[Frontiers in Bioscience 8, d55-68, January 1, 2003]

HPV DNA VACCINES

Michelle Moniz [1], Morris Ling [1], Chien-Fu Hung [1], and T.-C. Wu [1,2,3,4]

[1]Departments of Pathology, [2] Oncology, [3] Obstetrics and Gynecology, and [4]Molecular Microbiology and Immunology, The Johns Hopkins Medical Institutions, Baltimore, Maryland

TABLE OF CONTENTS

1. ABSTRACT

Human papillomaviruses (HPV), particularly HPV type 16, are the primary etiologic agent of cervical cancer. Thus, HPV-associated cervical malignancies might be prevented or treated by induction of the appropriate virus-specific immune responses in patients. HPV capsid proteins including L1 and L2 proteins have been shown to generate neutralizing antibodies against HPV particles in vaccinated individuals. Furthermore, HPV oncogenic proteins such as E6 and E7 proteins are important in the induction and maintenance of cellular transformation and are co-expressed in the majority of HPV-containing carcinomas. They represent ideal targets for the development of therapeutic vaccines against HPV infections and HPV-associated neoplasia. Vaccines targeting these proteins may provide an opportunity to control HPV-associated malignancies. Genetic immunization with naked DNA has emerged as an important strategy for vaccine development. Plasmid DNA encoding antigen of interest, such as capsid protein L1 and L2 (for preventive vaccines) and non-structural proteins E6 and E7 (for therapeutic vaccines) enters the host and stimulates an antigen-specific humoral and cellular immune response. Various strategies to enhance preventive and therapeutic effects of DNA vaccines are currently under active investigation. Should they fulfill their promise, these DNA vaccines may prevent HPV infection or control HPV-related cervical lesions.

2. INTRODUCTION

2.1. The Role of Human Papillomavirus in Cervical Cancers

Cervical cancer is the second leading cause of cancer death in women worldwide, with approximately 500,000 women developing the disease annually. Despite the availability of cervical cancer screening in the US, the

incidence of invasive cervical cancer does not seem to be diminishing (SEER Cancer Statistic Review). Over the past 15 years, epidemiologic/virologic data have identified a clear and consistent association of HPV infection with the development of cervical cancer. The evidence linking 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 (SIL), contain HPV DNA (1). Furthermore, molecular, biochemical, and cellular studies have demonstrated that E6 and E7, two HPV gene products that are consistently expressed in precursor lesions (SIL) and cervical cancer, lead to malignant transformation of epithelial cells (for review, see (2)). E6 binds, inactivates, and promotes the degradation of p53, while E7 binds and inactivates pRb. Although over a hundred HPV genotypes have been identified, about 80% of cervical cancer is associated with four "high risk" types of HPV (types 16, 18, 31, 45) and the remaining cases are associated with a dozen other oncogenic types (for review, see (2, 3)). HPV-16 is present in approximately 50% of all cervical cancers (4) and is consequently the focus of many recent HPV vaccine developments.

2.2. Events in the Progression from HPV Infection to Cervical Cancer

Clinical, pathologic, and virologic studies have defined a clear progression of events in the development of cervical cancer. The pathogenesis of cervical cancer is initiated by HPV infection of cervical epithelium during sexual intercourse. The majority of genital HPV infections are transient. However, a fraction of infections persist and initiate transformation events within the cervical epithelium. Initial changes in the cervical epithelium, pathologically classified as low grade squamous intraepithelial lesions (LSIL or CIN 1), are associated with continued viral replication and virus shedding (for review, see (5, 6)). In a study of 241 cytologically normal women recruited in a sexually transmitted disease clinic, the cumulative incidence of HSIL (CIN 2-3) at 2 years was 28% in HPV-positive women compared to 3% in HPV-negative women (7). The progression from HSIL to invasive cancer occurs at a high frequency. Progression is frequently 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 (5). 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 (5).

The well-defined virologic, genetic, and pathologic progression of HPV -- from initial infection to lesion formation to malignant tumor formation – and the limited number of well-defined foreign (viral) antigens provide an unique opportunity to evaluate intervention with therapeutic vaccines at various stages of HPV infection and tumorigenesis.

2.3. HPV Virus-Like Particles (VLPs) as Preventive Vaccine for HPV-Associated Lesions

HPV virus-like particles have emerged as an exciting candidate prophylactic HPV vaccine. Prophylactic vaccine development is complicated by the lack of animal models for the genital mucosatropic HPV types and by the difficulty in propagating the virus in culture. These difficulties have been partially overcome using cutaneous and mucosal animal papillomaviruses as models and by the development of virus-like particles (VLPs). The papillomavirus major capsid protein L1 spontaneously assembles to form empty capsids, known as virus-like particles (VLPs) when expressed in mammalian (8, 9), insect (10), yeast (11) or bacterial cells (12). Parenteral injection of these VLPs elicits high titers of serum neutralizing antibodies and protection from experimental challenge with infectious virus in several animal papillomavirus models, in the absence of the potentially oncogenic genome (13-17). Protection from experimental infection by cottontail rabbit papillomavirus (CRPV) or canine oral papillomavirus (COPV) following passive transfer of IgG from immunized animals to naive animals has been demonstrated in rabbits and dogs respectively (15, 16). This indicates that cell-mediated effector immune responses are not required for protection from experimental infection.

Although VLP vaccination provides immunity from experimental inoculation, it is unclear whether this extends to protection against natural transmission of genital HPV. To completely prevent sexual transmission of genital HPV infection, neutralizing antibodies must act at mucosal surfaces that are the natural site of infection. Antibodies both pass from plasma into genital secretions and are synthesised by local plasma cells (18, 19). The plasma cell precursors that migrate to the genital tract derive primarily from mucosal lymphoid tissues and predominantly secrete IgA. Induction of these cells requires direct immunization of the mucosa-associated lymphoid tissue and in several experimental systems, nasal instillation was found to be the most effective route of immunization to generate specific antibodies in genital secretions in mice and in monkeys (20, 21).

In previous studies, systemic immunization of mice with purified HPV VLPs induced no detectable mucosal IgA antibodies and low titers of IgG (20, 22). Further, low titers of VLP-specific IgG, and no IgA, were detected in cervicovaginal lavage of parenterally immunized monkeys (23). Although these experiments in monkeys showed that transudated IgG alone partially neutralized HPV-11 *in vitro*, the mucosal antibody response was short-lived (23). Local, sustained production of secretory IgA (sIgA) and/or specific IgG are likely required for long lasting sterilizing immunity. Only nasal immunization using HPV16 VLPs has been shown to induce significant and sustained titers of HPV16 neutralizing antibodies in both serum and mucosal secretions of mice (12, 20). Thus, the route of administration may be important for generating protective humoral immune responses.

2.4. HPV Oncogenic Proteins, E6 and E7, as Targets for Immunotherapy for HPV-Associated Cervical Malignancies

Since E6 and E7 are consistently expressed in most cervical cancers, they represent promising targets for

the development of antigen-specific therapeutic vaccines. While most tumor specific antigens are derived from normal or mutated proteins, E6 and E7 are completely foreign viral proteins, and therefore may harbor more antigenic peptides/epitopes than a mutant (i.e. p53) or reactivated embryonic protein (i.e. MAGE-1). Furthermore, since E6 and E7 are required for the induction and maintenance of malignant phenotype of cancer cells (24), cervical cancer cells cannot evade an immune response through antigen loss. Finally, studies in animal models suggest that vaccination targeting the papillomavirus early proteins such as E7 can generate therapeutic as well as protective effects (25). Therefore, E6 and E7 proteins represent good targets for developing antigen-specific immunotherapies or vaccines for cervical cancer. In our studies, we have focused on E7 because it is more abundantly expressed, better characterized immunologically and its sequence is more conserved than the E6 gene (26).

Various forms of vaccines, such as vector-based vaccines, tumor-based vaccines, DNA based vaccines and protein/peptide-based vaccines have been described in experimental systems targeting HPV-16 E6 and/or E7 proteins (27-31).

2.5. Importance of Cell-Mediated Immune Responses in Controlling Established HPV Infections and HPV-Associated Neoplasms

Findings from several studies suggest that cell-mediated immune responses are important in controlling both HPV infections and HPV-associated neoplasms (for review, see (32)). For example, the prevalence of HPV-related diseases (infections and neoplasms) is increased in transplant recipients (33) and human immunodeficiency virus (HIV) infected patients (34, 35), both of whom are known to have impaired cell-mediated immunity. Furthermore, animals immunized with non-structural viral proteins are protected from papillomavirus infection or the development of neoplasia. Immunization also facilitates the regression of existing lesions (36, 37). Infiltrating $CD4^+$ (T helper cells) and $CD8^+$ (cytotoxic T cells) T cells have been observed in spontaneously regressing warts (38); in addition, warts in patients who are on immunosuppressive therapy often disappear when treatment is discontinued (for review, see (39)). Therefore, effective HPV therapeutic vaccines should generate HPV-specific cell-mediated immune responses, particularly T cell-mediated immune responses.

2.6. Importance of Dendritic Cells in Mediating Immune Responses

DCs are the most potent professional APCs that prime helper and killer T cells *in vivo* (for review see (40-42)). DCs can stimulate T cells because of their high levels of MHC-I and MHC-II molecules, co-stimulatory molecules (i.e. B7), and adhesion molecules such as intercellular cell adhesion molecule-1 (ICAM-1), ICAM-3, and lymphocyte function-associated antigen-3. DCs perform a series of coordinated tasks in order to efficiently present antigens. Immature DCs develop from hematopoietic progenitors and are strategically located at body surfaces and in the interstitial spaces of many tissues. At these locations, DCs are equipped to capture antigens and to produce large numbers of immunogenic MHC–peptide complexes. In the presence of maturation-inducing stimuli such as inflammatory cytokines or stimulation via CD40 (43), DCs upregulate adhesion and costimulatory molecules to become more potent, terminally differentiated stimulators of T cell immunity. DCs also migrate to secondary lymphoid organs to select and stimulate antigen-specific T cells (44). Thus, many therapeutic vaccine strategies have focused on targeting antigen to professional APCs, such as DCs, and enhancing antigen processing and presentation in DCs in order to augment T-cell mediated immune responses.

