Cervical Cancer Vaccines: Progress and Prospects

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Cervical cancer remains a leading cause of cancer-related mortality in women, particularly in developing countries. The causal association between genital human papillomavirus (HPV) infection and cervical cancer has been firmly established and the oncogenic potential of certain HPV types has been clearly demonstrated. In recognition of the causal association of cervical cancer with this sexually transmitted viral infection, substantial interest has arisen to develop effective prophylactic and therapeutic vaccines. Prophylactic strategies currently under investigation focus on the induction of effective humoral and cellular immune responses that are potentially protective against subsequent HPV infection. Papillomavirus-like particles have been synthesized to induce neutralizing antibody responses, and impressive immunoprophylactic effects have been demonstrated in both animals and humans. For the treatment of existing HPV infection, techniques to augment cellular immunity by enhancing viral antigen recognition are under investigation. Vaccines targeting the oncogenic proteins E6 and E7 of HPV-16 and -18 are the focus of current clinical trials for cervical cancer patients. It is hoped that the development of successful HPV-specific vaccines will diminish the costs of existing cervical cancer screening programs and reduce the morbidity and mortality associated with the treatment of cervical neoplasias. (J Soc Gynecol Investig 2002;9:254–64) Copyright © 2002 by the Society for Gynecologic Investigation.

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Cervical cancer remains the second most common cancer among women worldwide, despite the implementation of cervical cytology screening programs over 50 years ago. Approximately 371,200 new cases are diagnosed each year, and nearly 200,000 deaths are attributable to the disease.[1] The widespread use of Papanicolaou smears has resulted in a 70% decline in the mortality from cervical cancer in the United States during the past 50 years.[2] However, cervical cancer remains a leading cause of cancer-related death in women in developing countries. Although successful screening has lowered the incidence of cervical cancer in developed countries, this comes at an estimated cost of nearly $6 billion annually in the United States alone.[3]

During the past quarter century, a growing body of evidence has demonstrated the etiologic association of the human papillomavirus (HPV) with a variety of anogenital cancers. Genital HPV infections are widespread among adults who have been sexually active and are estimated to have the highest incidence of any sexually transmitted disease (STD) in the United States.[4] HPV is a heterogeneous group of double-stranded closed circular deoxyribonucleic acid (DNA) viruses which consist of approximately 8 kilobases. The HPV genome encodes six early open reading frame proteins (E1, E2, E4, E5, E6, and E7) and two late open reading frame proteins (L1 and L2) on a single strand of DNA. The two "L" genes encode for viral capsid proteins, whereas the "E" genes encode proteins with a variety of regulatory functions. HPV infects at several sites in the body, including the skin, mouth, esophagus, larynx, and anogenital tract. Over 100 different HPV genotypes have been identified and approximately 20 have been shown to have a propensity to infect anogenital tract tissues.[5]

Extensive epidemiologic data have strongly associated HPV with a spectrum of anogenital neoplasms, including condylomata (genital warts), cervical dysplasia, and cervical carcinoma. HPV DNA is detected in more than 99% of all tumors of the uterine cervix.[6] Mucosotropic HPVs are grouped into low-risk or high-risk categories on the basis of each genotype's association with a benign or malignant disease process.[7] Low-risk HPV-6 and -11 are commonly detected in condyloma accuminata but are virtually never found in cervical carcinoma. In contrast, high-risk types 16 and 18 can be detected in nearly 70% of squamous cell carcinomas of the cervix.[5] Other high-risk types include HPV-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68. The E6 and E7 genes of high-risk HPV types encode for oncoproteins that can immortalize human keratinocytes.[8] This potential appears to be limited to high-risk types, because E6 and E7 from HPV-6 or -11 are nontransforming.[8,9] E6 and E7 alter cell growth regulation by inactivating the products of tumor suppressor genes p53 and pRB

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(retinoblastoma), respectively.[10,11] In contrast to the low-risk HPV-E6 and -E7 proteins, only high-risk HPV-E6 directs the ubiquitin degradation of p53 and only the high-risk HPV-E7 directly interferes with regulatory proteins of the cell cycle.[12] The continued expression of these E6 and E7 proteins appears to be necessary for maintaining the malignant phenotype of cells transformed by E6 and E7.[13] Because E6 and E7 are selectively retained and expressed in cervical tumors, they are attractive targets for immunotherapies. Since E6 and E7 are viral proteins with no appreciable sequence homology to human cellular proteins, the risk of inducing an autoimmune response by targeting E6 and E7 is theoretically eliminated. These observations provide the impetus for the development of novel vaccines to prevent or treat HPV-associated cervical cancer.

