Vaccination to Prevent and Treat Cervical Cancer

RICHARD B. S. RODEN, PHD, MORRIS LING, BS, AND T.-C. WU, MD, PHD

Human papillomaviruses (HPVs) are the primary etiologic agents of cervical cancer. Thus, cervical cancer and other HPV-associated malignancies might be prevented or treated by HPV vaccines. Transmission of papillomavirus may be prevented by the generation of antibodies to capsid proteins L1 and L2 that neutralize viral infection. However, because the capsid proteins are not expressed at detectable levels by infected basal keratinocytes or in HPV-transformed cells, therapeutic vaccines generally target non-structural early viral antigens. Two HPV oncogenic proteins, E6 and E7, are critical to the induction and maintenance of cellular transformation and are coexpressed in the majority of HPV-containing carcinomas. Thus, therapeutic vaccines targeting E6 and E7 may provide the best option for controlling HPV-associated malignancies. Various candidate therapeutic HPV vaccines are currently being tested whereby E6 and/or E7 are administered in live vectors, as peptides or protein, in nucleic acid form, as components of chimeric virus-like particles, or in cell-based vaccines. Encouraging results from experimental vaccination systems in animal models have led to several prophylactic and therapeutic vaccine clinical trials. If these preventive and therapeutic HPV vaccines prove successful in patients, as they have in animal models, then oncogenic HPV infection and its associated malignancies may be controllable by vaccination.

Approximately 500,000 women worldwide develop cervical cancer yearly, and cervical cancer is the third-leading cause of cancer death in women. Over the past 20 years, epidemiologic and virological data have identified a clear and consistent association of HPV infection with the development of cervical cancer. The evidence linking human papillomaviruses (HPVs) to cervical cancer comes from a wide variety of epidemiologic and laboratory studies. More than 99% of cervical cancers and their precursor lesions, squamous intraepithelial lesions (SILs), contain HPV DNA.1 Furthermore, molecular, biochemical, and cellular studies have unequivocally demonstrated that E6 and E7, HPV gene products that are consistently expressed in SILs and cervical cancer, lead to malignant transformation of epithelial cells.2 Although more than 100 HPV genotypes have been identified, about 80% of cervical cancer is associated with 4 "high-risk" types of HPV (types 16, 18, 31, and 45), and the remaining cases are associated with a dozen other oncogenic types.3,4 HPV-16 is present in approximately 50% of all cervical cancers3 and consequently has been the focus of many recent HPV vaccine developments.

Clinical, pathological, and virological studies have defined a progression of events in the development of cervical cancer. The pathogenesis of cervical cancer is initiated by HPV infection of the cervical epithelium during sexual intercourse. Most genital HPV infections are transient.5 However, a fraction of infections persist and initiate transformation events within cervical epithelium. The cervical epithelial changes associated with this initial set of events, pathologically classified as low-grade SILs (LSILs; cervical intraepithelial neoplasia [CIN] 1), are associated with continued viral replication and virus shedding. In a study of 241 cytologically normal women recruited in a sexually transmitted disease clinic, the cumulative incidence of high-grade SILs (HSILs; CIN 2-3) at 2 years was 28% in HPV-positive women, compared with 3% in HPV-negative women.6 The progression from HSIL to invasive cancer occurs at high frequency. In the majority of cases, progression is associated with conversion of the viral genome from an episomal form to an integrated form, along with deletion or inactivation of E2, a negative regulator of E6 and E7 expression. Development of invasive cancer requires additional genetic events facilitated by E6- and E7-mediated inactivation of the genome guardians p53 and pRb, genomic instability, and suppression of apoptosis.7

Several studies have demonstrated that virus-neutralizing antibodies mediate protection of animals from experimental papillomavirus infection.7,8 For example, passive transfer of sera from virus-like particle (VLP)-
vaccinated rabbits to naïve rabbits is sufficient for protection.\textsuperscript{7,8} 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.\textsuperscript{9}

Although antibody-mediated neutralization of virus has important preventive utility, several lines of evidence suggest that cell-mediated immune responses are important in controlling established HPV infections as well as HPV-associated neoplasms:\textsuperscript{10}

1. The prevalence of HPV-related diseases (infections and neoplasms) is increased in patients with impaired cell-mediated immunity, including transplant recipients\textsuperscript{1} and human immunodeficiency virus–infected patients.\textsuperscript{1,2,14}

2. Animals immunized with nonstructural viral proteins are protected from papillomavirus infection or the development of neoplasia. Immunization also facilitates the regression of existing lesions.\textsuperscript{13,16}

3. Infiltrating CD4+ (T-helper cells) and CD8+ (cytotoxic T cells) cells have been observed in spontaneously regressing warts.\textsuperscript{17}

4. Warts in patients receiving immunosuppressive therapy often disappear when treatment is discontinued.\textsuperscript{18}

The well-characterized foreign (viral) antigens and the well-defined virological, genetic, and pathological progression of HPV—from initial infection to lesion formation to malignant tumor formation—have provided a unique opportunity to evaluate interventions with antigen-specific immunotherapy. Conceptually, 2 different types of HPV vaccines can be designed: prophylactic (preventive) vaccines that prevent HPV infection, and therapeutic (curative) vaccines that induce regression of established HPV infection and its sequelae.