2.7. Advantage of DNA Vaccine Vectors

The strategy behind genetic immunization is simple: injection of a DNA plasmid encoding a desired protein into the skin or muscle of the host elicits high efficiency expression of the polypeptide antigen of interest; antigen presentation by transfected cells promotes a cellular and humoral immune response against the antigen.

There are a few essential features of DNA plasmids used for vaccination. DNA vaccines comprise an antigen gene of interest cloned into a bacterial plasmid engineered for optimal expression in eukaryotic cells. Plasmid DNA has a bacterial origin of replication, an antibiotic resistance gene to enable plasmid selection during bacteria culture, a eukaryotic promoter, and poly A sequences to stabilize mRNA transcripts. Constructs also contain unmethylated, palindromic sequences containing cytidine-phosphate-guanosine oligodinucleotides (CpG-ODN), to activate an antigen-independent immune response. Via the toll-like receptor (45-47), CpG motifs directly stimulate B cells to proliferate and secrete antibodies (48) and APCs to secrete cytokines (49-51), which can activate NK cells (52-55) and T cells (56-58). CpG motifs have exhibited adjuvant properties when administered with peptide (59) and human papillomavirus–like particles (60), and may contribute equally to the immunogenicity of DNA vaccines (61-63). The effects of CpG motifs can be maximized by optimal bases flanking the CpG dinucleotide, (possibly GTCGTT in humans (64)), a TpC dinucleotide on the 5' end and a pyrimidine rich 3' end (64-66). Studies have shown that the most immunogenic ODN have two or three CpG motifs spaced with at least two intervening bases, ideally, Ts (64-66). Additionally, eradication of suppressive motifs from the plasmid backbone may yield a significantly more immunogenic vaccine (67).

Various strategies to optimize the DNA vector are currently under investigation, including manipulation of gene regulatory elements, construction of multivalent vaccines, and manipulation of heterologous genes to modulate immune response (for review, see (68)).

3. DNA VACCINES for HPV

Although many types of vaccines are currently under investigation, DNA vaccination is an especially

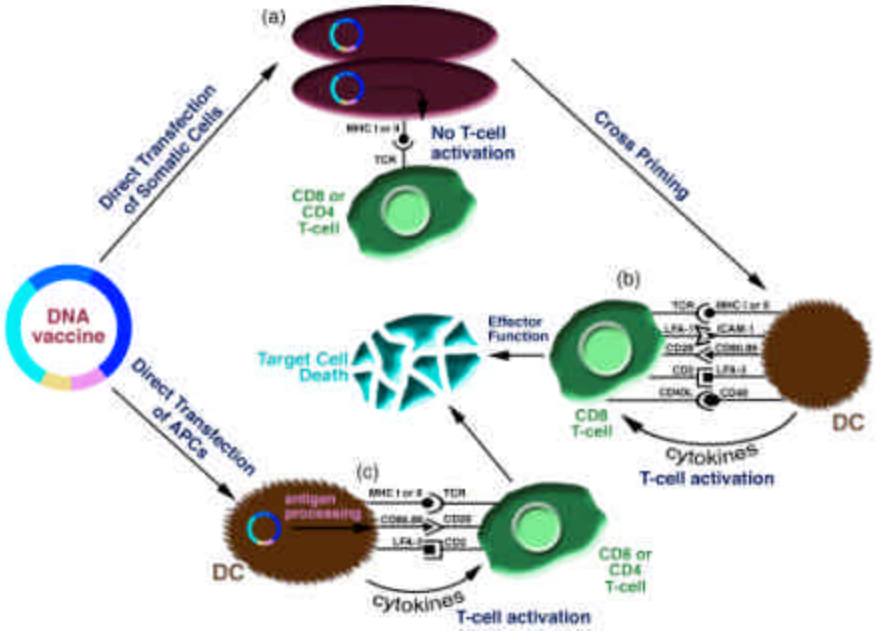

Figure 1. Mechanisms of DNA Vaccine Antigen Presentation. Antigen can be presented to T cells by APCs or somatic cells directly transfected with antigen. Conversely, these cells, particularly somatic cells, may deliver antigen to another APC, via secretion or uptake upon programmed cell death of the transfected cell. This APC may then present antigen of interest to $CD4^+$ and $CD8^+$ T cells.

attractive approach for HPV immunotherapy. In the past few years, several reports have shown that other antigen-specific cancer vaccines, in the form of cell-based vaccines, protein/peptide-based vaccines and viral vector-based vaccines are effective for the treatment of established tumors and metastases. However, production of autologous tumor cell-based vaccines is labor intensive and technically challenging. The application of peptide-based vaccines is limited by major histocompatibility complex (MHC) restriction and the necessity of defining CTL epitopes. As for viral vaccines, immune recognition of adenoviral and vaccinia vectors inhibits repeat vaccination with the same delivery system, while retroviral vectors display low *in vitro* infectivity and have potential virus-associated complications, including helper virus replication and insertional mutagenesis. Like other vaccines, DNA vaccines generate effective CTL and antibody responses by delivering foreign antigen to APCs that then stimulate $CD4^+$ and $CD8^+$ T cells. Unlike other vectors, naked plasmid DNA can easily be prepared in large scale with high purity, is relatively safe, and can be used for repeat administration. Additionally, DNA is highly stable relative to proteins and other biological polymers. Ease of preparation, storage and delivery make DNA vaccines attractive. Furthermore, DNA has the unique ability to integrate stably into the genome or to be maintained long term in an episomal form. This enables prolonged expression of the antigen of interest and enhancement of immunologic memory (for review, see (69, 70)).

3.1. Mechanism of Antigen Presentation

There are at least two mechanisms by which the antigen encoded by the foreign genes in the bacterial plasmid is processed and presented to the host immune system, direct priming and cross priming (see Figure 1 for details). As described earlier, overwhelming evidence suggests that DCs are the principal cells initiating an immune response after vaccination with naked DNA. DCs transfected with antigen can present antigen to T cells via direct priming. Additionally, DCs phagocytose and process peptides/proteins from apoptotic/necrotic somatic cells and other DCs (71). This process of DCs taking up antigen from somatic cells or other DCs and then processing the exogenous antigen via the MHC class I pathway for presentation to $CD8^+$ T cells is known as cross-priming.

3.2. Administration of DNA vaccines

DNA vaccines can be administered to the host by intramuscular injection, intradermal injection via hypodermic needle or via gene-gun (a ballistic device for delivering DNA-coated gold particles into the epidermis), intravenous injection, intranasal delivery or biojector delivery (for reviews, see (70, 72)). Intramuscular and intradermal injection have been shown to induce an immune response and antitumor effect (73-80). Intradermal DNA vaccination using via gene-gun has generated particularly effective antitumor immunity in several murine tumor models (81, 82).

Information about the mechanisms of DNA vaccination by intramuscular injection or gene gun delivery is accumulating. Following intramuscular injection, some of the myocytes are transfected by the DNA vaccines, and the transfected myocytes produce protein and transfer the antigens to the bone marrow-derived professional APC (cross-priming)(83). Alternatively, the injected DNA may

move as free DNA through blood to the spleen where DCs initiate responses (72). Following gene-gun delivery, the epidermal Langerhans cells are transfected by the DNA vaccine and then serve as APCs. The DCs of the skin carry the antigen from the skin to the draining lymph nodes, where the antigen-loaded DCs activate the naive T cells (84). The method of DNA inoculation (gene gun versus intramuscular injection) helps determine whether T-cell help will be primarily type 1 or type 2 (for review see (72)). The form of the DNA-expressed antigen (cell-associated versus secreted) may also influence T cell response (for review see (72)).

Recently, needle-free injection devices have emerged as effective, safe, less painful devices for delivering DNA vaccines. The Biojector (Bioject Inc., Portland OR) uses a CO_2 cartridge to propel vaccine intradermally or intramuscularly. This device has been shown to enhance antigen-specific antibodies in rabbits and rhesus monkeys (85-89). Observed increase in immunogenicity compared to needle injectors may result from the Biojector improving distribution of vaccine at the site of administration, spreading antigen to APCs in multiple tissue layers (90, 91). The Biojector is additionally advantageous because it uses disposable cartridges, which reduces the risk of spreading bloodborne pathogens by needle reuse, inadvertent needlestick, or improper needle disposal. The Biojector has been employed in clinical studies, which observed no adverse results or toxicity (86, 87, 92, 93). Thus, the Biojector may be a potent, safe, more patient-friendly mode of DNA vaccine administration.

Conceptually, HPV vaccines can be classified into two major categories: Preventive HPV vaccines and Therapeutic HPV vaccines.

3.3. Preventive HPV DNA vaccines

Immunoprophylactic vaccines induce a humoral immune response to prevent HPV infection. These vaccines generate neutralizing antibodies that can prevent viral particles from infecting host cells. Administration of DNA plasmid vaccines has been shown to induce a strong antibody response that is protective in a variety of animal models. DNA vaccination stimulates IgG, IgM, and IgA production, and the subclass of antibodies produced, IgG1 or IgG2a, depends on the route of DNA vaccination. Antibody production increases proportionally with dosage, in both a single injection and multiple injections by various routes of immunization (73, 94). Peak antibody responses typically occur 4-12 weeks post-vaccination (73, 94), but the magnitude and duration of antibody response to DNA vaccines is extremely variable, changing with the model system and vaccine used. Preventive DNA vaccine development has been complicated by the lack of animal models for the genital mucosatropic HPV types and by difficulty in propagating the virus in culture.