Several lines of independent evidence support the importance of the cellular immune response in the pathogenesis of cervical cancer. More than 60% of HPV-positive, mildly dysplastic lesions resolve spontaneously,[14] and immunodeficiency is associated with increased incidence of persistent HPV infection. For example, HIV-related immunosuppression has been correlated with increased risk of genital HPV infection,[15] and immunosuppressed renal transplant patients have an elevated risk of HPV-associated malignancies compared with the general immunocompetent population.[16] Studies using cervical carcinoma tissues have demonstrated high levels of mononuclear cell infiltrates consisting predominantly of $CD8^+$ cytotoxic T cells (CTLs).[17] Epidemiologic data indicate that the prevalence of genital HPV infections peaks soon after the onset of sexual activity in women and declines thereafter, suggesting that long-term protection is generally induced.[18] Taken together, these considerations underpin the effort to develop vaccine strategies to prevent or treat HPV infection.

IMMUNOLOGIC PRINCIPLES

Two divergent immunologic approaches have evolved for development of anti-HPV prophylactic and therapeutic vaccines. In general, immunoprophylactic vaccines elicit humoral immune response because they induce the production of antibodies capable of neutralizing a viral antigen before it enters the host cell. Therapeutic vaccines are intended to induce cellular components of the immune system to recognize and attack cells infected with HPV, including malignant tissues.

Immunoprophylactic vaccination is achieved by inducing antiviral neutralizing antibodies before viral infection. Historically, certain preventative vaccines have been enormously effective in preventing subsequent infection by other human viruses, including hepatitis B, measles, mumps, and polio. In contrast to the oral poliovirus vaccine, which is an attenuated form of the poliovirus, the development of attenuated HPV vaccine has been hampered because there is no effective culturing system to propagate HPV. The use of inactivated virus or crude viral extracts from infected humans has been impractical because of the paucity of available tissue, and this approach has the theoretic disadvantage of exposing normal subjects to viral oncogenes encoded by HPV DNA. Therefore,

prophylactic vaccine development for HPV has focused on recombinant subunit preparations consisting of the L1 and L2 virion structural proteins. The highly successful prophylactic vaccination program for hepatitis B virus[19] also relied on recombinant technology to permit the biosynthesis of the hepatitis B surface antigen despite the inability to culture and propagate hepatitis B effectively. The production of protective antibodies against the surface antigen of hepatitis B virus resulted from the immunization with recombinant hepatitis B vaccine, prevented the subsequent transmission of this virus,[19] and diminished the incidence of hepatitis B–associated hepatocellular carcinoma.[20]

For therapeutic vaccine development, attention has focused primarily on the fate of the intracellular viral proteins. These proteins are degraded into peptides consisting of 8–11 amino acids presented in association with MHC class I molecules on the cell's surface. The peptides are transported through the TAP 1 and TAP 2 proteins to the endoplasmic reticulum and, after the peptides associate with the class I heavy chain and β2-microglobulin, this trimeric structure is translocated and presented on the cell surface for recognition by $CD8^+$ CTL.[21] The processing and presentation of engulfed exogenous proteins generally occur in the context of MHC class II molecules. The class II molecule and invariant chain complex are formed in the endoplasmic reticulum (ER) and transported to a lysosomal compartment where the invariant chain is degraded, allowing for peptide loading of the class II molecule. At the cell's surface, the class II molecule and associated peptide are recognized by $CD4^+$ T cells.[21] For the treatment of established intracellular HPV infection, methods to improve cell-mediated immunity by enhancing viral antigen recognition are being studied.

Mechanisms of Escape From Immunologic Recognition

Therapeutic vaccines are subject to several important practical and theoretic limitations that could allow the viral peptides to go unrecognized by the cellular immune system. Peptide binding ensures structural stability for both MHC class I and II molecules. Without correct conformation, intracellular transport to the cell surface may be compromised. Tumor cells can also downregulate MHC class I expression or develop mutations of the β2-microglobulin gene, which partially accounts for the ability of tumor to evade immune recognition.[22,23] The downregulation of MHC class I molecule correlates with disseminated cervical cancer as determined by histopathologic features at diagnosis.[24] Several mechanisms of HLA class I molecule downregulation on tumor cells have been proposed. For instance, loss of TAP results in reduced levels of peptides in the ER, and in a reduced expression on the cell surface by destabilizing HLA class I molecules.[25] Tumors can also secrete Fas ligand to induce apoptosis of infiltrating CTLs.[26] Furthermore, induction of tolerance and deletion of T cell specificity rather than T cell activation has resulted from vaccination with peptides.[27]

Adjuvants and Immunostimulants

In practical applications, peptides and most cancer antigens are often poorly immunogenic.[28] To overcome this limitation, they can be altered to increase their immunogenicity by modification of the peptide amino acid sequence,[29,30] conjugation with immunostimulatory molecules,[31] and co-administration of adjuvants. Several T helper peptides have recently been identified,[32] and the covalent linking of one of these T helper epitopes (PADRE) and two palmitic acid residues to an HPV-16 E7 peptide epitope has recently been investigated in a phase I clinical trial.[33] Incomplete Freund's adjuvant (IFA) has been used clinically for several years, but it remains a nonspecific adjuvant and often induces local inflammatory responses.