### PREVENTIVE HPV VACCINES

Preventive HPV vaccine development has been complicated by a lack of animal models for the genital mucosatropic HPVs and by difficulty in propagating the virus in culture. These problems have been partially overcome using cutaneous and mucosal animal papillomaviruses as models and by the development of VLPs and pseudovirions.

The papillomavirus major capsid protein L1—overexpressed in mammalian,\textsuperscript{19,20} insect,\textsuperscript{21} yeast,\textsuperscript{22} and bacterial cells\textsuperscript{23}—spontaneously assembles to form VLPs that are devoid of the oncogenic viral genome. Parenteral injection of these VLPs, or even the pentameric L1 capsomer,\textsuperscript{24} elicits high titers of serum-neutralizing antibodies and protection from experimental challenge with infectious virus in several animal papillomavirus models.\textsuperscript{7,8,25} Genetic vaccination with an L1-expression vector also protects from experimental viral challenge.\textsuperscript{26-28} A summary of published L1-based prophylactic vaccine studies in animal models and humans is presented in Table 1.

### Table 1. Summary of Published L1-Based Prophylactic Vaccine Studies in Animal Models and Humans

<table>
<thead>
<tr>
<th>Study (et al)</th>
<th>Virus and animal</th>
<th>Dose and adjuvant</th>
<th>Vaccination schedule</th>
<th>ELISA Titer</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jarrett</td>
<td>BPV1,2,4,5,6: cow</td>
<td>Virus</td>
<td>0, 3 weeks</td>
<td>&gt;1000</td>
<td>+4 weeks</td>
</tr>
<tr>
<td>Bell</td>
<td>COPV: dog</td>
<td>2× inactivated virus</td>
<td>0, 2 weeks</td>
<td>N/D</td>
<td>+4 weeks</td>
</tr>
<tr>
<td>Donnelly</td>
<td>CRPV: rabbit</td>
<td>1 mg L1 DNA</td>
<td>0, 3 weeks</td>
<td>3000</td>
<td>+4 weeks</td>
</tr>
<tr>
<td>Stanley</td>
<td>CRPV: dog</td>
<td>9μg L1 DNA</td>
<td>0, 6, 12 weeks</td>
<td>320</td>
<td>+3 weeks</td>
</tr>
<tr>
<td>Lin</td>
<td>CRPV: rabbit</td>
<td>Vaccinia L1 10^6 pfu</td>
<td>0, 4 weeks</td>
<td>810–1080</td>
<td>+5 weeks</td>
</tr>
<tr>
<td>Beereburd</td>
<td>CRPV: rabbit</td>
<td>5× 50 μg; VLP: Alum</td>
<td>0, 2 weeks</td>
<td>5000</td>
<td>+1 week</td>
</tr>
<tr>
<td>Kirnbauer</td>
<td>BPV4: cow</td>
<td>2× 150 μg; VLP: alum</td>
<td>0, 4 weeks</td>
<td>1000</td>
<td>+2 weeks</td>
</tr>
<tr>
<td>Suzich</td>
<td>CRPV: dog</td>
<td>2× 20μg; VLP: None</td>
<td>0, 2 weeks</td>
<td>&lt;1000</td>
<td>+2 weeks</td>
</tr>
<tr>
<td>Christensen</td>
<td>CRPV: rabbit</td>
<td>3× 50μg; VLP: None</td>
<td>0, 4, 8 weeks</td>
<td>10,000</td>
<td>+2 weeks</td>
</tr>
<tr>
<td>Jansen</td>
<td>CRPV: rabbit</td>
<td>3× 1μg; VLP: none</td>
<td>0, 4, 8 weeks</td>
<td>10,000</td>
<td>+2 weeks</td>
</tr>
<tr>
<td>Yuan</td>
<td>COPV: dog</td>
<td>0.4μg L1; Pentamers: none</td>
<td>0, 2 weeks</td>
<td>&gt;100</td>
<td>+2 weeks</td>
</tr>
<tr>
<td>Harro</td>
<td>HPV16: human</td>
<td>3 × 10 or 50 μg; VLP: Alum</td>
<td>0, 1, 4 months</td>
<td>10,000</td>
<td>N/A</td>
</tr>
<tr>
<td>Evans</td>
<td>HPV11: human</td>
<td>3 × 3, 9, 30, 100 μg; VLP: Alum</td>
<td>0, 1, 4 months</td>
<td>2786–6400</td>
<td>N/A</td>
</tr>
<tr>
<td>Zhang</td>
<td>HPV6b: human</td>
<td>5×+×1, 5, 10 μg VLP</td>
<td>2 × month</td>
<td>&gt;100</td>
<td>N/A</td>
</tr>
<tr>
<td>Koutsky</td>
<td>HPV16: human</td>
<td>3 × 40 μg; VLP: alum</td>
<td>0, 2, 6 months</td>
<td>1510 μmolU/mL</td>
<td>Natural transmission</td>
</tr>
<tr>
<td>Dubin*</td>
<td>HPV16 and 18: human</td>
<td>3 × HPV16/18: VLP: 3-deacetylated monophosphoryl lipid A</td>
<td>0, 1, 6 months</td>
<td>Natural transmission</td>
<td></td>
</tr>
</tbody>
</table>

