopy, 19 patients out of 34 showed no lesion, in three patients the lesions were reduced by 85% to 90%, in eight others lesions were reduced by 60%, and in four more patients lesions were reduced by 25%. Histologic analysis showed total elimination of high-grade lesions in 20 of 34 patients after treatment with MVA E2. All patients developed antibodies against the MVA E2 vaccine and generated a specific cytotoxic response against papilloma-transformed cells. Conization eliminated the lesions in 80% of the patients, but patients did not develop cytotoxic activity specifically against cancer cells, and the vaccine did not eliminate the papillomavirus. In addition, three patients treated with conization had recurrence of lesions 1 year later. These results show that therapeutic vaccination with MVA E2 proved to be very effective in stimulating the immune system against papillomavirus and in generating regression of high-grade lesions.

Bacterial Vector Vaccines

Bacterial vector vaccines are highly immunogenic and can deliver engineered plasmids or express proteins. As with viral vaccines, safety concerns, preexisting immunity, and inhibited repeat immunization limit their clinical application.

Listeria monocytogenes is an intracellular bacterium that targets both MHC classes and affects the innate and acquired immune systems, including effects on tumor angiogenesis, antitumor suppressor factors, and cytokines [28]. An attenuated *Listeria* strain has been made (Lm-ActA-E7) that secretes a fusion protein containing E7. In mice, intraperitoneal administration of this vaccine resulted in generation of cytotoxic T cells and complete regression of implanted syngeneic tumors [29].

Lactobacillus lactus, a nonpathogenic, noninvasive bacterium, has been used to produce the HPV-16 E7 protein. This inducible bacterial form of the E7 protein was effective in inducing antigen-specific T-cell responses in mice after intranasal immunization [30]. Such mucosal vaccines may ultimately be useful in treatment or prevention of HPV lesions in humans.

Peptide Vaccines

Peptide vaccines have the advantages of safety and ease of production; however, their weak immunogenic properties and the need for HLA matching must be overcome. In a study by Muderspach et al. [31], 18 women with high-grade cervical or vulvar dysplasia who were positive for HPV-16 and HLA-A2 were treated with escalating doses of a vaccine consisting of a nine–amino acid peptide encoded by the *E7* gene emulsified with incomplete Freund's adjuvant. Only three of the 18 patients were free of dysplasia after vaccination, but an increased S100+ dendritic cell (DC) infiltrate was observed in six of six patients tested. Virologic assays revealed that 12 of 18 patients showed no virus in cervical scrapings by the fourth vaccine injection, but all biopsy samples were still positive by in situ RNA hybridization after vaccination. In addition to the three complete responders, six patients had partial colposcopically measured regression of their CIN lesions.

Table 3. Treating high-grade cervical intraepithelial neoplasia: comparison of ZYC101a and HspE7

Study	Garcia et al. [35•]	Einstein et al. [34]
	ZYC101a (MGI Pharmaceuticals)	HspE7 (Stressgen Biotechnologies)
Design	Randomized, double-blind controlled trial	Single-stage phase II
Age, *y*	18 or older	Not specified
Enrollees, *n*	127	31
Delivery system	Encapsulated polynucleotide	*Mycobacterium* bovis BCG heat-shock protein
Antigen	HPV-16 and -18 E6/E7	HPV-16 E7
Disease group	CIN 2/3	CIN 3
Vaccination schedule, *mo*	0, 3, 6	0, 1, 2
Follow-up, *mo*	6	4
Clinical outcome	67%–72% resolution of CIN2/3 in younger-than-25-y group (23% in placebo group)	32% resolution of CIN3; 39% PR, 29% stable disease
Adverse effects	Injection site pain, erythema, induration	Not specified

BCG—Bacille Calmette-Guérin; CIN—cervical intraepithelial neoplasia; HPV—human papillomavirus; PR—partial response.

Protein Vaccines

Whereas peptide vaccines exhibit MHC restriction, protein-based vaccines can bypass this restriction and thus are less dependent on the HLA type of the patient (Table 2). The potency of HPV-16 E7 peptide-based vaccines may be further enhanced through the use of adjuvants or fusion proteins.

A fusion protein vaccine including the bacillus Calmette-Guérin HSP65 and the HPV-16 E7 sequence (HSPE7; Stressgen, Collegeville, PA) has been tested in patients with anal intraepithelial neoplasia, recurrent respiratory papillomatosis, and CIN [32]. HspE7 induced partial responses in patients with anal intraepithelial neoplasia and reduced the requirement for surgical treatment in pediatric patients with recurrent respiratory papillomatosis [33]. In an ongoing phase II HspE7 trial (National Cancer Institute protocol #5850) in women with CIN 3, all patients underwent a loop electrosurgical excision procedure or cone biopsy ablation at 4 months. Ten of 31 patients (32%) had complete pathologic regression, 12 (39%) had partial regression, and nine (29%) had stable disease. The overall response rate was 71% (95% CI, 55%–87%). No patient experienced progression (Table 3) [34]. All three trials revealed responses that were CD4 T cell–count independent and were not HPV type specific.