Other immunostimulants under investigation include immune stimulating complexes (ISCOMs), cytokines, and co-stimulatory molecules. ISCOM, containing a cholesterol matrix into which protein antigens are incorporated, has been shown to elicit both humoral and cell-mediated responses in immunized animals.[34,35] Fernando et al immunized mice with glutaraldehyde-polymerized HPV-16 E7 peptides incorporated into an ISCOM vaccine. In response to the immunization, mice developed an antigen-specific immune response.[36] In contrast, free peptide was not immunogenic, indicating that ISCOM increased the immunogenicity of the peptides. Other ISCOM adjuvants consist of cagelike microspheres and are prepared by mixing Quillaia, cholesterol, and a phospholipid.[37] Protein or peptide antigens can be incorporated into the microspheres or mixed with the adjuvant. By incorporating the Quillaia into the ISCOM particles, the local reactivity of Quillaia is reduced and the immunostimulating properties are enhanced. The particles are taken up rapidly by the immune system and are transported to the regional lymph nodes. It is believed that the ISCOM particles deliver antigens into the MHC class I pathway.

PREVENTIVE VACCINES

The L1 (major capsid protein) organizes itself into papillomavirus-like particles (VLPs) when it is expressed at high levels in eukaryotic cells,[38] but L1 has also been successfully expressed in attenuated bacterial vectors such as Salmonella typhimurium.[39] Although L1 alone is sufficient for assembly of VLPs,[40] the co-expression of L2 (minor capsid protein) provides for greater capsid production.[41] The VLPs are morphologically indistinguishable from the authentic virion, are noninfectious, and lack oncogenic DNA. VLPs have also proven to be effective in generating papillomavirus type-specific protection from viral challenge in animal papillomavirus models. In in vitro assays, L1 and L1/L2 VLPs are capable of inducing high titer neutralizing antibodies to L1 epitopes.[40] In vivo, inoculation with VLP derived from canine oral papillomavirus (COPV) induced the production of circulating antibodies against COPV and protection against subsequent challenge with high doses of COPV.[42] Similar findings have been described using the cottontail rabbit papillomavirus model[43] and the bovine papillomavirus model.[40] More recently, anti-HPV-16 neutralizing

antibodies were induced in a cohort of humans inoculated with an HPV-16 VLP consisting of the L1 protein (Table 1).[44]

Several important questions remain to be answered before an effective prophylactic vaccine becomes a clinical reality. For instance, it remains uncertain whether immunoreactivity can be induced against all other HPV types, or at least the major oncogenic HPV ones. In addition, it is hoped that the results of current field trials will determine whether robust antibody responses are protective from the development of HPV-associated lesions on genital mucosal surfaces when subjects are subsequently challenged with authentic virion. Immunity in VLP-based vaccines is likely to be primarily genotype specific,[45] although divergent variants of HPV genotypes are serologically cross-reactive.[46] Therefore, a multivalent vaccine should be considered for clinical trials. Because about 80% of HPV-associated cervical cancers contain either HPV-16, -18, -31, or -45,[5] it would be desirable to include VLPs of at least these four genotypes in a multivalent vaccine.

Several studies indicate that the route of VLP administration is an important consideration when the aim is to induce effective mucosal immune responses. Clearly, protection from natural genital HPV infection will require robust local antibody responses in the mucosal epithelium. For instance, systemic immunization of monkeys with HPV-11 VLPs induced neutralizing immunoglobulin (Ig)G, but not IgA, antibodies in the serum and genital mucosal secretions.[47] Similarly, mucosal but not parenteral immunization with purified HPV-16 VLPs have been shown to induce neutralizing titers of antibodies throughout the estrous cycle of mice.[48] Enduring titers of anti-HPV-16 VLP antibodies in mucosal secretions have been described after the intranasal administration of HPV-16 VLPs in mice, including IgA and IgG in saliva and genital secretions.[49] Preliminary data from a recent clinical trial using an HPV-6b VLP vaccine to treat patients with genital warts resulted in acquisition of VLP-specific antibodies, a delayed-type hypersensitivity response to L1 protein, and regression of genital warts in over 50% of subjects.[50]

Recent data suggest that cell-mediated immune responses may also confer immunoprotection even though immunoprophylactic vaccines are traditionally thought to elicit humoral immune responses that are protective when the host is later challenged with the live virion. Studies in mice immunized with immunodominant HPV peptides appear to be protected when they are subsequently challenged with lethal doses of HPV-induced tumors.[51] This observation suggests that the cellular immune response may have an important role in prophylactic as well as therapeutic vaccine development (see below).