Choice of antigen is important for prophylactic vaccines. Early gene-based vaccines have been shown to generate only partial protection in studies with prophylactic DNA vaccines administered via gene gun to rabbits (95-98), although prophylactic immunity has been enhanced by priming DNA vaccination sites with a GM-CSF-exressing vector (99). The relatively weak prophylactic effects observed after E6 and E7 vaccine administration are probably a result of the absence of E6 and E7 on the viral surface. Conversely, L1 and L2, capsid proteins expressed on the viral surface, have generated highly potent humoral responses when administered in DNA vaccines, and are therefore the antigens generally employed in prophylactic vaccines for HPV.

Many prophylactic studies involving the intramuscular injection of capsid protein L1-encoding DNA have successfully generated an L1-specific immune response. For example, BALB/c (100, 101) and C57BL/6 mice (102) intramuscularly immunized with HPV L1-expressing plasmid DNA have been shown to develop specific anti-L1 antibodies. However, the route of administration seems to be important for the generation of mucosal immunity. Schreckenberger *et al.* reported that vaginal immunization of rabbits with HPV 6bL1 DNA induced 6bL1 virus-like particle-specific lgA antibodies in vaginal secretions and no mucosal immune response was detected in vaginal secretions of rabbits immunized intramuscularly or intrarectally (103).

The L1-specific humoral immune responses generated by L1 DNA vaccines have led to preventive effects in some animal models. For example, subcutaneous (104) or intramuscular (105) vaccination of rabbits with the L1 gene of cottontail rabbit papillomavirus (CRPV) generates antibodies capable of protecting all vaccinated rabbits (compared to no vaccination control group animals) against CRPV challenge. In another study, Stanley *et al.* immunized beagle dogs via particle-mediated DNA delivery (PMDD) of a plasmid encoding the canine oral papillomavirus (COPV) L1 gene to cutaneous and oral mucosal sites (106). Immunized animals developed a moderate L1 serum antibody response, and while five of six animals in the control group developed COPV papillomas, all animals vaccinated with the COPV L1 gene were protected against disease. Thus, studies in animal models elucidate the usefulness of L1-encoding DNA for generating L1-specific antibody responses that may contribute to protection from the formation of papillomas or tumors.

Although prophylactic vaccines have elicited protective immune responses in various models, several issues remain to be addressed. It is unclear whether sexual transmission is due to free viral particles or to particles still enclosed in detached squamous cells that might protect that virus from neutralizing antibody. Furthermore, the frequent and intimate nature of sexual contact implies a potentially large HPV inoculum, allowing for the potential breakthrough infection that escapes antibody-mediated neutralization. On the basis of observations made in both humans and mammals, it appears that HPV-specific antibodies are insufficient for clearing preexisting papillomavirus infection. Although HPV L1 vaccines has been shown to generate L1-specific $CD8^+$ T cell immune responses (107, 108), it is expected that such immunization

will be unable to generate significant therapeutic effects for established or breakthrough HPV infections that have escaped antibody-mediated neutralization. This is probably because capsid genes are only expressed upon terminal differentiation in the upper strata of the epidermis, but not in basal keratinocytes. In addition, cells with HPV integration usually exhibit deletion of L1, rendering these cells ineffective as a target for HPV L1-specific $CD8^+$ T cells. Pre-existing HPV infection is highly prevalent and responsible for considerable morbidity and mortality. Evidence suggests that cellular immunity, particularly antigen-specific T cell-mediated immunity, is required for treatment of established HPV infection (39). It is therefore important to develop vaccines that induce cell-mediated immune responses specific for early viral proteins in order to effect regression of established lesions and malignant tumors.

3.4. Therapeutic HPV DNA Vaccines

Therapeutic vaccines induce specific cell-mediated immunity, which can hinder the development of pre-existing lesions and malignant tumors, and even eliminate them. Theoretically, cell-mediated immunity can directly target HPV viral products, HPV-induced cellular products, or a combination of both. Since little is known about which cellular products can serve as targets for specific cell-mediated immune responses and there is also a concern for autoimmune responses, most of the experimental vaccination systems use carcinoma-associated HPV proteins, particularly E6 and E7, as targets for specific cellular immunity.

E6 and E7 are consistently expressed in most cervical cancers and their precursor lesions but absent from normal tissues. Thus, these viral oncoproteins represent promising targets for the development of antigen-specific vaccines to treat HPV associated cervical malignancies and their precursor lesions. While many tumor-specific antigens are derived from normal or mutated proteins, E6 and E7 are completely foreign viral proteins, and may therefore harbor more antigenic peptides/epitopes than a mutant cellular protein. Furthermore, since E6 and E7 are required for the induction and maintenance of the malignant phenotype of cancer cells (24), cervical cancer cells are unlikely to evade an immune response through antigen loss. Finally, studies in animal models suggest that vaccination targeting papillomavirus early proteins such as E7 can generate therapeutic as well as protective effects (25). Although E5- and L1- specific immune responses have been observed in certain HPV vaccine studies (107, 109), it is believed that the effectiveness of such strategies against HPV are limited since these proteins are frequently deleted in cervical neoplasia and carcinoma. Therefore, E6 and E7 proteins represent the most useful targets for developing antigen-specific immunotherapies or vaccines for cervical cancer.

Researchers typically focus on vaccines targeting E7 since it is more abundantly expressed and better characterized immunologically. Furthermore, its sequence is more conserved than that of the E6 gene (26). Recently, naked DNA vaccines have emerged as important therapeutic HPV vaccines because of their stability. DNA vaccines allow for sustained expression of antigen on MHC-peptide complexes compared to 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. Since DNA vaccines targeting different HPV types can be administered together, DNA vaccines may be effective for treating a variety of HPV-associated infections and tumors. These advantages have spurred interest in the development of DNA vaccines to treat cancers. 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 biojector delivery (for review, see (70, 72)). Thus, in the current review, we will focus on therapeutic HPV DNA vaccines.

3.4.1. DNA Vaccines and Activation of T Cells

Studies have investigated the mechanisms involved with intramuscular injection or gene gun delivery of DNA vaccines. Following intramuscular injection, myocytes can uptake DNA, allowing them to produce protein and transfer antigen to bone marrow-derived professional APCs (110). Cross priming, the processing of exogenous antigen transferred from another cell (i.e. secreted from DCs or in apoptotic bodies) via the MHC class I pathway (111, 112), provides an explanation for the transfer of antigen from cells initially transfected by intramuscular immunization (i.e. myocytes) to professional APCs.

After gene gun delivery, epidermal Langerhans cells uptake DNA and function as APCs. DCs in the skin carry antigen from the skin to the draining lymph nodes, where the antigen-loaded DCs activate naïve T cells (84). Intradermal vaccination with DNA facilitates direct priming, whereby antigen expressed in DCs is directly processed within the cell and presented on MHC class I molecules to CD8+ T cells. (113). The method of DNA inoculation (gene gun versus intramuscular injection) and the form of the DNA-expressed antigen (cytoplasmic versus secreted) can also influence the type of T cell help (Th1 or Th2)(for review see (114)).

3.4.2. Intracellular Targeting Strategies to Enhance MHC class I-restricted $CD8^+$ and MHC class II-restricted $CD4^+$ T Cell Responses

The delivery of DNA vaccines intradermally via gene gun allows for direct targeting of genes of interest into professional APCs *in vivo.* Gene gun immunization has been used to test several intracellular targeting strategies that enhance MHC class I and/or class II presentation of antigen. For example, MHC class I presentation of HPV-16 E7 can be significantly enhanced by linkage with *Mycobacterium tuberculosis* heat shock protein 70 (HSP70) (115), calreticulin (116) or the translocation domain (domain II) of *Pseudomonas aeruginosa* exotoxin A (ETA(dII)) (117) in the context of a DNA vaccine. The linkage of these molecules to E7 results in augmentation of

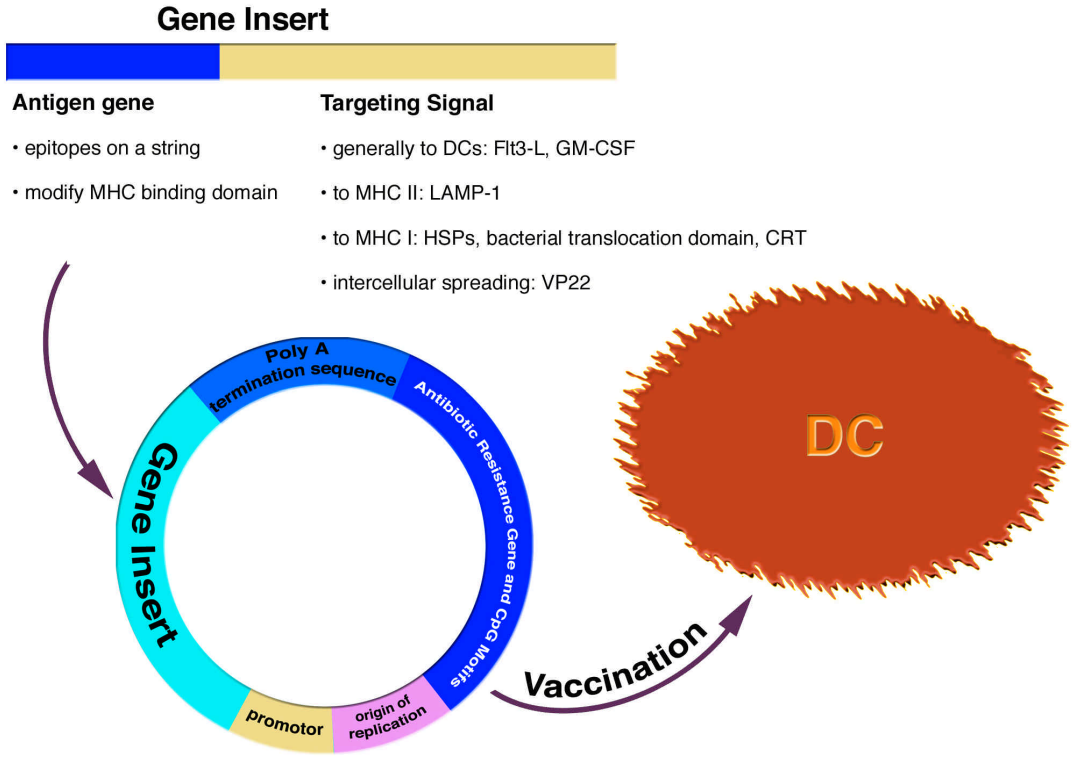

Figure 2. Strategies for Optimizing DNA Vaccines. Various strategies for enhancing the potency of DNA vaccines are currently under investigation. This figure summarizes popular sequences included in the genes insert to ensure DNA vaccine optimization.