*21st International Papillomavirus Conference, Mexico City, 2004; Abstract #412.
Although VLP vaccination provides immunity from experimental inoculation, protection against sexual transmission of HPV requires neutralizing antibodies acting at mucosal surfaces. Serum-neutralizing IgG induced by parenteral VLP vaccination may enter via transudation into the genital tract and maintain a sufficient level to provide sterilizing immunity across the menstrual cycle. Neutralizing antibodies may either transudate from plasma into genital secretions or be synthesized by local plasma cells. Induction of plasma cells requires direct immunization of the mucosa-associated lymphoid tissue, but nasal instillation of VLPs was found to be efficient in generating specific antibodies, including IgG in serum and IgG and IgA in mucosal secretions of mice. More recently, oral vaccination with HPV VLPs in mice has been shown to induce systemic virus-neutralizing antibodies, suggesting that HPV VLPs may be antigenically stable in the environment of the gastrointestinal tract. These studies provide the possibility of vaccinating large populations with HPV VLP without using syringes.

In recent clinical data, intramuscular vaccination of women with HPV-16 VLPs was found to induce significant antibody titers in cervical secretions. A comparison by the same researchers of nasal spray or aerosol for mucosal delivery of 50 μg of HPV-16 VLPs to humans revealed that aerosol was effective at inducing an antibody response, with IgG found predominantly in serum and both IgG and IgA found in cervical secretions.

Early phase I/II clinical trials using HPV L1 VLP delivered intramuscularly have demonstrated the immunogenicity and safety of this vaccine. Importantly, Koutsy et al recently reported data from a clinical trial of HPV 16 L1 VLPs indicating for the first time that a vaccine strategy can be implemented in humans to prevent HPV-16 infections and HPV-16-associated premalignant lesions. A similar vaccine strategy is being tested with HPV-11 VLPs, and VLP vaccination also may be extended to other oncogenic HPV types. Vaccination of a single animal with multiple HPV VLP types does not detract from the response to the individual types. A quadrivalent HPV VLP vaccine (types 6, 11, 16, and 18) from Merck is currently in a clinical trial; preliminary results have found that the vaccine is well tolerated and generates neutralizing antibody titers. These HPV VLP vaccines may be useful in the prevention not only of cervical cancer, but also of other HPV-associated malignancies, including a subset of head and neck squamous cell carcinoma, vulvar cancers, and other anogenital cancers.

A clearer picture of the long-term effects, including duration of antibody titers and side effects, will likely emerge several years after this study and other parallel studies of HPV VLP vaccines have concluded. Concern has been raised that widespread HPV-16 vaccination may eventually exert evolutionary pressure, selecting outgrowths of other types of HPV and/or some HPV-16 variants that carry mutation(s) on the L1 gene and thus are unaffected by the vaccine. But this is improbable, given the slow evolution of HPV and evidence of cross-neutralization of variants by immune sera. Developing nations tend to have the highest prevalence of HPV-associated lesions and cervical cancer–related cancer death. Although the VLP vaccine shows great promise for HPV control in industrialized nations, it is probably not ready for use in developing countries due to its cost. Many developing countries do not have sufficient resources for routine Pap smear testing, let alone preparation and parenteral administration of VLPs. Another difficulty is the instability of the preventive HPV VLP vaccine at room temperature over long periods. The VLP vaccine requires sophisticated equipment for production and refrigerated storage facilities. Future vaccine research should work toward a new generation of HPV vaccines that are cheaper, more easily administered, and more stable to be applicable worldwide. Naked DNA vaccines that express L1 are effective in animals and may be cheaper than using purified VLP, particularly in the formulation of polymeric vaccines based on multiple HPV genotypes.

There are at least 15 oncogenic HPV genotypes, and in vitro neutralization studies suggest that they may represent an equivalent number of serotypes, although limited cross-reactivity can occur between L1s of different genotypes with >85% sequence identity. The multitude of distinct HPV serotypes has probably evolved from evolutionary pressure to evade neutralizing antibodies. Indeed, limited animal studies suggest that vaccination with L1 VLP of a heterologous genotype is not protective, although this has not been tested for more closely related genotypes. Therefore, protection against infection by all of these types with a VLP-based vaccine would likely require a highly polymeric vaccine that would be difficult to generate. Another potential alternative for comprehensive protection is to vaccinate against L2. L2 is immunologically subdominant to L1, because immunization with virions or capsids composed of L1 and L2 elicits almost exclusively L1-specific antibody. However, induction of in vitro neutralizing antibody and protection of animals from experimental infection by vaccination with recombinant L2 alone has demonstrated the promise of L2 as a prophylactic vaccine (Table 2).