THERAPEUTIC VACCINES

Because most sexually active women have already been exposed to HPV, it is unlikely that humoral immunity induced from a VLP-based HPV vaccine will be effective in treating women who have already been infected. Therapeutic vaccines are administered to reduce or eradicate existing disease or infection by targeting cells expressing tumor-associated or tu-

mor-specific antigens on their surface. For cervical cancer, the viral peptides derived from high-risk HPV-E6 and -E7 oncoproteins are the tumor-specific antigens.[18] T lymphocytes express receptors specific for these antigens and are of major importance as the primary effectors of tumor rejection.[52] This type of immune surveillance is important in the defense against many viral infections and virally induced tumors.[53,54]

Antigen-presenting cells (APCs) are important for the initiation of an effective immune response. APCs consist of a heterogeneous population of leukocytes including Langerhans cell, macrophages, B cells, and dendritic cells (DCs).[55] The APCs can engulf exogenous proteins and present the processed peptides to T cells in an MHC-restricted manner. These APCs efficiently present antigens along with MHC, adhesion, and costimulatory molecules on their cell surface. DCs have been generated from peripheral blood specimens or bone marrow stem cells and can stimulate antitumor T lymphocytes by co-incubation with immunologically relevant tumor or viral peptide antigens. Peptide-pulsed DCs have already been successfully used to generate CTL immune responses in animal and human models.[56,57] Antigens not presented by APCs may avoid T cell recognition. This is particularly true for a noncytopathic virus such as HPV, which has a persistent nonlytic infectious life cycle. Therefore, the relevant peptide antigens are not available for presentation by APCs and specific CTLs are not induced.[58]

A wide variety of immunologic approaches are candidates for antitumor vaccines, including the adoptive transfer of APCs or inactivated whole cancer cells. In general, there are four broad categories of therapeutic vaccine strategies: peptide-based, protein-based, nucleic acid–based, and cell-based (Table 1). Adoptive immunotherapies usually involve the ex vivo expansion of specific CTLs that are then infused back into the patients. This approach may include the use of tumor or viral peptides, gangliosides, and heat-shock proteins.[59] Expression vectors encoding the gene for a specific tumor antigen can also be used to elicit immune responses.[60]

Peptides

Peptides are attractive vaccine candidates because they can be synthesized in large quantities inexpensively and are relatively nontoxic. To exploit this strategy for therapeutic tumor vaccination, it is necessary to identify the immunologically relevant target peptides. The majority of CTL responses thus far described have been HLA-A*0201-restricted, reflecting the prevalence of this class I allele among humans in general and whites in particular (nearly 50% of whites express HLA-A2).[61]

Several HLA class I–restricted epitopes of HPV-16 and HPV-18 E6 and E7 have been identified that elicit specific CTL responses.[51,62–64] Human CTLs induced by using the HLA-A*0201-restricted, HPV-16 E7 peptides 11–20 (E7$_{11-20}$) or 86–93 (E7$_{86-93}$) are capable of recognizing and lysing CaSki cervical cancer cells which contain HPV-16.[51,65] In animal models, CTL can be generated using E6 and E7 peptide-based vaccines that are protective against subsequent challenge with lethal doses of E6- and E7-containing tumors.[66]

Stimulation of peripheral blood lymphocytes from patients with HPV-16–positive cervical cancer with synthetic HPV-16 E7$_{11-20}$ peptide generates specific CTLs capable of tumor recognition and lysis of cervical cancer cells.[65]