DNA Vaccines

DNA vaccines allow for sustained expression of antigen on MHC–peptide complexes, compared with peptide or protein vaccines. One strategy to improve delivery and antigenicity of HPV DNA vaccines is the use of encapsulation. Garcia et al. [35•] reported on the use of encapsulated plasmid DNA-encoding fragments derived from E6 and E7 of HPV-16 and HPV-18 in biodegradable particles (ZYC101a). Their study population included women with biopsy-confirmed CIN 2 or CIN 3 (Table 3). Approximately 6 months after study entry, all patients underwent an excisional procedure of the cervix, most often a loop electrosurgical excision procedure. Patients were monitored with periodic colposcopic evaluations, cytology, and HPV testing. There was a statistically significant higher rate of CIN 2 or CIN 3 resolution in vaccinated women younger than 25 years. However, no difference in resolution rates was observed between vaccine (either dosage) and placebo in the group aged younger than 25 years. Neither immune parameters nor other variables, such as tobacco use or infection with specific HPV types, correlated with response or lack thereof. This vaccine has been acquired by MGI Pharmaceuticals (Bloomington, MN; http://www.mgipharma.com), and further studies are planned.

Recently, Lin et al. [36] developed a codon-optimized HPV-16 E6 DNA vaccine (pNGVL4a-E6/opt) and characterized the E6-specific CD8+ T-cell immune responses as well as the protective and therapeutic antitumor effects in vaccinated C57BL/6 mice. Their data indicates that transfection of human embryonic kidney cells with pNGVL4a-E6/opt resulted in highly efficient translation of E6. In addition, this vaccine significantly enhanced E6-specific CD8+ T-cell immune responses in C57BL/6 mice. Vaccinated mice were able to generate potent protective and therapeutic antitumor effects against challenge with the E6-expressing tumor cell line, TC-1. Thus, DNA vaccines encoding a codon-optimized HPV-16 E6 may be a promising strategy for improving the potency of prophylactic and therapeutic HPV vaccines with potential clinical implications.

Cell-based Vaccines

Studies performed by several groups have established the key role played by DCs in the immune system and provide a rationale for using DCs as natural adjuvants for human

immunotherapy [37]. DCs are highly potent professional antigen-presenting cells of bone marrow origin that can stimulate both primary and secondary T- and B-cell responses [38]. The in vitro establishment and standardization of human DC cultures from the peripheral blood of patients have facilitated their use as immunotherapeutic agents for the treatment of infectious diseases and a variety of human tumors.

In a study by Santin et al. [39•], autologous monocyte-derived DCs were pulsed with recombinant HPV-16 E7 or HPV-18 E7 oncoproteins and administered to four patients with cervical cancer. Vaccinations were followed by subcutaneous administration twice daily of low doses of human recombinant interleukin-2 (1×106 IU/m^2) from day 3 to day 7. Three out of four patients were found to be significantly immunocompromised before starting the vaccination treatment, as assessed by delayed-type hypersensitivity (DTH) with a panel of recall antigens. Specific humoral and cellular CD4+ T-cell responses to the E7 vaccine were detected in two patients, and increased numbers of E7-specific interferon-γ–secreting CD8+ T cells were detected in all patients after vaccination. Swelling and induration (ie, positive DTH response) to the intradermal injection of HPV E7 oncoprotein, irradiated autologous tumor cells, or both were detected in two patients after six vaccinations. No objective clinical responses were observed. However, both patients who developed a positive DTH to the vaccine experienced a slow tumor progression (ie, 13 months survival), whereas DTH-unresponsive patients died within 5 months from the beginning of therapy. The investigators concluded that autologous DCs pulsed with HPV-16/18 E7 proteins can induce systemic B- and T-cell responses in patients who are unresponsive to standard treatment modalities. However, treatment-induced immunosuppression may impose severe limitations on the efficacy of active vaccination strategies in patients with late-stage cervical cancer. DC-based vaccination trials are warranted in immunocompetent cervical cancer patients with early-stage disease, limited tumor burden, or both and those at significant risk for tumor recurrence or disease progression.

Combined Approaches

Therapeutic vaccination may be useful in the treatment of premalignant lesions in conjunction with prophylactic strategies. It has been shown that VLPs can activate DCs, and that HPV-16 VLP-E7 chimera vaccines can generate useful T-cell responses to E7 [40] as well as neutralizing antibodies to viral capsids. This approach could provide a means to treat incident HPV infection.

Conclusions

The ability to generate VLPs by synthesis and self-assembly of the major virus capsid protein L1 has paved the way for development of prophylactic HPV vaccines. These vaccines are immunogenic and safe, and data from proof of principle efficacy trials suggest strongly that they will protect against persistent HPV infection, cervical dysplasia, and cervical cancer. However, the duration of protection provided by these vaccines is not known, the induced antibody responses are HPV type specific, and immunization must occur before exposure to the virus.

The ideal therapeutic vaccine would eliminate established HPV-induced cervical lesions without affecting normal cells. It should elicit a sustained and robust cytotoxic T-cell response, while being cost effective and safe. Despite these challenges, there is growing confidence in several therapeutic strategies using high-risk HPV *E6* and *E7* oncogenes in different delivery systems. In clinical trials these vaccines are immunogenic and safe but show limited efficacy; further scientific developments are needed before they can be deployed in clinical practice.

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