Indeed, L2-specific cross-neutralizing polyclonal sera and monoclonal antibodies have been demonstrated. A recent study by Embers et al indicated that protection by L2 peptide vaccination is likely mediated by neutralizing antibody. Vaccination of patients with fusion proteins containing L2 has proven safe. As seen in animal models, the humoral immune responses of patients to L2 are much weaker than the responses to L1 VLP. Therefore, if the relatively weak immunogenicity of L2 can be overcome, then vaccination against L2 holds great potential for prophylaxis against infection by a broad range of HPV types and their associated cancers.
Table 2. Summary of Published L2 Vaccine Studies in Animal Models and Humans

<table>
<thead>
<tr>
<th>Study (et al)</th>
<th>Virus and host</th>
<th>Dose and adjuvant</th>
<th>Vaccination schedule (weeks)</th>
<th>Challenge (weeks)*</th>
<th>Neutralizing (ELISA) titer</th>
<th>Protected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Christensen 1991</td>
<td>CRPV: rabbit</td>
<td>4× gelpure: CFA</td>
<td>0, 4, 8, 12</td>
<td>+2</td>
<td>32</td>
<td>Yes</td>
</tr>
<tr>
<td>Lin 1992</td>
<td>CRPV: rabbit</td>
<td>4× 250 µg: RIBI</td>
<td>0, 3, 6, 9</td>
<td>+2</td>
<td>10 (1000)</td>
<td>Yes</td>
</tr>
<tr>
<td>Embers 2002</td>
<td>CRPV, CRPV: rabbit</td>
<td>3× 500 µg peptide/KLH: -CFA/IFA</td>
<td>0, 4, 8</td>
<td>+4-12</td>
<td>1000</td>
<td>Yes</td>
</tr>
<tr>
<td>Campo 1993; Chandrachud 1995; Gaukroger 1996</td>
<td>BPV4: cattle</td>
<td>2× 100 µg: alum</td>
<td>0, 4</td>
<td>+2</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td>Jarret 1991</td>
<td>BPV2: cattle</td>
<td>2× 50 µg: none</td>
<td>0, 4</td>
<td>+2</td>
<td>(100)</td>
<td></td>
</tr>
<tr>
<td>Thompson 1999; Lacey 1999</td>
<td>HPV6: human</td>
<td>2× 1 mg: alum</td>
<td>0, 4</td>
<td>+2</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Kanda§</td>
<td>HPV, human</td>
<td>HPV-16 L2 peptide amino acids I08-I20 500 mg, 100 mg</td>
<td>0, 4, 12</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Experimental challenge with the homologous papillomavirus type.
†A 5-fold dilution of serum was the only serum concentration tested.
‡Cattle were not protected, but warts were eventually cleared in the vaccine group. This may reflect reduced inoculum due to partial neutralization or clearance, perhaps as a bystander effect. This enhanced clearance was not described in any other L2 vaccine study.
§19th Papillomavirus Meeting, 2001; Abstract #0103.
Abbreviation: ND, not done.

THERAPEUTIC HPV VACCINES

Although vaccination with preventive HPV vaccines is able to generate high titers of serum-neutralizing antibodies in animals and in humans, such immunization may not be able to generate significant therapeutic effects for established or breakthrough HPV infections that have escaped antibody-mediated neutralization. Preexisting HPV infection is highly prevalent and is responsible for considerable morbidity and mortality. Therapeutic vaccines should induce specific cell-mediated immunity that prevents the development of lesions and eliminates preexisting lesions or even malignant tumors. Early viral antigens (E1, E2, E5, E6, and E7) have potential as therapeutic vaccine antigens. However, unlike E6 and E7, neither E1 nor E2 is consistently expressed in carcinoma. E5 shows limited immunogenicity and has not been extensively studied as a vaccine antigen. Likewise, E4 and the L1 and L2 capsid proteins are unlikely to be suitable targets for therapeutic vaccine development, because these proteins are not detectably expressed in basal epithelial cells of benign lesions or in abnormal proliferative cells of premalignant and malignant lesions. Although recent studies indicate that vaccination with VLPs may generate a capsid protein–specific cytotoxic T lymphocyte (CTL) response, such a response against L1 or L1/L2 VLPs alone may not be able to generate a significant therapeutic effect.

Whereas most tumor-specific antigens are derived from normal or mutated proteins, E6 and E7 are completely foreign viral proteins, and thus they may harbor more antigenic peptides/epitopes than a mutant cellular protein. Furthermore, because E6 and E7 are required for the induction and maintenance of the malignant phenotype of cancer cells, cervical cancer cells are unlikely to evade an immune response through antigen loss. Thus, although care must be taken, given the oncogenic nature of these genes, E6 and E7 proteins represent good targets for developing antigen-specific immunotherapies or vaccines for cervical cancer.

