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HPV PROTEIN/PEPTIDE VACCINES: FROM ANIMAL MODELS TO CLINICAL TRIALS

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1. ABSTRACT

Human Papillomaviruses are viruses that infect the epithelial layers of the oral, rectal, vaginal, and cervical mucosa. There is a causal link between the development of cervical cancer and some other cancers and high-risk human papillomavirus infection (1, 2). Currently there is no prophylactic or therapeutic vaccine available against human papillomavirus infection and its associated lesions. In addition, there exists a high degree of species specificity associated with papillomavirus infection precluding human papillomavirus's use in animal models. Therefore, multiple researchers have utilized a variety of homologous animal papillomaviruses and animal model systems for the development of vaccine strategies against papillomaviruses. The goal of their efforts is to identify vaccine strategies that are efficacious in the animal model systems and translate these strategies into human clinical trials against human papillomavirus. The development of such a vaccine would ultimately result in a reduction in the incidence of cervical cancer and some other HPV-linked cancers and may provide therapy for individuals harboring papillomavirus lesions. This review discusses the advances in papillomavirus vaccinology using proteins/peptides, from the work completed in the animal models to the results of the early human clinical trials.

2. INTRODUCTION

Human Papillomaviruses (HPV) are the causative agent for several benign, proliferative, epithelial lesions and are one of the main risk factors associated with the development of cervical cancer and other cancers like penile, anal, and head and neck cancers. One percent of women infected with the virus suffer from squamous intra-epithelial lesions, the precursor of cervical cancer. Cervical cancer is the third most common cancer among women worldwide, with approximately 370,000 newly diagnosed cases every year (3, 4). However, with the increasingly widespread use of the Papanicolaou (Pap) smear, a 70% decline in the mortality from cervical cancer has occurred in the last 50 years (5). In developing countries, where routine examinations are not available, the extent of the problem is more severe. Cervical cancer is the leading cause of cancer-related deaths among women in these countries. Therefore, the development of an effective and easily administered vaccine against HPV would greatly contribute to the health of individuals around the world.

HPV is a non-enveloped, double-stranded DNA virion of the papillomaviradae family. The more than 100 HPV genotypes are divided into two groups, based on the lesions that develop. HPV of the low-risk genotype (e.g. HPV 6 and 11) cause benign lesions such as venereal warts whereas DNA from high-risk genotypes, such as HPV 16, 18, 31, 33, and 45 is detected in nearly all cases of cervical carcinoma (1). The only viral proteins expressed in cervical cancers are the non-structural viral proteins E6 and E7 and their expression is required for the maintenance of malignant transformation (6). A direct correlation exists between the E6/E7 expression levels and the transformation potential of the virus (7). The E6 and E7 proteins account

for the transforming capabilities of the high-risk HPV genotypes by binding to and inactivating the tumor-suppressor gene products p53 and pRb, respectively (8-10). As a result of the constitutive expression of the viral E6 and E7 proteins in cancerous lesions, these proteins are potential targets for the development of T cell-mediated vaccine strategies against high-risk HPV.

The only methods currently available to reduce papillomas and cervical tumors are radiotherapy and/or chemotherapy and surgery. Radiotherapy and/or chemotherapy are effective only during the first stages of tumor development, whereas in later stages, surgery is the patients' only option. Patients infected with HPV who have undergone radiotherapy and/or chemotherapy and are therefore immunocompromised, exhibit more persistent HPV-associated lesions and have a high degree of lesion recurrences (11-13). This indicates that the immune system is important in the control of papillomavirus lesions. In addition, the spontaneous regression of papillomavirus lesions is associated with type-specific lymphocyte infiltration into lesions and destruction of infected basal epithelial cells (14, 15). Even in immunocompetent papillomavirus-infected individuals, a spectrum of responses is seen. Responses ranging from total clearance of the virus with no clinical disease, to persistent, untreatable lesions are observed. Therefore, the vaccination strategies in development attempt to boost the patients' immune system against the virus and its associated lesions.