the E7-specific $CD8^+$ T cell immune response in vaccinated mice. Furthermore, the use of DNA encoding a signal sequence linked to E7 and the sorting signal of the lysosome associated membrane protein (LAMP-1) to create the Sig/E7/LAMP-1 chimera can enhance MHC class II antigen processing (118). Expression of this DNA vaccine *in vitro* and *in vivo* targets E7 to endosomal and lysosomal compartments and enhances MHC class II presentation to $CD4^+$ T cells compared to DNA encoding wild-type E7 (119). While chimeric E7/HSP70, ETA(dII)/E7 or CRT/E7 DNA generates potent CD8+ T cell responses through enhanced MHC class I presentation, other constructs that target antigen to MHC class II presentation pathways may provide enhanced $CD4^+$ T cell responses. This realization raises the notion of co-administration of vaccines such as E7/HSP70 and Sig/E7/LAMP-1 in a synergistic fashion. Such an approach may directly enhance both MHC class I and class II presentation of E7 and lead to significantly enhanced E7-specific $CD4^+$ and $CD8^+$ T cell responses and potent antitumor effects.

Although DNA vaccines employing intracellular targeting strategies can significantly enhance MHC class I and class II presentation of antigen in transfected DCs, they may only generate a limited number of antigen-expressing DCs since naked DNA vaccines lack the intrinsic ability to amplify and spread *in vivo*. This significantly limits the potency of DNA vaccines. Therefore, a strategy that facilitates the spread of antigen to more DCs may significantly enhance the potency of naked DNA vaccines.

3.4.3. Intercellular Spreading to Enhance DNA Vaccine Potency

The potency of DNA vaccines may be enhanced through the use of herpes simplex virus (HSV-1) VP22, an HSV-1 tegument protein capable of intercellular transport and useful in spreading protein to surrounding cells (120). HSV-1 VP22 (HVP22) is capable of enhancing intercellular spreading of the linked protein. Furthermore, mice vaccinated with HVP22/E7 DNA generate a significantly greater number of E7 specific $CD8^+$ T cell precursors (117, 121, 122) and a stronger antitumor effect than wild-type E7 DNA (117). The success of the chimeric HSV-1 VP22/E7 DNA vaccine warrants the consideration of other proteins with similar trafficking properties.

At least two other proteins with purported intercellular spreading properties have been reported, including bovine herpesvirus VP22 (BVP22) (123) and Marek's disease virus VP22 (MVP22) (124), both of which are VP22 homologues. Bovine herpesvirus VP22 shares about 22% amino acid identity to human herpesvirus VP22 (123). A previous study found that BVP22 trafficking may be more efficient than human VP22 trafficking after endogenous synthesis (123). Marek's disease virus VP22 (MVP22) shares about 17% amino acid identity to human herpesvirus VP22 and may be capable of intercellular transport after exogenous application (125). The linkage of MVP22 to HPV-16 E7 in a context of DNA vaccine is capable of generating a significantly greater number of E7 specific $CD8^+$ T cell precursors and a stronger antitumor

effect than wild-type E7 DNA in vaccinated mice (126). Since different degrees of intercellular spreading of linked antigen encoded by DNA vaccine may influence the generation of antigen-specific immunity and antitumor effects in vaccinated mice, a head-to-head comparison of VP22 molecules may elucidate their relative ability to generate intercellular spreading of linked antigen and to influence vaccine potency. This strategy may also be combined with intracellular targeting strategies, such as HSP70, to generate even greater enhancement of antigen-specific immunity and antitumor effects.

3.4.4. Prolonging DC Survival to Enhance Immune Responses to DNA Vaccines

Although E7-specific T cell-mediated immune responses and antitumor effects can be enhanced by the vaccine strategies mentioned in **Section 3.4.2 and 3.4.3**, vaccine efficiency may be limited since transduced dendritic cells may themselves become targets of effector cells through apoptosis via perforin/granzyme B or death receptor pathways. One potential strategy to overcome this problem is to utilize DNA encoding inhibitors of apoptosis delivered to DCs in order to enhance the survival of DCs and prolong their ability to present the antigen of interest. Kim *et al.* have tested a variety of anti-apoptotic factors for their ability to enhance DC survival and E7-specific $CD8^+$ T cell immune responses including bclxL and bcl2, members of the bcl2 family of proteins; X-linked inhibitor of apoptosis protein (XIAP); and dominant negative mutants (dn) of caspases such as dn caspase-9 and dn caspase-8, which lack key functional components and serve as inhibitors of apoptosis (Kim, personal communication). An examination of E7-specific immune responses, the antitumor effect, and the characteristics and kinetics of dendritic cell involvement, revealed that co-administration of E7-containing DNA with DNA encoding anti-apoptotic proteins enhanced DNA vaccine potency and efficacy. In addition, the anti-apoptotic strategy may be used in conjunction with other intracellular targeting strategies or intercellular spreading strategies to generate synergistic effects in enhancing DNA vaccine potency (Kim *et al.*, unpublished observations).

3.4.5. Improving DNA Vaccine Potency with Cytokines and Costimulatory Adjuvants

It is well-known that costimulators are required to generate a CTL response. Methods that employ cytokines or costimulatory molecules may enhance the potency of DNA vaccines (82, 127, 128). Leachman *et al.* demonstrated that priming the E6 DNA vaccination site with a GM-CSF-expressing vector greatly enhances the effects of cottontail rabbit papillomavirus (CRPV) E6 vaccination, increasing tumor regression frequency and probability of rabbits remaining disease free after CRPV challenge (99). Tan *et al.* have shown that administration of IL-12 at the vaccination site of gene gun-administered plasmid DNA encoding E7 increased vaccine induced therapeutic efficacy (129). Thus, cytokines and costimulatory molecules may act as useful adjuvants for HPV DNA vaccines.

3.4.6. Improving DNA Vaccine Potency by Enhancing Protein Degradation

The immune response elicited by DNA vaccines may be augmented by manipulating pathways for intercellular protein degradation. Ubiquitin, a small protein cofactor, targets conjugated protein for recognition and degradation within the proteasome. Velders *et al.* have shown that a multi-epitope vaccine for HPV protected 100% of vaccinated mice against challenge with tumor cell line expressing HPV 16 E7, when ubiquitin and certain flanking sequences were included in the gene insert (130). Similarly, Liu *et al.* observed enhancement of E7-specific CTL activity and protection against E7-expressing tumors in mice given a DNA vaccine with a ubiquitinated L1-E7 gene insert (131). Although ubiquitin may be a useful molecule for expediting protein degradation and antigen processing, it is not the only means of enhancing intercellular protein degradation. Shi *et al.* engineered mutations into two zinc-binding motifs of an HPV-16 E7 DNA vaccine to generate a rapidly degraded E7 protein (132). This mutated E7 protein elicited a significantly enhanced E7-specific CTL response and better protection compared to a wild type E7 DNA vaccine (132). These studies suggest that the enhancement of intercellular degradation of the antigen of interest may increase the immunogenicity of DNA vaccines.

3.4.7. Safety of DNA Vaccines

Although the efficacy of DNA vaccination is important, safety is also a critical issue. DNA present in the vaccine may integrate into the host genome, potentially inactivating tumor suppresser genes or activating oncogenes, thereby inducing malignant transformation of the host cell. Fortunately, it is estimated that the frequency of integration is much lower than that of spontaneous mutation and integration should not pose any real risk (133). A second issue concerns potential risks associated with the presence of HPV-16 E7 protein in host cells. E7 is an oncoprotein that disrupts cell cycle regulation by binding to pRb, a tumor suppressor protein in nuclei (134). The presence of E7 in the nuclei may lead to accumulation of genetic aberrations and eventual malignant transformation of the host cells. To avoid such problems, strategies such as the endosomal/lysosomal-targeting Sig/E7/LAMP-1 DNA vaccine may be employed to divert E7 away from the nucleus to regions such as the endosomal and lysosomal compartments to physically separate E7 from pRb. In addition, detailed mutational analysis of E7 has led to the identification of a number of mutations that abrogate the transformation activity of E7 (135-138). One recent study demonstrated that a DNA vaccine encoding E7 with a mutation which inactivate Rb-binding site was able to enhance CTL activity and E7-specific antitumor effects compared to wild-type E7 (132). DNA vaccines using a shuffled E7 gene may also alleviate concerns of oncogenicity associated with E7 (121). Another strategy to avoid the problem of the oncogenicity of E7 is to use a DNA vaccine encoding a string of multiple epitopes flanked by defined spacer sequences; this may be safe and promising for tumor protection and therapy, particularly if epitopes are targeted to the protein degradation pathway (130). Ultimately, DNA vectors employed in human

clinical trials may use a minimally mutated E7 gene or multiepitope gene approach in which critical epitopes are preserved while potential oncogenic activity is eliminated.