Various forms of HPV vaccines, including vector-based vaccines, peptide-based vaccines, protein-based vaccines, DNA-based vaccines, chimeric VLP-based vaccines, and cell-based vaccines, have been described in experimental systems targeting HPV-16 E6 and E7 proteins. Most studies focus on E7, because it is more abundantly expressed and better characterized immunologically. Furthermore, its sequence is more conserved than that of the E6 gene. A summary of therapeutic HPV vaccines, their advantages, and their disadvantages is provided in Table 3.

The following sections describe each of the therapeutic HPV vaccines outlined in Table 3 (or combined approaches).

Live Vector Vaccines

Viral Vector Vaccines

Viral vectors, such as vaccinia virus and adenovirus, have been evaluated as tools for HPV vaccine development. Modified or replication-deficient forms of viruses, such as adenovirus or vaccinia vectors, have been used to generate a potent immunogenic response with minimal toxicity.

Several studies have shown that immunotherapy targeting E6 and/or E7 using vaccinia vectors generates strong CTL activity and antitumor responses in preclinical studies. Strategies that affect the intracellular
sorting or localization of antigen may be able to improve the in vivo therapeutic potency of recombinant vaccinia vaccines against cervical cancer. Results of phase I/II clinical trials using recombinant vaccinia virus encoding HPV-16 and 18 E6/E7 (also called TA-HPV) indicated that some patients with advanced cervical cancer, CIN 3, or early invasive cervical cancer developed T-cell immune responses after vaccination.

Studies using modified adenovirus (Adv)-expressing HPV-16 E6 or E7 for vaccination or for ex vivo preparation of dendritic cell (DC)-based vaccines (Adv-E7 targeted to CD40 using bispecific Abs) have demonstrated enhancement of antigen-specific T-cell immune responses and antitumor effects. Replication-defective adeno-associated virus, a parvovirus that is nonpathogenic in humans, encoding HPV-16 E7 fused to heat-shock protein 70 (HSP70) was found to induce CD4- and CD8-dependent CTL activity and antitumor effects in vitro.

Alphavirus replicon packaging cell lines (ie, from Sindbis or Venezuelan equine encephalitis virus) can be used to produce replication-defective alphavirus replicon particles that are free of detectable replication-competent virus yet can efficiently deliver the antigen. Vaccination of mice with a replication-defective Venezuelan equine encephalitis virus replicon particle vector containing HPV16 E7 RNA can enhance E7-specific CD8+ T-cell immune responses and eliminate established tumors. Another study found that replication-defective Sindbis virus replicon particles encoding herpes simplex virus type 1 (HSV-1) VP22, linked to HPV-16 E7-generated improved E7-specific CD8+ T-cell immune responses and a potent antitumor effect in vaccinated mice.

Nonreplicative pseudovirions are formed from naked DNA encapsulated in papillomavirus capsids using various expression systems, including vaccinia virus, Semliki Forest virus, and baculovirus, or Saccharomyces cerevisiae systems. Pseudovirions can protect encapsulated DNA from nuclease activity, efficiently deliver the DNA, act as an adjuvant, and induce DC maturation. Studies have demonstrated higher frequency of gene transfer with HPV pseudovirions than with DNA alone or with liposome and induction of mucosal and systemic E7-specific CTL responses. Vaccine efficacy may be further improved by inclusion of L2.

**Table 3. Characteristics of HPV Vaccines Used to Treat Cervical Cancer**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector-based: Viral (e.g., vaccinia, Adv, AAV, alphavirus)</td>
<td>Highly immunogenic; different immunologic properties of viruses; versatility of construction</td>
</tr>
<tr>
<td>Bacterial (e.g., Listeria, Salmonella, BCG)</td>
<td>Highly immunogenic; can deliver engineered plasmids or express protein</td>
</tr>
<tr>
<td>HPV pseudovirions</td>
<td>Highly immunogenic; can induce DC maturation</td>
</tr>
<tr>
<td>Peptide-based</td>
<td>Ease of production; safety</td>
</tr>
<tr>
<td>Protein-based (including VLPs)</td>
<td>Ease of production; multiple known adjuvants; no HLA restriction</td>
</tr>
<tr>
<td>DNA</td>
<td>Ease of production, storage, and transport; versatility in ability to add targeting and/or costimulatory genes; capable of multiple immunizations</td>
</tr>
<tr>
<td>RNA</td>
<td>No risks of integration or cellular transformation; capable of multiple immunizations</td>
</tr>
<tr>
<td>DC-based</td>
<td>Highly immunogenic; multiple methods of antigen loading; gene transduction or cytokine treatment to enhance potency</td>
</tr>
<tr>
<td>Tumor cell-based</td>
<td>Useful if tumor antigen is unknown; gene transduction or cytokine treatment to enhance potency</td>
</tr>
</tbody>
</table>