These peptides have been used in phase I/II clinical trials. The results of a pilot study described the subcutaneous administration of a lipidated peptide derived from HPV-16 E7 (E7$_{86-93}$) as a candidate vaccine for patients with recurrent or refractory cervical cancer.[33] Preclinical animal data demonstrated that by linking two palmitic acid molecules and a helper epitope to the CTL-restricted viral epitope, substantially enhanced immunogenicity resulted, with induction of memory CTLs from a single injection that persisted for more than 1 year.[31] Administration of the HPV lipopeptide resulted in no appreciable toxicities, and primary E7$_{86-93}$–specific CTL responses were observed in 3 of 12 patients. One of the three patients in whom a primary CTL response was observed subsequently achieved a clinical complete response in the absence of additional treatment during 52 months of follow-up (manuscript in preparation). This patient also demonstrated a robust CTL response as measured by a standard interferon-gamma release enzyme-linked immunosorbent assay. Another trial performed in the Netherlands involved the administration of two HPV-16 E7 peptide epitopes emulsified in incomplete Freund's adjuvant. This treatment was well tolerated as no significant toxicities were noted, even at the highest vaccine dose,[67] but no clinical responses were observed and subsequent analysis revealed no evidence of specific cellular immune responses to the HPV peptides.[68] In patients with preinvasive anogenital neoplasia of the cervix or vulva, another clinical trial using a peptide-based vaccine was performed.[69] Eighteen patients with high-grade dysplasia were vaccinated with either an HPV-16 E7 peptide (E7$_{12-20}$) or a lipidated E7 peptide (E7$_{86-93}$). Ten of 16 subjects mounted primary CTL responses to the E7 peptides, and a complete clinical response was observed in three subjects. Because established tumors are generally a heterogeneous mixture of different malignant cell populations, it is likely that variant tumor clones within a tumor may not express the target antigen or will possess defects in their antigen-presenting mechanism. Therefore, vaccine therapy may ultimately be better suited for the treatment of preinvasive disease, where these less advanced lesions are generally more genetically stable.

Proteins

Full-length viral E6 and/or E7 proteins are under investigation for use in therapeutic cervical cancer vaccine development. The use of proteins has the advantage of including all of the protein's putative immunogenic epitopes for every MHC haplotype. Protein-based strategies require APC engulfment of the protein and presentation of its peptide fragments in the context of MHC class I or class II molecules. Several investigators have demonstrated that denatured proteins can be endocytosed by APCs with subsequent processing and presentation inducing effective CD8$^+$ CTLs.[70,71] This approach surmounts one limitation of peptide-based vaccines: namely, that it is not neces-

sary to have a priori knowledge of the patient's HLA haplotype to choose appropriate peptides compatible with that particular haplotype. Therefore, a more potent immune response is possible, involving the presentation of epitopes from all restriction elements (the molecules expressed by both of the HLA-A, both of the HLA-B, and both of the HLA-C alleles for any given subject). Because no patient would be excluded owing to HLA haplotype restrictions, protein-based vaccines may be readily translated into clinical application.

The successful induction of specific, MHC class I–restricted CTL responses after protein-based vaccination has been demonstrated in several investigations. One study used an HPV-16 E7 protein fused to glutathione-S-transferase to protect mice against subsequent lethal challenge with an E7-expressing tumor cell line.[72] Another study mixed the E7 protein with the adjuvant PROVAX and demonstrated the protection of rodents against subsequent tumor challenge and the inhibition of established tumor growth.[73] Vaccination with HPV-16 L2, E6, and E7 as a single fusion protein has been shown to elicit HPV-16–specific CTLs, T helper cells, and antibodies in a mouse model.[74] These immune responses effectively prevented outgrowth of HPV-16–positive tumor cells in a prophylactic setting as well as in a minimal residual disease setting. Another fusion protein vaccine protected mice from subsequent lethal tumor challenge and had therapeutic efficacy in rejecting established tumors.[75] This vaccine consisted of HPV-16 E7 fused to the heat-shock protein Hsp65 of *Mycobacterium bovis*.

Protein-based vaccinations have been used in early-phase clinical trials. HPV-16 E6/E7 fusion protein mixed with a saponin-based adjuvant ISCOMATRIX (CSL Ltd., Melbourne, Australia) is presently being used in a phase I, multicenter, placebo-controlled, dose escalation study in women with HPV-16–associated high-grade cervical squamous intraepithelial neoplasia. A vaccine consisting of HPV-6 L2 E7 has been investigated for the treatment of genital warts. Studies in 42 healthy male volunteers indicated that the vaccine was well tolerated with minimal toxicity, and was also immunogenic[76]; a subsequent phase II study of 27 patients with genital warts reported five complete responses with vaccine alone, and 19 of 25 subjects mounted proliferative immune responses after vaccination.[77] The hsp65-HPV-16 E7 fusion protein vaccine is also under investigation in both phase I and phase II studies.[78]

Chimeric Papillomavirus Virus–Like Particles

Although HPV VLPs have been developed as a candidate prophylactic vaccine, chimeric VLPs have recently been synthesized to increase the number of viral antigen targets for cell-mediated immune responses.[79] Chimeric VLPs are produced by expressing the major (L1) and minor (L2) capsid proteins along with a nonstructural HPV protein (eg, E6 or E7). These chimeric VLPs are morphologically indistinguishable from their parental VLPs and also elicit high titers of neutralizing antibodies. In contrast to the parental VLPs, the chimeric VLPs induce CD8$^+$, E7-specific CTL responses that were also protective against subsequent tumor challenge.