There is no prophylactic or therapeutic vaccine strategy available for the treatment of cervical cancer. Cell culture systems for propagating HPV do not exist, which further hampers vaccine development. Prophylactic vaccines attempt to prevent HPV infection and suppress viral replication in infected individuals by initiating humoral anti-viral immune responses. As a result of the intracellular location of E6 and E7, humoral responses are ineffective for clearance of established lesions. Therefore, therapeutic vaccines target previously established virus-induced lesions and cancers by initiating cellular immune responses.

The presence of an in vitro or in vivo animal model system is crucial for the development of prophylactic and therapeutic vaccine strategies against HPV. Animal models offer the advantages of allowing comparisons of the efficacy of various vaccines in the same experimental system and being able to optimize vaccine strategies prior to human clinical trials. Animal models of mucosal papillomas allow investigation into the papilloma life cycle, from infection through resolution. Papillomavirus animal model systems also afford insight into the host organism's immune reaction to its natural pathogen. These interactions are likely to represent those taking place in nature and are biologically significant. Three different papillomavirus animal model systems have been used for these purposes: Cottontail Rabbit Papillomavirus (CRPV), Bovine Papillomavirus (BPV), and Canine Oral Papillomavirus (COPV). Even though papillomavirus infections are highly species-specific, the genomes of HPV and the various animal papillomavirus types are conserved, with their genes encoding proteins with homologous functions. In addition, the regulatory mechanisms of transcription and replication are common between the different species (16-24). The homology between the different papillomaviruses allows conclusions drawn from research in one species to be translated to another. The HPV-mouse model system has also been used for the testing of vaccine strategies against HPV, despite the fact that HPV does not infect mouse cells.

The purpose of this review is to address how research in the various animal models has led to the testing of initial vaccines in early stage human clinical trials. Also, this review will discuss the prophylactic and therapeutic protein or peptide vaccines presently in development.

3. CRPV

CRPV was the first DNA tumor virus to be isolated and characterized (25). CRPV induces cutaneous papillomas in cottontail rabbits under natural and experimental conditions. The domestic rabbit-CRPV model system is easy to manipulate and the nature of this model system allows for highly quantifiable and repeatable data (26). However, CRPV only infects haired skin, and therefore, may not be the best model system for mucosa-tropic HPV. Systemic regression of CRPV-induced lesions, which precludes tumor progression, occurs in a variable proportion of rabbits as a result of a specific T cell-mediated immune response. Therefore, regression is a consequence of the host's immunosurveillance of the oncogenic capabilities of CRPV, similar to HPV (27-29). For these reasons, the rabbit-CRPV model system represents a good model system for the use of testing various prophylactic and therapeutic vaccination strategies that can be translated to HPV.

3.1. CRPV Protein/Peptide Vaccines

Immunizations of rabbits with either of the CRPV structural proteins, L1 or L2, protected rabbits from CRPV challenge (30). Neutralizing antibodies were generated by vaccination with either of the structural proteins, with the strongest response found with the L1 protein vaccination. The epitopes responsible for this effect were mapped and immunization of rabbits with L1 subfragments resulted in seroconversion, but no neutralizing antibodies were generated (31). Furthermore, the subfragment immunized rabbits were not protected from virus challenge. This indicates that CRPV whole protein vaccinations, which contain all peptide epitopes, may be better than single epitope vaccines.

4. BPV

The BPV-bovine model is another animal papillomavirus model system used for the identification of efficacious vaccine strategies. Most types of BPV infect the cutaneous epithelium of cattle. However, BPV-4 infects the mucosal epithelium of the upper alimentary canal, which can lead to squamous cell carcinomas (32, 33). Therefore, like HPV, BPV can infect either skin or mucosa, and progression to cancerous lesions does

occur. Infectious BPV is available in large amounts from warts of infected cattle and there is an almost endless supply of host tissue, bovine skin (34). This overcomes some of the fundamental problems associated with the use of human tissue for experiments, availability and variability of tissue. However, the housing requirements for such a large animal preclude their use by many investigators.