**Bacterial Vector Vaccines**

Attenuated bacteria (eg, Listeria monocytogenes, Salmonella, Shigella, Escherichia coli) can serve as bacterial carriers to deliver either plasmids encoding genes of interest or proteins of interest to antigen-presenting cells (APCs). L. monocytogenes produces listeriolysin O, which allows escape into the cytoplasm after phagocytosis by macrophages and facilitates delivery of antigens into both the major histocompatibility complex (MHC)-I and MHC-II pathways. Recent studies have found that intraperitoneal or oral vaccination with recombinant L. monocytogenes secreting HPV-16 E7 can lead to regression of preexisting E7-expressing murine tumors.

Attenuated Salmonella has been used to deliver HPV-16 E7 or E7 epitopes harbored in hepatitis B virus core antigen particles or HPV-16 VLPs as therapeutic HPV vaccines. Bacille Calmette-Guerin (BCG; Mycobacterium bovis) is a safe bacterial vaccine vector (ie, for tuberculosis vaccination to induce prolonged immune responses) that has been used to develop a vaccine encoding HPV-16 L1 and E7 and has been found...
to induce E7-specific antibody and cytotoxic immune responses. The future of live viral or bacterial vectors for HPV vaccine delivery is likely in the development and refinement of modified, replication-deficient, or otherwise attenuated forms of these vectors with the goal of boosting immunogenicity while minimizing toxicity.

Peptide/Protein Vaccines

**Peptide Vaccines**

The identification and characterization of murine (H-2Db) and human (HLA-A.2) CTL epitopes for HPV-16 has promoted the development of peptide vaccines against cervical cancer. 

Peptides relevant to other HPV types (ie, HPV-18) and other HLA backgrounds (ie, HLA-B18), as well as human MHC-II–restricted T-helper cell epitopes, have also been investigated. Vaccination with lipiddated HPV-16 E7 peptide was found to generate CTL responses in some patients with HPV-associated cancer. Clinical data from another study demonstrate T-helper immune responses in humans in response to peptide vaccination with minimal adverse side effects. In another study, 10 patients with HPV-16– and HLA-A.2–positive high-grade cervical or vulvar intraepithelial neoplasia vaccinated with an E7-specific peptide vaccine exhibited measurable enhancement in cytokine release and cytolysis mediated by CTLs derived from peripheral blood mononuclear cells, and some even demonstrated partial clearance of virus and regression of lesions.

**Protein Vaccines**

Protein-based HPV vaccines have been tested in humans. Whereas peptide vaccines exhibit MHC restriction, protein-based vaccines can bypass this restriction and thus are less dependent on the patient’s HLA type. TA-GW fusion protein, which consists of HPV-6 L2 fused to E7 protein, has been tested for clinical treatment of genital warts, and TA-CIN fusion protein, which consists of HPV-16 L2/E6/E7, can induce E7-specific CD8+ T-cell immune responses and tumor protection in mice. TA-CIN was well tolerated by patients and induced both humoral and T-cell–mediated immune responses.

The potency of HPV-16 E7 peptide-based vaccines may be further enhanced through the use of adjuvants, fusion proteins (ie, HSPs), or anchor-modified peptide epitopes. Vaccination with HPV65 fused to E7 protein led to CD8-dependent and CD4-independent regression of HPV-16 E7-expressing tumors in mice, and this vaccine is now in clinical trials with Stresgen Biotechnologies for HPV-associated anal dysplasia and the National Cancer Institute for HPV-associated cervical cancer. In another fusion approach, vaccination of women with E7 fused to Hemophilus influenzae protein D (D16E7) and administered in GlaxoSmithKline adjuvant AS02B was well tolerated, leading to enhanced T-cell activity and regression of lesions in some patients.

Chimeric HPV VLPs containing E2 and/or E7 antigen can induce high-tier neutralizing antibodies in the serum, activate DCs, and prime T-cell–mediated immune responses. Currently, clinical grade HPV-16 L1/L2-E2-E7 chimeric VLPs, which contain 4 HPV-encoded proteins (L1, L2, E2, and E7) as target antigens, are being prepared for a phase I clinical trial (D. Lowy and J. Schiller, personal communication). Likewise, chimeras of L1 VLP or pentamers and E7 will soon be tested in the clinic.

**Nucleic Acid Vaccines**

**DNA Vaccines**

DNA vaccines allow for sustained expression of antigen on MHC–peptide complexes compared with peptide or protein vaccines. Furthermore, the MHC restriction of peptide-based vaccines may be bypassed with approaches that directly transduce DNA coding for antigen to APCs so that synthesized peptides can be presented by the patient’s own HLA molecules. DNA vaccines can be administered to the host by intramuscular injection, intradermal injection via hypodermic needle or gene gun (a ballistic device for delivering DNA-coated gold particles into the epidermis), intravenous injection, intranasal delivery, or biojetor delivery.