Other investigators have successfully used chimeric VLPs containing a truncated E7 protein,[80] an E7 peptide epitope,[81] or a string of CTL epitopes.[82] All of these findings suggest that VLPs may be useful as vehicles for generating cell-mediated immune responses and that the chimeric VLPs may increase the therapeutic potential of VLP-based vaccines.

Gene Therapies

Various gene transfer techniques permit the introduction of E6 and/or E7 DNA into target cells. Vaccinia virus is a useful vehicle because it is stable, and it has already been studied in humans. A disadvantage of using the wild-type E6 or E7 oncogenes is their potential for tumorigenesis. Theoretically, this problem is overcome by using mutant forms of E6 or E7, but these mutations may compromise immunogenicity in some cases. Unfortunately, the viral expression vectors themselves may cause substantial morbidity, especially in immunosuppressed patients.

VIRAL VECTORS. Viral expression vectors represent another avenue of approach for immunotherapy. Genes encoding relevant antigens can be spliced into recombinant expression vectors allowing for increased cellular production of antigen and induction of cellular and humoral immune responses. Vaccinia virus is perhaps the most widely studied expression vector for immunotherapeutic strategies. The vaccinia virus has the capacity to accept large gene insertions and is efficient at inducing immunity.[83] Recombinant vaccinia-expressing modified E6 and E7 genes have been shown to protect against subsequent tumor challenge in a variety of experimental systems.[84–86] Adenovirus, fowlpox virus, and Avipox virus are other vectors currently under investigation.

Recombinant vaccinia virus has also been used to express a synthetic oligonucleotide encoding the minimal determinant peptide of a known tumor-associated antigen.[87] By coupling the peptide minigene with an endoplasmic reticulum signal sequence, CD8$^+$ CTLs were elicited that could recognize endogenously processed peptide on target tumor cells in vitro and eradicate established tumors in vivo. This kind of vaccine construct avoids the dangers associated with the expression of full-length genes which encode for viral or cellular proteins that are potentially oncogenic.

Augmentation of helper T cell activity by potentiation of CD4$^+$ T cell responses has also been explored using the lysosomal-associated membrane protein (LAMP)-1 system.[88] Mice inoculated with a vaccinia viral vector encoding an E7/LAMP-1 fusion protein were protected against subsequent challenge with an HPV-induced model tumor.[89] Furthermore, treatment with this vaccine cured mice with small-established tumors, whereas the wild-type E7 vaccinia showed no effect on this established tumor burden.[90] Additional investigations using this novel vaccine strategy in humans are under consideration.

A vaccinia-based HPV vaccine has been tested clinically in a phase I trial of eight patients with advanced HPV-16–positive cervical cancer.[91] In that study, a recombinant vaccinia

Table 1. Summary of Cervical Cancer Vaccine Strategies

Approach	Molecular targets	Strategy	Description
Prophylactic	High-risk HPV-L1, -L2	Virus-like particles	Noncogenic viral capsid proteins to induce humoral immune response
Prophylactic and therapeutic	High-risk HPV-L1, -L2, -E6, -E7	Chimeric virus-like particles	VLPs encapsidate high-risk HPV-E7 protein or E6/E7 minigenes. Induces both humoral and cellular immune responses
Therapeutic	High-risk HPV-E6, -E7	Peptide	Alone, with adjuvant, or loaded onto dendritic cells to elicit specific CTLs
Therapeutic	High-risk HPV-E6, -E7	Protein	Stimulate APCs with full length protein alone or with adjuvant to elicit specific CTLs
Therapeutic	High-risk HPV-E6, -E7	Viral vectors	Incorporate DNA into a recombinant viral vector (eg, vaccinia or adenovirus) or immunize with the relevant oligonucleotide sequence
Therapeutic	High-risk HPV-E6, -E7	Nucleic acid: naked DNA and naked DNA minigenes	Administered intravenously, subcutaneously, intramuscularly, or intradermally results in DNA uptake by APCs and other cells as well as expression of the DNA-encoded antigen
Therapeutic	High-risk HPV-E6, -E7	Nucleic acid: RNA	Launches self-replicating RNA replicons that are also self-limiting
Therapeutic	High-risk HPV-E6, -E7	Cell-based: dendritic cells	Professional APCs are loaded with HPV peptide, protein, or nucleic acid encoding HPV epitopes.
Therapeutic	High-risk HPV-E6, -E7	Cell-based: tumor-dendritic cell fusions	Fusion of a DC with an autologous tumor cell
Therapeutic	High-risk HPV-E6, -E7	Cell-based: modified tumor cells	Genetic modification of tumor cells from cancer-bearing patients to express immunostimulatory molecules to enhance the tumor's immunogenicity