4.1. BPV Protein/Peptide Vaccines

The L1 and L2 proteins of BPV, produced as ?-galactosidase fusions in Escherichia coli, were used to vaccinate calves prophylactically and therapeutically (35). L1 vaccination protected calves prophylactically, whereas L2 vaccination protected calves regardless of whether it was administered before or after viral challenge. L1-vaccinated calves responded by rapidly producing neutralizing antibodies, while L2-vaccination resulted in lesion-infiltrating CTL. Calves immunized with the N-terminus of L2 were completely protected from viral challenge (36) and produced virus-neutralizing antibodies (37). Three B cell epitopes located in the N-terminus of L2, when administered together, were found to be responsible for disease prevention (38). In contrast to CRPV where an L1 vaccine was the most efficacious, in BPV an L2 vaccine is the most efficacious indicating that in each model system different vaccine strategies are optimal.

As stated previously, in HPV vaccinology a vaccine that harbors the viral E7 protein may be the best therapeutic vaccine as L1 and L2 are not expressed in HPV-containing cancerous lesions. Therefore to test E7-based vaccine strategies using the BPV animal model system, the E7 protein of BPV was administered to calves and the B and T cell responses were measured (39, 40). The E7 protein vaccine resulted in slow papilloma growth after challenge and promoted early lesion regression. High titres of BPV E7-specific IgG antibodies could be detected in the serum of vaccinated calves 10 weeks after viral challenge, whereas E7 antibodies could not be detected until 13 weeks post-viral challenge in control, non-vaccinated animals. Major B cell epitopes were mapped to the N and C termini of the E7 protein, and a minor epitope was found in the middle of the protein (39). High levels of T cells specific for BPV E7 could be detected in vaccinated calves, whereas nonvaccinated calves showed E7-specific CTL only at late stages of papilloma development (40). These data indicate that the E7 protein of BPV can provide both B and T cell responses in vaccinated animals that result in the retardation of lesion growth and aids in lesion regression.

5. COPV

COPV induces warts on the oral mucosa of domestic dogs and wild canines similar to those induced by low-risk genital HPV subtypes. The development of lesions is highly reproducible, and only a few papillomas progress to higher-grade lesions such as oral squamous cell carcinomas. Low-grade lesions produce large amounts of infectious virus that can be easily isolated. There is significant overlap of dog cells with human cells in cell surface antigen markers and cellular function of both lymphocytes and antigen presenting cells (41, 42). This overlap has allowed the dog to be used as a model system for the development of vaccines against low-risk HPV.

Four to 8 weeks after infection, COPV-induced lesions develop, followed by immune-mediated regression within an additional 4-8 weeks. An influx of lymphocytes into the papilloma is seen during wart regression, which peaks during resolution and returns to pre-infection levels after the lesion has resolved, similar to HPV-induced papillomas (41, 43). COPV-neutralizing IgG antibodies can be isolated from dogs that have spontaneously regressing papillomas (44). In addition, passive transfer of serum from regressor hosts is able to protect against COPV-induced papillomas. This indicates that circulating anti-COPV IgG antibodies protect against intra-oral infection by COPV. As with HPV, immunosuppressed animals develop more severe disease (45). A non-regressing COPV infection with a new tropism not restricted to oral mucosa, extending to haired skin and remote cutaneous sites, has been described. The dog infected with this variant was found to be immuno-compromised further supporting the conclusion that the immune system is important in COPV lesion development and regression.

5.1. COPV Protein Vaccinations

A Glutathione S-transferase (GST)-COPV L1 fusion protein protected dogs from high-dose COPV infection of the oral mucosa similar to COPV-L1 VLP (46). The fusion protein was produced in bacteria and did not form intact VLP, but did form L1 pentamers, as determined by L1 conformation-specific antibodies. The GST-L1 fusion can be produced economically in relation to VLP, as a result of the GST-tag, which allows for simple purification. The simple purification methods and high immunogenicity of this vaccine may indicate that this vaccine will be efficacious in large scale production.