4. PERSPECTIVE

Recently, significant progress has been made in the field of HPV naked DNA vaccine development. The determination that HPV is an etiological 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 HPV-associated malignancies and its potentially lethal consequences. An understanding of the molecular progression of cervical cancer has led to the realization that HPV L1 and L2 are suitable targets for preventive vaccines, while E6 and E7 are important targets for the development of HPV therapeutic vaccines for the control of established HPV infections and HPV-associated lesions. The emergence of DNA vaccines and various strategies to engineer potent anti-HPV immune responses represents an important advance in cervical cancer immunotherapy. Early clinical application of DNA vaccines against HPV is underway, and may pave the way for more advanced clinical investigation. 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 immunological parameters will help define protective immune mechanisms for controlling HPV infections and HPV-related disease. Comprehensive information on these protective immune mechanisms in humans will facilitate the development of more effective anti-HPV DNA vaccines to fight cervical cancer.

5. ACKNOWLEDGEMENTS

This review is not intended to be an encyclopedic one and we apologize to the authors not cited. We would like to thank Drs. Keerti V. Shah, Robert J. Kurman, Drew M. Pardoll and Ken-Yu Lin for their helpful discussions. We would also like to thank Drs. Richard Roden and Liang Qiao for their critical review of the manuscript.

6. REFERENCES

1. Walboomers, J. M., M. V. Jacobs, M. M. Manos, F. X. Bosch, J. A. Kummer, K. V. Shah, P. J. Snijders, J. Peto, C. J. Meijer, and N. Munoz: Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 189, 12-19 (1999)

2. zur Hausen, H.: Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2, 342-350 (2002)

3. Stoler, M. H.: Human papillomaviruses and cervical neoplasia: A model for carcinogenesis. *Int J Gynecol Pathol* 19, 16-28. (2000)

4. Bosch, F. X., M. M. Manos, N. Munoz, M. Sherman, A. M. Jansen, J. Peto, M. H. Schiffman, V. Moreno, R. Kurman, and K. V. Shah: Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer Inst* 87, 796-802 (1995)

5. Howley, P. M. (1996). Papillomavirinae: The viruses and their replication. In Fields Virology, B. N. Fields, D. M. Knipe, and P. M. Howley, Eds. (Philadelphia, Lippincott-Raven Publishers), pp. 2045-2076.

6. Shah, K. V., and P. M. Howley (1996). Papillomaviruses. In Fields Virology, B. N. Fields, D. M. Knipe, and P. M. Howley, Eds. (Philadelphia, Lippincott-Raven Publishers), pp. 2077-2109.

7. Koutsky, L. A., K. K. Holmes, C. W. Critchlow, C. E. Stevens, J. Paavonen, A. M. Beckmann, T. A. DeRouen, D. A. Galloway, D. Vernon, and N. B. Kiviat: A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection. *N Engl J Med* 327, 1272-1278 (1992)

8. Zhou, J., X. Y. Sun, H. Davies, L. Crawford, D. Park, and I. H. Frazer: Definition of linear antigenic regions of the HPV16 L1 capsid protein using synthetic virion-like particles. *Virology* 189, 592-599 (1992)

9. Hagensee, M. E., N. Yaegashi, and D. A. Galloway: Self-assembly of human papillomavirus type 1 capsids by expression of the L1 protein alone or by coexpression of the L1 and L2 capsid proteins. *J Virol* 67, 315-322 (1993)

10. Kirnbauer, R., F. Booy, N. Cheng, D. R. Lowy, and J. T. Schiller: Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. *Proc Natl Acad Sci U S A* 89, 12180-12184 (1992)

11. Sasagawa, T., P. Pushko, G. Steers, S. E. Gschmeissner, M. A. Hajibagheri, J. Finch, L. Crawford, and M. Tommasino: Synthesis and assembly of virus-like particles of human papillomaviruses type 6 and type 16 in fission yeast Schizosaccharomyces pombe. *Virology* 206, 126-135 (1995)

12. Nardelli-Haefliger, D., R. B. Roden, J. Benyacoub, R. Sahli, J. P. Kraehenbuhl, J. T. Schiller, P. Lachat, A. Potts, and P. De Grandi: Human papillomavirus type 16 virus-like particles expressed in attenuated Salmonella typhimurium elicit mucosal and systemic neutralizing antibodies in mice. *Infect Immun* 65, 3328-3336 (1997)

13. Jansen, K. U., M. Rosolowsky, L. D. Schultz, H. Z. Markus, J. C. Cook, J. J. Donnelly, D. Martinez, R. W. Ellis, and A. R. Shaw: Vaccination with yeast-expressed cottontail rabbit papillomavirus (CRPV) virus-like particles protects rabbits from CRPV-induced papilloma formation. *Vaccine* 13, 1509-1514 (1995)

14. Christensen, N. D., C. A. Reed, N. M. Cladel, R. Han, and J. W. Kreider: Immunization with viruslike particles induces long-term protection of rabbits against challenge with cottontail rabbit papillomavirus. *J Virol* 70, 960-965 (1996)

15. Breitburd, F., R. Kirnbauer, N. L. Hubbert, B. Nonnenmacher, C. Trin-Dinh-Desmarquet, G. Orth, J. T. Schiller, and D. R. Lowy: Immunization with viruslike particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. *J Virol* 69, 3959-3963 (1995)

16. Suzich, J. A., S. J. Ghim, F. J. Palmer-Hill, W. I. White, J. K. Tamura, J. A. Bell, J. A. Newsome, A. B. Jenson, and R. Schlegel: Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. *Proc Natl Acad Sci U S A* 92, 11553-11557 (1995)

17. Kirnbauer, R., L. M. Chandrachud, B. W. O'Neil, E. R. Wagner, G. J. Grindlay, A. Armstrong, G. M. McGarvie, J. T. Schiller, D. R. Lowy, and M. S. Campo: Virus-like particles of bovine papillomavirus type 4 in prophylactic and therapeutic immunization. *Virology* 219, 37-44 (1996)
18. Parr, M. B., and E. L. Parr: Immunohistochemical localization of immunoglobulins A, G and M in the mouse female genital tract. *J Reprod Fertil* 74, 361-370 (1985)
19. Thapar, M. A., E. L. Parr, and M. B. Parr: Secretory immune responses in mouse vaginal fluid after pelvic, parenteral or vaginal immunization. *Immunology* 70, 121-125 (1990)
20. Balmelli, C., R. Roden, A. Potts, J. Schiller, P. De Grandi, and D. Nardelli-Haefliger: Nasal immunization of mice with human papillomavirus type 16 virus-like particles elicits neutralizing antibodies in mucosal secretions. *J Virol* 72, 8220-8229 (1998)
21. Russell, M. W., Z. Moldoveanu, P. L. White, G. J. Sibert, J. Mestecky, and S. M. Michalek: Salivary, nasal, genital, and systemic antibody responses in monkeys immunized intranasally with a bacterial protein antigen and the cholera toxin b subunit. *Infect Immun* 64, 1272-1283 (1996)
22. Hagensee, M. E., J. J. Carter, G. C. Wipf, and D. A. Galloway: Immunization of mice with HPV vaccinia virus recombinants generates serum IgG, IgM, and mucosal IgA antibodies. *Virology* 206, 174-182 (1995)
23. Lowe, R. S., D. R. Brown, J. T. Bryan, J. C. Cook, H. A. George, K. J. Hofmann, W. M. Hurni, J. G. Joyce, E. D. Lehman, H. Z. Markus, M. P. Neeper, L. D. Schultz, A. R. Shaw, and K. U. Jansen: Human papillomavirus type 11 (HPV-11) neutralizing antibodies in the serum and genital mucosal secretions of African green monkeys immunized with HPV-11 virus-like particles expressed in yeast. *J Infect Dis* 176, 1141-1145 (1997)
24. Crook, T., J. P. Morgenstern, L. Crawford, and L. Banks: Continued expression of HPV-16 E7 protein is required for maintenance of the transformed phenotype of cells co-transformed by HPV-16 plus EJ-ras. *Embo J* 8, 513-519 (1989)
25. Campo, M. S., G. J. Grindlay, B. W. O'Neil, L. M. Chandrachud, G. M. McGarvie, and W. F. Jarrett: Prophylactic and therapeutic vaccination against a mucosal papillomavirus. *J Gen Virol* 74, 945-953 (1993)
26. Zehbe, I., E. Wilander, H. Delius, and M. Tommasino: Human papillomavirus 16 E6 variants are more prevalent in invasive cervical carcinoma than the prototype. *Cancer Res* 58, 829-833 (1998)
27. Meneguzzi, G., C. Cerni, M. P. Kieny, and R. Lathe: Immunization against human papillomavirus type 16 tumor cells with recombinant vaccinia viruses expressing E6 and E7. *Virology* 181, 62-69 (1991)
28. Chen, L., S. Ashe, W. A. Brady, I. Hellstrom, K. E. Hellstrom, J. A. Ledbetter, P. McGowan, and P. S. Linsley: Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. *Cell* 71, 1093-1102 (1992)
29. Feltkamp, M. C., H. L. Smits, M. P. Vierboom, R. P. Minnaar, J. B. de, J. W. Drijfhout, S. J. ter, C. J. Melief, and W. M. Kast: Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. *Eur J Immunol* 23, 2242-2249 (1993)
30. Lin, K.-Y., F. G. Guarnieri, K. F. Staveley-O'Carroll, H. I. Levitsky, T. August, D. M. Pardoll, and T.-C. Wu: Treatment of established tumors with a novel vaccine that enhances major histocompatibility class II presentation of tumor antigen. *Cancer Research* 56, 21-26. (1996)
31. Greenstone, H. L., J. D. Nieland, K. E. de Visser, M. L. De Bruijn, R. Kirnbauer, R. B. Roden, D. R. Lowy, W. M. Kast, and J. T. Schiller: Chimeric papillomavirus virus-like particles elicit antitumor immunity against the E7 oncoprotein in an HPV16 tumor model. *Proc Natl Acad Sci U S A* 95, 1800-1805 (1998)
32. Wu, T. C.: Immunology of the human papilloma virus in relation to cancer. *Curr Opin Immunol* 6, 746-754. (1994)
33. Halpert, R., R. G. Fruchter, A. Sedlis, K. Butt, J. G. Boyce, and F. H. Sillman: Human papillomavirus and lower genital neoplasia in renal transplant patients. *Obstet Gynecol* 68, 251-258 (1986)
34. Schafer, A., W. Friedmann, M. Mielke, B. Schwartlander, and M. A. Koch: The increased frequency of cervical dysplasia-neoplasia in women infected with the human immunodeficiency virus is related to the degree of immunosuppression. *Am J Obstet Gynecol* 164, 593-599 (1991)
35. Laga, M., J. P. Icenogle, R. Marsella, A. T. Manoka, N. Nzila, R. W. Ryder, S. H. Vermund, W. L. Heyward, A. Nelson, and W. C. Reeves: Genital papillomavirus infection and cervical dysplasia--opportunistic complications of HIV infection. *Int J Cancer* 50, 45-48 (1992)
36. Brandsma, J. L. (1994). Animal models for HPV vaccine development. In Papillomavirus Report, pp. 105-111.
37. Selvakumar, R., L. A. Borenstein, Y. L. Lin, R. Ahmed, and F. O. Wettstein: Immunization with nonstructural proteins E1 and E2 of cottontail rabbit papillomavirus stimulates regression of virus-induced papillomas. *J Virol* 69, 602-605 (1995)
38. Tagami, H.: Regression phenomenon of numerous flat warts--an experiment on the nature of tumor immunity in man. *Int J Dermatol* 22, 570-571 (1983)
39. Benton, C., H. Shahidullah, and J. A. A. Hunter (1992). Human papillomavirus in the immunosuppressed. In Papillomavirus Report, pp. 23-26.
40. Steinman, R. M.: The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* 9, 271-296 (1991)
41. Cella, M., F. Sallusto, and A. Lanzavecchia: Origin, maturation and antigen presenting function of dendritic cells. *Curr Opin Immunol* 9, 10-16 (1997)
42. Liu, Y. J.: Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. *Cell* 106, 259-262 (2001)
43. van Kooten, C., and J. Banchereau: Functional role of CD40 and its ligand. *Int Arch Allergy Immunol* 113, 393-399 (1997)
44. Austyn, J. M.: New insights into the mobilization and phagocytic activity of dendritic cells. *J Exp Med* 183, 1287-1292 (1996)
45. Krug, A., A. Towarowski, S. Britsch, S. Rothenfusser, V. Hornung, R. Bals, T. Giese, H. Engelmann, S. Endres, A. M. Krieg, and G. Hartmann: Toll-like receptor expression reveals CpG DNA as a unique microbial

stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur J Immunol* 31, 3026-3037 (2001)
46. Kadowaki, N., S. Ho, S. Antonenko, R. W. Malefyt, R. A. Kastelein, F. Bazan, and Y. J. Liu: Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* 194, 863-869 (2001)
47. Bauer, S., C. J. Kirschning, H. Hacker, V. Redecke, S. Hausmann, S. Akira, H. Wagner, and G. B. Lipford: Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc Natl Acad Sci U S A* 98, 9237-9242 (2001)
48. Krieg, A. M., A. K. Yi, S. Matson, T. J. Waldschmidt, G. A. Bishop, R. Teasdale, G. A. Koretzky, and D. M. Klinman: CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* 374, 546-549 (1995)
49. Jakob, T., P. S. Walker, A. M. Krieg, M. C. Udey, and J. C. Vogel: Activation of cutaneous dendritic cells by CpG-containing oligodeoxynucleotides: a role for dendritic cells in the augmentation of Th1 responses by immunostimulatory DNA. *J Immunol* 161, 3042-3049 (1998)
50. Stacey, K. J., M. J. Sweet, and D. A. Hume: Macrophages ingest and are activated by bacterial DNA. *J Immunol* 157, 2116-2122 (1996)
51. Hartmann, G., G. J. Weiner, and A. M. Krieg: CpG DNA: a potent signal for growth, activation, and maturation of human dendritic cells. *Proc Natl Acad Sci U S A* 96, 9305-9310 (1999)
52. Cowdery, J. S., J. H. Chace, A. K. Yi, and A. M. Krieg: Bacterial DNA induces NK cells to produce IFN-gamma *in vivo* and increases the toxicity of lipopolysaccharides. *J Immunol* 156, 4570-4575 (1996)
53. Yamamoto, S., E. Kuramoto, S. Shimada, and T. Tokunaga: *In vitro* augmentation of natural killer cell activity and production of interferon-alpha/beta and -gamma with deoxyribonucleic acid fraction from Mycobacterium bovis BCG. *Jpn J Cancer Res* 79, 866-873 (1988)
54. Yamamoto, S., T. Yamamoto, S. Shimada, E. Kuramoto, O. Yano, T. Kataoka, and T. Tokunaga: DNA from bacteria, but not from vertebrates, induces interferons, activates natural killer cells and inhibits tumor growth. *Microbiol Immunol* 36, 983-997 (1992)
55. Yamamoto, T., S. Yamamoto, T. Kataoka, and T. Tokunaga: Ability of oligonucleotides with certain palindromes to induce interferon production and augment natural killer cell activity is associated with their base length. *Antisense Res Dev* 4, 119-122 (1994)
56. Bendigs, S., U. Salzer, G. B. Lipford, H. Wagner, and K. Heeg: CpG-oligodeoxynucleotides co-stimulate primary T cells in the absence of antigen-presenting cells. *Eur J Immunol* 29, 1209-1218 (1999)
57. Sun, S., X. Zhang, D. F. Tough, and J. Sprent: Type I interferon-mediated stimulation of T cells by CpG DNA. *J Exp Med* 188, 2335-2342 (1998)
58. Lipford, G. B., M. Bauer, C. Blank, R. Reiter, H. Wagner, and K. Heeg: CpG-containing synthetic oligonucleotides promote B and cytotoxic T cell responses to protein antigen: a new class of vaccine adjuvants. *Eur J Immunol* 27, 2340-2344 (1997)
59. Gerard, C. M., N. Baudson, K. Kraemer, C. Bruck, N. Garcon, Y. Paterson, Z. K. Pan, and D. Pardoll: Therapeutic potential of protein and adjuvant vaccinations on tumour growth. *Vaccine* 19, 2583-2589 (2001)
60. Gerber, S., C. Lane, D. M. Brown, E. Lord, M. DiLorenzo, J. D. Clements, E. Rybicki, A. L. Williamson, and R. C. Rose: Human papillomavirus virus-like particles are efficient oral immunogens when coadministered with Escherichia coli heat-labile enterotoxin mutant R192G or CpG DNA. *J Virol* 75, 4752-4760 (2001)
61. Klinman, D. M., G. Yamshchikov, and Y. Ishigatsubo: Contribution of CpG motifs to the immunogenicity of DNA vaccines. *J Immunol* 158, 3635-3639 (1997)
62. Davis, H. L., R. Weeratna, T. J. Waldschmidt, L. Tygrett, J. Schorr, A. M. Krieg, and R. Weeranta: CpG DNA is a potent enhancer of specific immunity in mice immunized with recombinant hepatitis B surface antigen. *J Immunol* 160, 870-876 (1998)
63. Brazolot Millan, C. L., R. Weeratna, A. M. Krieg, C. A. Siegrist, and H. L. Davis: CpG DNA can induce strong Th1 humoral and cell-mediated immune responses against hepatitis B surface antigen in young mice. *Proc Natl Acad Sci U S A* 95, 15553-15558 (1998)
64. Hartmann, G., R. D. Weeratna, Z. K. Ballas, P. Payette, S. Blackwell, I. Suparto, W. L. Rasmussen, M. Waldschmidt, D. Sajuthi, R. H. Purcell, H. L. Davis, and A. M. Krieg: Delineation of a CpG phosphorothioate oligodeoxynucleotide for activating primate immune responses *in vitro* and *in vivo*. *J Immunol* 164, 1617-1624 (2000)
65. Yi, A. K., M. Chang, D. W. Peckham, A. M. Krieg, and R. F. Ashman: CpG oligodeoxyribonucleotides rescue mature spleen B cells from spontaneous apoptosis and promote cell cycle entry. *J Immunol* 160, 5898-5906 (1998)
66. Hartmann, G., and A. M. Krieg: Mechanism and function of a newly identified CpG DNA motif in human primary B cells. *J Immunol* 164, 944-953 (2000)
67. Krieg, A. M., T. Wu, R. Weeratna, S. M. Efler, L. Love-Homan, L. Yang, A. K. Yi, D. Short, and H. L. Davis: Sequence motifs in adenoviral DNA block immune activation by stimulatory CpG motifs. *Proc Natl Acad Sci U S A* 95, 12631-12636 (1998)
68. Gurunathan, S., D. M. Klinman, and R. A. Seder: DNA vaccines: immunology, application, and optimization*. *Annu Rev Immunol* 18, 927-974 (2000)
69. Pardoll, D. M., and A. M. Beckerleg: Exposing the immunology of naked DNA vaccines. *Immunity* 3, 165-169 (1995)
70. Donnelly, J. J., J. B. Ulmer, J. W. Shiver, and M. A. Liu: DNA vaccines. *Annu Rev Immunol* 15, 617-648 (1997)
71. Albert, M. L., S. F. Pearce, L. M. Francisco, B. Sauter, P. Roy, R. L. Silverstein, and N. Bhardwaj: Immature dendritic cells phagocytose apoptotic cells via alphavbeta5 and CD36, and cross-present antigens to cytotoxic T lymphocytes. *J Exp Med* 188, 1359-1368 (1998)
72. Robinson, H. L., and C. A. Torres: DNA vaccines. *Semin Immunol* 9, 271-283 (1997)
73. Robinson, H. L., and C. A. Torres: DNA vaccines. *Semin Immunol* 9, 271-283 (1997)
74. Tuting, T., A. Gambotto, P. D. Robbins, W. J. Storkus, and A. B. DeLeo: Co-delivery of T helper 1-biasing cytokine genes enhances the efficacy of gene gun