viral vector encoding the E6 and E7 genes of HPV-16 and -18 was used. Immune responses of antivaccinia IgG antibody occurred in all eight patients, and three patients produced antibodies to HPV-18 E7. The fact that anti-HPV-18 antibodies were detected suggests that the immune responses resulted from the vaccinations because none of the patients in that study were found to have HPV-18 in their tumors. HPV-specific CTLs were detected in one patient and no complications were observed in response to the vaccination. This vaccine was subsequently used as an adjuvant in patients with early-stage cervical cancer, and clinical data will require several years to mature.

Recombinant adenovirus vaccines have been engineered such that they are replication-deficient, and encouraging results have been reported for the induction of protective antitumor responses after vaccination. An E7-expressing adenovirus vector has been used successfully to induce E7-specific CTL responses,[92] and a polyepitope construct has also been described.[93]

NUCLEIC ACIDS. Naked DNA has emerged as an attractive vaccination strategy, in part because it is inexpensive, stable, and relatively simple to produce. The DNA can be administered by a variety of routes, including intravenously, subcutaneously, intramuscularly, and intradermally, all of which result in DNA uptake by APCs and other cells as well as expression of the DNA-encoded antigen. E7 plasmid DNA vaccines have been shown to induce CTL responses and protect mice against subsequent tumor challenge.[94,95]

Augmentation of $CD4^+$ helper T cell responses represents another therapeutic vaccine approach currently under investigation. By increasing the presentation of antigenic HPV-16 E7 peptides by MHC class II molecules, $CD4^+$ T cell responses are potentiated. For this purpose, E7 is concentrated into the MHC class II processing pathway by using the sorting signal of LAMP-1. When this chimeric DNA, designated Sig/E7/LAMP-1, was tested in mice, enhanced E7-specific $CD4^+$ and $CD8^+$ T cell responses were observed, and not only were mice protected from subsequent tumor challenge, but established tumors were rejected.[96,97] The fusion of heat-shock protein Hsp70 from M bovis with the E7 gene has also been shown to elicit $CD8^+$ T cell responses.[98]

Naked DNA minigenes are also under investigation as a candidate vaccine strategy. HLA-specific HPV epitopes are encoded for by these minigenes, and they can be linked together to encompass multiple epitopes suitable for use in patients with diverse HLA haplotypes. Using recombinant DNA technologies, it is possible to develop multiepitope vaccines rapidly and efficiently. A construct containing CTL, Th cell, and B cell epitopes of the HPV-16 has recently been described[99] in which this gene gun-mediated vaccine protected 100% of the vaccinated mice against a lethal tumor challenge.

RNA-based HPV vaccines represent another attractive vaccine strategy under active investigation. This approach launches self-replicating RNA replicons that are also self-

limiting.[100] This technology is being adapted for the development of an HPV-specific vaccine.[78]

Cell-Based Vaccines

DENDRITIC CELLS (DCs). Preclinical models have shown that the administration of peptide alone is not efficient[31] and the choice of an appropriate adjuvant capable of enhancing the immunogenic stimulus is important. DCs have a critical role in antigen presentation in vivo[101] and may represent a useful adjuvant. DCs are professional APCs with a unique ability to initiate primary immune responses. Although our understanding of DC biology is still in its infancy, DC-based immunotherapy protocols have been developed to elicit immunity against cancer and infectious diseases. Sallusto and Lanzavecchia showed that monocytes derived from the peripheral circulation can be readily differentiated into DCs by culturing them in vitro for 5 to 7 days in media containing granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4).[102] Studies have shown that peptide-pulsed DCs are significantly more efficient in inducing antitumor protection than immunization with peptide alone[103,104] or peptide emulsified in IFA.[105] DCs can also be loaded with full-length purified protein antigens or nucleic acids. Indeed, even crude tumor lysates derived from tumor tissues have been used to load DCs with tumor antigens for induction of tumor-specific CTLs.[106]