Due to the weak intrinsic potency of naked DNA vaccines, various strategies have been developed to enhance their immunogenicity. Because DCs are the primary mediators of DNA vaccine–induced immune responses, vaccines that modify intracellular or intercellular movement of antigen or other DC properties are able to enhance DNA vaccine potency. Intracellular targeting strategies are able to enhance MHC-I and/or -II presentation of antigen. Enhancement of MHC-I presentation of E7 can be achieved by linkage of E7 to M. tuberculosis Hisp70, calreticulin, the translocation domain (domain II) of Pseudomonas aeruginosa exotoxin A, or γ-tubulin in the context of a DNA vaccine, leading to potent CD8-dependent antitumor immune responses. Enhanced MHC-II presentation of E7 and antitumor immunity has been demonstrated by linkage of E7 to a signal sequence (Sig, at the N-terminus of E7) and lysosome-associated membrane protein (LAMP-1, at the C-terminus), which routes antigen to the endosome/lysosomal compartments.

Intercellular spreading of E7 is facilitated by linkage of E7 to HSV-1 VP22, or one of its homologues (bovine herpesvirus VP22 or Marek’s disease virus VP22), unique viral tegument proteins that can mobilize antigen for intercellular transport to neighboring cells. Mice vaccinated with HVP22/E7 DNA generate a significantly greater number of E7-specific CD8+ T-cell precursors and a stronger antitumor effect than wild-type E7 DNA. BVP22/E7 DNA (Hung, unpublished data) and MVP22/E7 DNA are similarly potent.

Because DCs have a limited lifespan, a method to
prolong in vivo DC survival may help improve DNA potency. Co-administration of E7-containing DNA with DNA-encoding antiapoptotic proteins (Bcl-xL, Bcl-2, X-linked inhibitor of apoptosis protein, and dominant negative caspase-9 or dominant negative caspase-8) is able to enhance E7-specific immune responses, tumor treatment, and DC survival. Other DNA-based strategies for improving E7-specific CD8+ T-cell immune responses include co-administration of E7 DNA with cytokines or costimulatory molecules, enhancing intracellular degradation of E7 protein, and codon optimization to enhance E7 antigen presentation.

Currently, a plasmid DNA vaccine encoding multiple HLA-A2-restricted epitopes derived from the HPV-16 E7 protein has been tested in a phase I clinical trial in patients with high-grade anal intraepithelial lesions. The vaccine (ZYC101) is composed of plasmid DNA encapsulated in biodegradable polymer microparticles. The initial results of the trial suggest that the DNA vaccine was well tolerated in all subjects at all dosage levels tested. Furthermore, 10 of 12 subjects demonstrated increased immune response to the peptide epitopes encoded within the DNA vaccine.

**RNA Replicon Vaccines**

Although RNA is typically less stable than DNA and often has lower transfection efficiency, self-replicating RNA replicon vectors may have improved immunogenicity. These noninfectious, self-replicating, and self-limiting RNA can be launched into RNA or DNA form, followed by transcription into RNA replicons and expression of the antigen of interest at high levels for an extended period. Studies have demonstrated that the potency of HPV-16 E7-specific self-replicating RNA vaccines can be enhanced by applying the LAMP-1 targeting strategy, the M. tuberculosis HSP70 strategy, or the HSV-1 VP22 strategy. Hsu et al. recently used DNA-launched replicons for the development of HPV vaccines and demonstrated significant E7-specific CTL activity and antitumor effects.

**Cell-Based Vaccines**

**Dendritic Cell-Based Vaccines**

Greater understanding about the origin of DCs, their antigen-uptake mechanisms, and the signals that stimulate their migration and maturation into immunostimulatory APCs has aided the development of DC-based vaccines, which include DCs pulsed with E6 and/or E7 peptides/proteins and DCs transduced with DNA, RNA, or viral vectors encoding E6 and/or E7.

Presentation of HPV E6 and/or E7 peptides to the immune system by DCs is a promising method for circumventing tumor-mediated immunosuppression. HPV-18 E7-pulsed DCs have also been used in a human cervical cancer case. Transfer of the E7 gene into DCs can be accomplished by various methods, including DNA, RNA, and viral vectors. The route of administration may be important for the efficacy of DC-based vaccines. Wang et al. transduced the HPV-16 E7 gene into a DC line by electroporation using an E7-expressing DNA plasmid and demonstrated that intramuscular administration of DC-E7–generated greater antitumor immunity than subcutaneous and intravenous administration, eliciting the highest levels of E7-specific antibody and greatest numbers of E7-specific CD4+ T-helper and CD8+ T-cell precursors.