6. MOUSE MODELS FOR HPV

Although the CRPV-rabbit, BPV-bovine, COPV-canine animal models have proved invaluable as papillomavirus infection models, the mouse model has greatly aided the development of vaccination strategies against HPV. Even though HPV does not infect mouse cells and there is no known mouse papillomavirus, the mouse model system is a genetically well-characterized system that overcomes some of the limitations of using other animal models. Mice are easy to breed and as a result of their small size, mice are easy to house and care for. Biological reagents are readily available and tumor cell lines transfected with various HPV genes have been generated to determine the effects of various vaccination strategies on tumor growth (47). Many of the vaccine strategies found to work in each of the other papillomavirus animal models have been used in the HPV-mouse model.

6.1. Protein/Peptide Vaccinations in Mice

An HPV16-E7 peptide was used for the generation of epitope-specific CTL to protect against an

HPV-expressing cell line (48, 49). The epitope of HPV16-E7, comprising amino acids 49-57 (RAHYNIVTF), was the dominant high-affinity MHC-binding (H2-Db) epitope in C57BL/6 mice (48). HPV 16-E7 H2-Kb binding epitopes were also found to be immunogenic in vivo (50). The highest affinity HLA-A*0201 binding epitopes of HPV E7 were the most immunogenic indicating that the binding affinity of the peptide has a major impact on its immunogenicity (51). The identification of these high-affinity HPV epitopes has led to further epitope-based vaccine testing in the mouse model and in clinical trials.

Adjuvants are often used to boost an immune response against an antigen when utilizing protein or peptide vaccinations. Adjuvants can either be vehicles for the delivery of antigen or immunostimulatory agents that stimulate the innate immune system to enhance the immune response. HPV16-E7 protein when given in Quil-A (a saponin adjuvant), but not complete Freund's adjuvant or Algammulin, induced a Th1-type response against E7 (52). This was shown by enhanced CTL activity against a tumor cell line transfected with HPV16-E7 and E7-specific antibodies. The adjuvant monophosphoryl lipid A (MPL), a detoxified form of lipopolysaccharide, increased the immunogenicity of an L2E7 fusion protein (53). The data indicates that this vaccine enhanced T-cell proliferative responses, IFN-gamma production, and DTH responses without increasing its reactogenicity. A soluble HPV16-E7 protein in the adjuvant PROVAX was injected into mice previously challenged with an E7-transfected, metastatic melanoma cell line (54). A significant inhibition of tumor growth was observed, where E7-specific CD8+ cells were the major effector cells mediating the anti-tumor immunity. E7-specific CTL were also found in vitro after vaccination. An HPV16-E7 protein in the adjuvants SBAS 1 and SBAS2 provided therapy against mice bearing another E7-expressing tumor cell line (55). Vaccinated mice rejected the tumor and induced a strong systemic E7-specific T helper 1 immune response. The immuno-stimulants MPL and QS21 were used to boost a HPV16-E7 protein vaccine's efficacy against an E7-expressing tumor cell line when injected into mice (56). The results indicated that the enhanced regression of the tumors was accompanied by the induction of E7-specific antibodies and CTL. A long peptide vaccine that harbored a CTL epitope and a T-helper epitope in IFA generated a more robust immune response than the minimal CTL epitope alone in IFA in vaccinated mice (57). The inclusion of an antigen-presenting cell activating agent, oligodeoxynucleotide CpG, with the long peptide vaccine dramatically increased the potency of the vaccine. These studies with adjuvants, when taken together, indicate that multiple different adjuvants are available that are capable of enhancing epitope-specific CTL and antibody generation against HPV proteins and peptides.