By using IL-4 and GM-CSF cultured DCs, in vitro PBMCs from cervical cancer patients have been sensitized against the MHC class I (HLA-A*0201)-restricted epitope $E7_{86-93}$ by exposure of responder cells to the relevant epitope in the presence of a low number of autologous DCs.[33] In other studies, specific anti-HPV-16 $CD8^+$ CTLs were successfully induced in vitro using autologous DCs from a healthy subject to process and present a recombinant HPV E6/E7 fusion protein.[107] Other investigators have also reported successful CTL induction using HPV-E7 protein and DCs from cervical cancer patients.[108] These results suggest that DCs are capable of engulfing extracellular viral proteins which can then undergo intracellular processing in the class I pathway to permit the peptide fragments to become available for $CD8^+$ T-lymphocyte recognition. Because autologous DCs are used to induce the CTLs, this approach permits all possible epitopes of the E6/E7 fusion protein to be processed and presented for CTL recognition. Thus, the limitations of peptide-based vaccines, such as a priori knowledge of the patient's HLA haplotype to choose appropriate peptides compatible with that particular haplotype, can be overcome and may therefore permit a more potent immune response involving the presentation of epitopes from all possible restriction elements. Both peptide-pulsed and protein-pulsed DC protocols are currently being investigated for the treatment of patients with cervical cancer.

Nucleic acids encoding tumor peptides or antigens can be transduced into DCs for induction of specific CTL responses. Using electroporation of E7 DNA into an immortalized DC

cell line, E7-specific CTL responses have been induced[109]; another study has described the use of the gene gun for DNA transduction.[110] Successful CTL induction has also been described using DCs transfected with E6 and E7 RNA.[111]

TUMOR-DENDRITIC CELL FUSIONS AND MODIFIED TUMOR CELLS. Another novel method under investigation is the fusion of a DC with an autologous tumor cell. This hybridoma has been shown to elicit novel antitumor CTL responses.[112] Genetic modification of tumor cells from cancer-bearing patients to express immunostimulatory molecules to enhance the tumor's immunogenicity is also under investigation. Various cyotokines and immunostimulatory molecules are under investigation, including IL-2, IL-12, GM-CSF, CD27, and CD49 ligand.[78] Before these approaches become clinically applicable, numerous technical obstacles must be overcome.

CONCLUSIONS

During the past 25 years, there has been an explosion of new knowledge relating to HPV-induced cervical cancer and immunologic responses to HPV antigens. A wide variety of immunologic vaccine strategies are under investigation for the prevention or treatment of HPV infection or cervical neoplasia. Among the most attractive candidate vaccine strategies, VLP-based vaccines hold enormous potential for the prophylaxis of HPV-associated mucocutaneous diseases, and the chimeric VLPs also hold great promise for safe and effective therapeutic vaccine development. VLP-based vaccines are also relatively safe, easy to deliver, and inexpensive to prepare. Other innovative approaches ranging from peptide or protein-based vaccines to cell-based and tumor-dendritic cell fusion vaccines also remain theoretically promising.

Several practical issues will require careful consideration before anti-HPV vaccines become a clinical reality. For instance, cervical cancer does not develop in the vast majority of women, even in unscreened populations,[113] even though epidemiologic data indicate that female genital HPV infection occurs commonly, with an estimated lifetime risk of HPV infection approximating 80% in some populations.[114,115] It follows that few women are likely to benefit from the protective effects of a prophylactic HPV vaccine because the disease (cervical cancer) is relatively rare yet HPV infection occurs commonly. On the other hand, the cost of expensive screening and surveillance programs might be dramatically reduced if an effective vaccine were to become widely available, and there would be a clear benefit in the unscreened population.

The timing of vaccine delivery represents an important consideration because HPV infection is in large part sexually transmitted (see Brinton[116] for review), and a successful prophylactic vaccine will be effective only when it is administered to women before they acquire HPV infection through sexual activity. Like the hepatitis B vaccine, should a prophylactic HPV vaccine be administered universally during childhood? To enhance overall effectiveness and diminish transmission rates, should the vaccine also be administered to males, even

though they rarely manifest HPV-associated diseases? Would this be cost-effective? The answers to these questions must take into account that cervical cancer remains a leading cause of cancer death worldwide,[117] and that the cost of its prevention is nearly $6 billion annually in the United States alone.[3]

Because of the large lag-time from incident HPV infection to the development of invasive cervical cancer, the effect of an effective vaccine to prevent cervical cancer will take several years before it can be clinically appreciated. For example, in North American women, sexual relations are initiated (and incident genital HPV infection occurs) on average during the late teenage years,[118] yet the mean age of women diagnosed with cervical cancer is 53.[119] Therefore, despite the encouraging results of initial field trials using VLP-based prophylactic vaccines,[44] it is likely to take many years before a decline in the incidence of cervical cancer is observed.

The future of vaccine development for cervical cancer remains bright. As clinical experience continues to accumulate, progress toward the development of an effective vaccine seems increasingly realistic. It is hoped that the true effectiveness of anti-HPV vaccines and real impact on HPV-associated diseases will be demonstrated after well-done, long-term clinical studies.

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