immunization of mice: studies with the model tumor antigen beta-galactosidase and the BALB/c Meth A p53 tumor-specific antigen. *Gene Ther* 6, 629-636 (1999)
75. Bright, R. K., B. Beames, M. H. Shearer, and R. C. Kennedy: Protection against a lethal challenge with SV40-transformed cells by the direct injection of DNA-encoding SV40 large tumor antigen. *Cancer Res* 56, 1126-1130 (1996)
76. Schirmbeck, R., W. Bohm, and J. Reimann: DNA vaccination primes MHC class I-restricted, simian virus 40 large tumor antigen-specific CTL in H-2d mice that reject syngeneic tumors. *J Immunol* 157, 3550-3558 (1996)
77. Geissler, M., G. Wands, A. Gesien, S. de la Monte, D. Bellet, and J. R. Wands: Genetic immunization with the free human chorionic gonadotropin beta subunit elicits cytotoxic T lymphocyte responses and protects against tumor formation in mice. *Lab Invest* 76, 859-871 (1997)
78. Syrengelas, A. D., T. T. Chen, and R. Levy: DNA immunization induces protective immunity against B-cell lymphoma. *Nat Med* 2, 1038-1041 (1996)
79. Rosato, A., A. Zambon, G. Milan, V. Ciminale, D. M. D'Agostino, B. Macino, P. Zanovello, and D. Collavo: CTL response and protection against P815 tumor challenge in mice immunized with DNA expressing the tumor-specific antigen P815A. *Hum Gene Ther* 8, 1451-1458 (1997)
80. Conry, R. M., A. F. LoBuglio, F. Loechel, S. E. Moore, L. A. Sumerel, D. L. Barlow, and D. T. Curiel: A carcinoembryonic antigen polynucleotide vaccine has *in vivo* antitumor activity. *Gene Ther* 2, 59-65 (1995)
81. Ciernik, I. F., J. A. Berzofsky, and D. P. Carbone: Induction of cytotoxic T lymphocytes and antitumor immunity with DNA vaccines expressing single T cell epitopes. *J Immunol* 156, 2369-2375 (1996)
82. Irvine, K. R., J. B. Rao, S. A. Rosenberg, and N. P. Restifo: Cytokine enhancement of DNA immunization leads to effective treatment of established pulmonary metastases. *J Immunol* 156, 238-245 (1996)
83. Tighe, H., M. Corr, M. Roman, and E. Raz: Gene vaccination: plasmid DNA is more than just a blueprint. *Immunol Today* 19, 89-97 (1998)
84. Condon, C., S. C. Watkins, C. M. Celluzzi, K. Thompson, and L. D. Falo, Jr.: DNA-based immunization by *in vivo* transfection of dendritic cells. *Nat Med* 2, 1122-1128 (1996)
85. Rogers, W. O., J. K. Baird, A. Kumar, J. A. Tine, W. Weiss, J. C. Aguiar, K. Gowda, R. Gwadz, S. Kumar, M. Gold, and S. L. Hoffman: Multistage multiantigen heterologous prime boost vaccine for Plasmodium knowlesi malaria provides partial protection in rhesus macaques. *Infect Immun* 69, 5565-5572 (2001)
86. Jackson, L. A., G. Austin, R. T. Chen, R. Stout, F. DeStefano, G. J. Gorse, F. K. Newman, O. Yu, and B. G. Weniger: Safety and immunogenicity of varying dosages of trivalent inactivated influenza vaccine administered by needle-free jet injectors. *Vaccine* 19, 4703-4709 (2001)
87. Wang, R., J. Epstein, F. M. Baraceros, E. J. Gorak, Y. Charoenvit, D. J. Carucci, R. C. Hedstrom, N. Rahardjo, T. Gay, P. Hobart, R. Stout, T. R. Jones, T. L. Richie, S. E. Parker, D. L. Doolan, J. Norman, and S. L. Hoffman: Induction of CD4(+) T cell-dependent CD8(+) type 1 responses in humans by a malaria DNA vaccine. *Proc Natl Acad Sci U S A* 98, 10817-10822 (2001)
88. Williams, J., L. Fox-Leyva, C. Christensen, D. Fisher, E. Schlicting, M. Snowball, S. Negus, J. Mayers, R. Koller, and R. Stout: Hepatitis A vaccine administration: comparison between jet-injector and needle injection. *Vaccine* 18, 1939-1943 (2000)
89. Aguiar, J. C., R. C. Hedstrom, W. O. Rogers, Y. Charoenvit, J. B. Sacci, Jr., D. E. Lanar, V. F. Majam, R. R. Stout, and S. L. Hoffman: Enhancement of the immune response in rabbits to a malaria DNA vaccine by immunization with a needle-free jet device. *Vaccine* 20, 275-280 (2001)
90. Reis, E. C., R. M. Jacobson, S. Tarbell, and B. G. Weniger: Taking the sting out of shots: control of vaccination-associated pain and adverse reactions. *Pediatr Ann* 27, 375-386 (1998)
91. Lemon, S. M., R. M. Scott, and W. H. Bancroft: Subcutaneous administration of inactivated hepatitis B vaccine by automatic jet injection. *J Med Virol* 12, 129-136 (1983)
92. Marshall, J. L., R. J. Hoyer, M. A. Toomey, K. Faraguna, P. Chang, E. Richmond, J. E. Pedicano, E. Gehan, R. A. Peck, P. Arlen, K. Y. Tsang, and J. Schlom: Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J Clin Oncol* 18, 3964-3973 (2000)
93. Marshall, J. L., M. J. Hawkins, K. Y. Tsang, E. Richmond, J. E. Pedicano, M. Z. Zhu, and J. Schlom: Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J Clin Oncol* 17, 332-337 (1999)
94. Deck, R. R., C. M. DeWitt, J. J. Donnelly, M. A. Liu, and J. B. Ulmer: Characterization of humoral immune responses induced by an influenza hemagglutinin DNA vaccine. *Vaccine* 15, 71-78 (1997)
95. Sundaram, P., R. E. Tigelaar, W. Xiao, and J. L. Brandsma: Intracutaneous vaccination of rabbits with the E6 gene of cottontail rabbit papillomavirus provides partial protection against virus challenge. *Vaccine* 16, 613-623 (1998)
96. Han, R., N. M. Cladel, C. A. Reed, X. Peng, and N. D. Christensen: Protection of rabbits from viral challenge by gene gun-based intracutaneous vaccination with a combination of cottontail rabbit papillomavirus E1, E2, E6, and E7 genes. *J Virol* 73, 7039-7043 (1999)
97. Han, R., C. A. Reed, N. M. Cladel, and N. D. Christensen: Intramuscular injection of plasmid DNA encoding cottontail rabbit papillomavirus E1, E2, E6 and E7 induces T cell-mediated but not humoral immune responses in rabbits. *Vaccine* 17, 1558-1566 (1999)
98. Han, R., C. A. Reed, N. M. Cladel, and N. D. Christensen: Immunization of rabbits with cottontail rabbit papillomavirus E1 and E2 genes: protective immunity induced by gene gun-mediated intracutaneous delivery but not by intramuscular injection. *Vaccine* 18, 2937-2944 (2000)
99. Leachman, S. A., R. E. Tigelaar, M. Shlyankevich, M. D. Slade, M. Irwin, E. Chang, T. C. Wu, W. Xiao, S. Pazhani, D. Zelterman, and J. L. Brandsma: Granulocyte-macrophage colony-stimulating factor priming plus