**Tumor Cell-Based Vaccines**

Due to patient perceptions of safety, tumor cell–based vaccination may be a feasible treatment strategy only for advanced HPV-associated cancer. Transduction of tumor cells with genes encoding costimulatory molecules or cytokines may enhance immunogenicity, leading to T-cell activation and antitumor effects after vaccination. Vaccines using HPV-transformed tumor cells transduced with cytokine genes, such as interleukin-12, interleukin-2, or GM-CSF, can generate strong antitumor effects in mice.

**Combined Approaches**

Combining vaccine vehicles using a prime-boost regimen primes the immune system, then (ideally) augments and maintains a long-term immune response, making it an attractive approach for cancer immunotherapy. A study conducted by Chen et al. to test various combinations of viral vectors and nucleic acids in a prime-boost regimen concluded that priming with a DNA vaccine followed by a recombinant vaccinia booster provided the most potent antitumor effects. Kowalczyk et al. found that priming with intramuscularly delivered L1-expressing DNA and boosting with adenoviral HPV-16 L1 induces antibodies in vaccinated mice, with Ig2a and Ig2b isotypes predominating in sera.

Another combinational approach involves the simultaneous administration of DNA vaccines and other antiviral or anticancer agents. Combined administration of intralesional antiviral treatment and immune stimulation may be a feasible means of providing a long-lasting cure to persistent HPV infections. Christensen et al. topicaly administered the antiviral agent cidovor and intracutaneously administered (via a gene gun) a DNA vaccine encoding CRPV genes. Cures of large established CRPV-induced rabbit papillomas and reduced incidence of lesion recurrence were observed, suggesting that this combination may also be useful in treating persistent HPV infections.

**Summary**

The next 5 years in the field of HPV vaccinology will be very exciting. During this period, we should gain a clearer understanding of the protective efficacy of HPV VLP-based vaccines. We also anticipate preliminary results from trials of “second-generation” prophylactic vaccines that target neutralizing epitopes in both L1 and L2, as well as therapeutic
antigens. They will be designed for simplicity of preparation and administration, and, importantly, for increasing the spectrum of oncogenic HPV genotypes covered. Our understanding of the adaptive immune system and vaccines continues to improve at a dramatic pace, as does the number of novel strategies for enhancing the immunogenicity of HPV antigens. As vaccinology becomes less empirical and the multitude of new therapeutic HPV vaccines are tested in the clinic, great strides will be made toward the elimination of HPV-associated malignancy. We are particularly excited about the prospect of human trials of DNA-based vaccines in both the therapeutic and prophylactic settings. The development of better, standardized assays for cellular immunity and surrogates for clinical efficacy will be critical to the rational development of vaccines targeting HPV, and these lessons will be translated to other viral systems.

CONCLUSION

In the past decade, dramatic progress has been made in the field of HPV vaccine development. The recognition of high-risk HPV as the primary etiologic agent for cervical cancer and its precursor lesions has paved the way for the development of preventive and therapeutic HPV vaccines that may lead to the control of cervical cancer and other HPV-associated malignancies. HPV VLPs show promise as protective vaccines capable of generating neutralizing antibodies to prevent HPV infection in patients. A quarter of a century after the development of the hepatitis B vaccine, these prophylactic vaccines could prove to be the second major vaccine for the prevention of a human cancer. However, such a preventative vaccine would not benefit those with established oncogenic HPV infections. Given the prevalence of HPV infection, it is therefore critical to continue in parallel the development of therapeutic vaccine approaches. Many experimental HPV vaccine strategies including vector-based vaccines, peptide-based vaccines, protein-based vaccines, nucleic acid-based vaccines, chimeric VLP-based vaccines, cell-based vaccines, pseudovirions, and RNA replicons have been shown to enhance virus-specific immune cell activity and antitumor responses in murine tumor systems. Several clinical trials are currently underway, based on encouraging preclinical results from these therapeutic HPV vaccines. A head-to-head comparison of these vaccines will help identify the most potent therapeutic HPV vaccine with minimal negative side effects. Clinical HPV vaccine trials provide a unique opportunity to identify the characteristics and mechanisms of the immune response that best correlate with clinical vaccine potency. Such immunologic parameters will help define protective and therapeutic immune mechanisms for controlling HPV infections and HPV-related disease. Rational development of more effective vaccines for HPV infections would be greatly facilitated by comprehensive information on these protective immune mechanisms in humans. With continued endeavors in HPV vaccine development, we may soon be able to implement a variety of safe and effective strategies for the eventual control of HPV-associated cervical cancer.

Acknowledgment. This review is not intended to be an encyclopedic one, and we apologize to any authors not cited. We thank Drs. Keerit V. Shah, Michelle Moniz, and Robert J. Kurman for their helpful discussions. We also thank Chien-Fu Hung for his critical review of the manuscript.

REFERENCES

munity in healthy volunteers through vaccination with TACIN, an HPV16 L2E7E6 fusion protein vaccine. Vaccine 20:3456-3464, 2002


141. Cid-Arregui A, Juarez V, zur Hausen H: A synthetic E7 gene of human papillomavirus type 16 that yields enhanced expression of