papillomavirus E6 DNA vaccination: effects on papilloma formation and regression in the cottontail rabbit papillomavirus--rabbit model. *J Virol* 74, 8700-8708 (2000)
100. Sun, X., L. Si, Z. Cao, Y. Wang, T. Liu, and J. Guo: Immune response to plasmid DNA encoding HPV16-L1 protein. *Chin Med J (Engl)* 113, 277-280 (2000)
101. Matsumoto, K., K. Kawana, H. Yoshikawa, Y. Taketani, K. Yoshiike, and T. Kanda: DNA vaccination of mice with plasmid expressing human papillomavirus 6 major capsid protein L1 elicits type-specific antibodies neutralizing pseudovirions constructed *in vitro*. *J Med Virol* 60, 200-204 (2000)
102. Rocha-Zavaleta, L., J. E. Alejandre, and A. Garcia-Carranca: Parenteral and oral immunization with a plasmid DNA expressing the human papillomavirus 16-L1 gene induces systemic and mucosal antibodies and cytotoxic T lymphocyte responses. *J Med Virol* 66, 86-95 (2002)
103. Schreckenberger, C., P. Sethupathi, A. Kanjanahaluethai, M. Muller, J. Zhou, L. Gissmann, and L. Qiao: Induction of an HPV 6bL1-specific mucosal IgA response by DNA immunization. *Vaccine* 19, 227-233 (2000)
104. Sundaram, P., R. E. Tigelaar, and J. L. Brandsma: Intracutaneous vaccination of rabbits with the cottontail rabbit papillomavirus (CRPV) L1 gene protects against virus challenge. *Vaccine* 15, 664-671 (1997)
105. Donnelly, J. J., D. Martinez, K. U. Jansen, R. W. Ellis, D. L. Montgomery, and M. A. Liu: Protection against papillomavirus with a polynucleotide vaccine. *J Infect Dis* 173, 314-320 (1996)
106. Stanley, M. A., R. A. Moore, P. K. Nicholls, E. B. Santos, L. Thomsen, N. Parry, S. Walcott, and G. Gough: Intra-epithelial vaccination with COPV L1 DNA by particle-mediated DNA delivery protects against mucosal challenge with infectious COPV in beagle dogs. *Vaccine* 19, 2783-2792 (2001)
107. De Bruijn, M. L., H. L. Greenstone, H. Vermeulen, C. J. Melief, D. R. Lowy, J. T. Schiller, and W. M. Kast: L1-specific protection from tumor challenge elicited by HPV16 virus- like particles. *Virology* 250, 371-376 (1998)
108. Rudolf, M. P., J. D. Nieland, D. M. DaSilva, M. P. Velders, M. Muller, H. L. Greenstone, J. T. Schiller, and W. M. Kast: Induction of HPV16 capsid protein-specific human T cell responses by virus-like particles. *Biol Chem* 380, 335-340 (1999)
109. Liu, D. W., Y. P. Tsao, C. H. Hsieh, J. T. Hsieh, J. T. Kung, C. L. Chiang, S. J. Huang, and S. L. Chen: Induction of CD8 T cells by vaccination with recombinant adenovirus expressing human papillomavirus type 16 E5 gene reduces tumor growth. *J Virol* 74, 9083-9089 (2000)
110. Corr, M., D. J. Lee, D. A. Carson, and H. Tighe: Gene vaccination with naked plasmid DNA: mechanism of CTL priming. *J Exp Med* 184, 1555-1560 (1996)
111. Huang, A. Y., P. Golumbek, M. Ahmadzadeh, E. Jaffee, D. Pardoll, and H. Levitsky: Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. *Science* 264, 961-965 (1994)
112. Albert, M. L., B. Sauter, and N. Bhardwaj: Dendritic cells acquire antigen from apoptotic cells and induce class I- restricted CTLs. *Nature* 392, 86-89 (1998)
113. Porgador, A., K. R. Irvine, A. Iwasaki, B. H. Barber, N. P. Restifo, and R. N. Germain: Predominant role for directly transfected dendritic cells in antigen presentation to CD8+ T cells after gene gun immunization. *J Exp Med* 188, 1075-1082 (1998)
114. Robinson, H. L.: Nucleic acid vaccines: an overview. *Vaccine* 15, 785-787 (1997)
115. Chen, C.-H., T.-L. Wang, C.-F. Hung, Y. Yang, R. A. Young, D. M. Pardoll, and T.-C. Wu: Enhancement of DNA vaccine potency by linkage of antigen gene to an HSP70 gene. *Cancer Res* 60, 1035-1042 (2000)
116. Cheng, W. F., C. F. Hung, C. Y. Chai, K. F. Hsu, L. He, M. Ling, and T. C. Wu: Tumor-specific immunity and antiangiogenesis generated by a DNA vaccine encoding calreticulin linked to a tumor antigen. *J Clin Invest* 108, 669-678 (2001)
117. Hung, C. F., W. F. Cheng, C. Y. Chai, K. F. Hsu, L. He, M. Ling, and T. C. Wu: Improving vaccine potency through intercellular spreading and enhanced MHC class I presentation of antigen. *J Immunol* 166, 5733-5740 (2001)
118. Wu, T.-C., F. G. Guarnieri, K. F. Staveley-O'Carroll, R. P. Viscidi, H. I. Levitsky, L. Hedrick, K. R. Cho, T. August, and D. M. Pardoll: Engineering an intracellular pathway for MHC class II presentation of HPV-16 E7. *Proc Natl Acad Sci* 92, 11671-11675 (1995)
119. Ji, H., T.-L. Wang, C.-H. Chen, C.-F. Hung, S. Pai, K.-Y. Lin, R. J. Kurman, D. M. Pardoll, and T.-C. Wu: Targeting HPV-16 E7 to the endosomal/lysosomal compartment enhances the antitumor immunity of DNA vaccines against murine HPV-16 E7-expressing tumors. *Human Gene Therapy* 10, 2727-2740. (1999)
120. Elliott, G., and P. O'Hare: Intercellular trafficking and protein delivery by a herpesvirus structural protein. *Cell* 88, 223-233 (1997)
121. Osen, W., T. Peiler, P. Ohlschlager, S. Caldeira, S. Faath, N. Michel, M. Muller, M. Tommasino, I. Jochmus, and L. Gissmann: A DNA vaccine based on a shuffled E7 oncogene of the human papillomavirus type 16 (HPV 16) induces E7-specific cytotoxic T cells but lacks transforming activity. *Vaccine* 19, 4276-4286 (2001)
122. Michel, N., W. Osen, L. Gissmann, T. N. Schumacher, H. Zentgraf, and M. Muller: Enhanced immunogenicity of HPV 16 E7 fusion proteins in DNA vaccination. *Virology* 294, 47-59 (2002)
123. Harms, J. S., X. Ren, S. C. Oliveira, and G. A. Splitter: Distinctions between bovine herpesvirus 1 and herpes simplex virus type 1 VP22 tegument protein subcellular associations. *J Virol* 74, 3301-3312 (2000)
124. Koptidesova, D., J. Kopacek, V. Zelnik, N. L. Ross, S. Pastorekova, and J. Pastorek: Identification and characterization of a cDNA clone derived from the Marek's disease tumour cell line RPL1 encoding a homologue of alpha- transinducing factor (VP16) of HSV-1. *Arch Virol* 140, 355-362 (1995)
125. Dorange, F., S. El Mehdaoui, C. Pichon, P. Coursaget, and J. F. Vautherot: Marek's disease virus (MDV) homologues of herpes simplex virus type 1 UL49 (VP22) and UL48 (VP16) genes: high-level expression and characterization of MDV-1 VP22 and VP16. *J Gen Virol* 81 Pt 9, 2219-2230. (2000)
126. Hung, C. F., L. He, J. Juang, T. J. Lin, M. Ling, and T. C. Wu: Improving DNA vaccine potency by linking Marek's disease virus type 1 VP22 to an antigen. *J Virol* 76, 2676-2682 (2002)

127. Corr, M., H. Tighe, D. Lee, J. Dudler, M. Trieu, D. C. Brinson, and D. A. Carson: Costimulation provided by DNA immunization enhances antitumor immunity. *J Immunol* 159, 4999-5004 (1997)
128. Tuting, T.: The immunology of cutaneous DNA immunization. *Curr Opin Mol Ther* 1, 216-225 (1999)
129. Tan, J., N. S. Yang, J. G. Turner, G. L. Niu, H. F. Maassab, J. Sun, M. L. Herlocher, A. E. Chang, and H. Yu: Interleukin-12 cDNA skin transfection potentiates human papillomavirus E6 DNA vaccine-induced antitumor immune response. *Cancer Gene Ther* 6, 331-339 (1999)
130. Velders, M. P., S. Weijzen, G. L. Eiben, A. G. Elmishad, P. M. Kloetzel, T. Higgins, R. B. Ciccarelli, M. Evans, S. Man, L. Smith, and W. M. Kast: Defined flanking spacers and enhanced proteolysis is essential for eradication of established tumors by an epitope string DNA vaccine. *J Immunol* 166, 5366-5373 (2001)
131. Liu, W. J., K. N. Zhao, F. G. Gao, G. R. Leggatt, G. J. Fernando, and I. H. Frazer: Polynucleotide viral vaccines: codon optimisation and ubiquitin conjugation enhances prophylactic and therapeutic efficacy. *Vaccine* 20, 862-869 (2001)
132. Shi, W., P. Bu, J. Liu, A. Polack, S. Fisher, and L. Qiao: Human papillomavirus type 16 E7 DNA vaccine: mutation in the open reading frame of E7 enhances specific cytotoxic T-lymphocyte induction and antitumor activity. *J Virol* 73, 7877-7881 (1999)
133. Nichols, W. W., B. J. Ledwith, S. V. Manam, and P. J. Troilo: Potential DNA vaccine integration into host cell genome. *Annals of NY Academy of Science* 772, 30-39. (1995)
134. Lukas, J., H. Muller, J. Bartkova, D. Spitkovsky, A. A. Kjerulff, D. P. Jansen, M. Strauss, and J. Bartek: DNA tumor virus oncoproteins and retinoblastoma gene mutations share the ability to relieve the cell's requirement for cyclin D1 function in G1. *J Cell Biol* 125, 625-638 (1994)
135. Edmonds, C., and K. H. Vousden: A point mutational analysis of human papillomavirus type 16 E7 protein. *J Virol* 63, 2650-2656 (1989)
136. Heck, D. V., C. L. Yee, P. M. Howley, and K. Munger: Efficiency of binding the retinoblastoma protein correlates with the transforming capacity of the E7 oncoproteins of the human papillomaviruses. *Proc Natl Acad Sci U S A* 89, 4442-4446 (1992)
137. Jewers, R. J., P. Hildebrandt, J. W. Ludlow, B. Kell, and D. J. McCance: Regions of human papillomavirus type 16 E7 oncoprotein required for immortalization of human keratinocytes. *J Virol* 66, 1329-1335 (1992)
138. Phelps, W. C., K. Munger, C. L. Yee, J. A. Barnes, and P. M. Howley: Structure-function analysis of the human papillomavirus type 16 E7 oncoprotein. *J Virol* 66, 2418-2427 (1992)

Key Words: Cervical Cancer, Vaccine, Plasmid DNA, Immunotherapy, Tumor Specific Antigens, Cytotoxic T Lymphocyte And Tumor Immunology, Virus, HPV, Review

Send correspondence to: T.-C. Wu, M.D., Ph.D., Associate Professor, Department of Pathology, The Johns Hopkins University, 720 Rutland Avenue, Ross Building 512, Baltimore, MD 21205, USA, Tel: 410-614-4906, Fax: 443-287-4295, E-mail: wutc@jhmi.edu