Viral proteins in the context of fusions can also offer protective immunity in mouse models. The hepatitis core antigen was fused to HPV 16-E7 epitopes (58). The chimeric protein formed particles that, when administered to mice, induced strong E7 epitope-specific antibody responses. Lymph node cells from immunized mice produced IL-2 and IL-4 after restimulation in vitro. This indicates that both Th1 and Th2 immune responses are generated by this vaccine strategy. The addition of a lipid tail to a CTL peptide greatly increased CTL induction over that seen with the native peptide in vaccinated mice (59). The easily isolatable fusion protein HPV16-E7-GST was injected into mice as a prophylactic vaccine (60). This vaccine protected mice against challenge with an HPV16-E7 transfected tumor cell line. An HPV16 L2, E6, E7 fusion protein was constructed and injected into mice and this fusion elicited HPV16-specific CTL, T-helper cells, and antibodies (61). This prophylactic vaccine was able to prevent growth of HPV16 tumor cells in vaccinated mice. Another group expressed the HPV16-E7 gene in a Nicotiana benthamiana plant using a potato virus vector (62). Foliar extracts that harbored the E7 protein were simply fed to mice and these mice were protected from tumor development via both humoral and cell-mediated immune responses. These fusion proteins offer effective alternatives for HPV vaccines without the unwanted side effects of oil-based adjuvants.

Recombinant bacterial strains and fusions of HPV early genes to bacterial factors are other vaccine strategies being developed to boost the immune responses of the encoded protein/peptide. Heat shock proteins enhance the production of specific CTL sufficient to reject tumors expressing the fusion partner (63, 64). Fusing the E7 gene to the Mycobacterium bovis heat shock protein 65 gene protected mice from HPV16-expressing tumors and also protected the mice against re-challenge with higher doses of tumor cells (65). Listeria monocytogenes is an intracellular pathogen that secretes the listeriolysin O (LLO) protein, which perforates the phagosomal membrane and allows the bacteria access the cytoplasm of antigen presenting cells. Proteins secreted by the bacterium, when it resides intracellularly, are targeted to the cellular immune system (66). HPV16-E7 fused to LLO was secreted from a recombinant L. monocytogenes strain (67). The LLO-E7 fusion induced both CD4+ and CD8+ cells that mediated regression of the E7-expressing tumor, TC-1, in mice. Fusion of HPV16-E7 to the translocation domain of Pseudomonas aeruginosa exotoxin A led to enhanced intracellular translocation of E7 into the MHC class I presentation pathway in vitro. Vaccination of mice with the exotoxin A-E7 fusion protein led to an increase in the number of E7-specific CTL and provided protection and therapy against HPV-expressing tumors (68). Fusing a 19mer peptide containing B cell and CTL epitopes of HPV E7 to an integral membrane protein of Escherichia coli, TraT, which is known to stimulate Th1 and Th2 responses, protected mice from E7-transfected tumor cells (69). These results, when taken together, indicate that recombinant bacterial strains and recombinant fusion proteins/peptides can be used to induce systemic and mucosal anti-tumor immune responses capable of offering prophylactic and therapeutic immunity against HPV-expressing tumors in mice better than vaccinations with HPV proteins/peptides alone.

The presentation of antigen by dendritic cells is regarded as the rate-limiting step in the generation of an anti-tumor immune response. Therefore, dendritic cells are

often used to boost the immune response against vaccinated antigens. A single injection of dendritic cells pulsed with a HPV16 E7 peptide protected mice from challenge with an E7-transfected tumor cell line (70). Dendritic cells pulsed with HPV16-E7 protein are able to be recognized in vitro by E7-specific CTL and are able to induce E7-specific CTL responses in vivo. The E7-pulsed dendritic cell vaccine protected mice from challenge with an HPV16 tumor cell line (71). Dendritic cells pulsed with an MHC Class I-binding E7 peptide protected against lethal E7-expressing tumor challenge. The vaccination resulted in specific CTL responses that resulted in sustained tumor regression in more than 80% of cases (72). Therefore, not only is HPV-E7 protein/peptide in adjuvant able to stimulate tumor-specific CTL, but also E7 protein/peptide-pulsed dendritic cells can initiate protective immunity against HPV16-expressing tumors.

DNA vaccinations of genes encoding disrupted HPV genes or immunogenic HPV epitopes are another avenue explored in the mouse model system. However vaccination with the transforming E6 or E7 genes is precarious. Mutations rendering the E6 or E7 genes nontransforming may result in a loss of immunogenicity of encoded peptide epitopes. Mutating the two zinc-binding motifs of HPV16-E7 resulted in the loss of E7's transforming capability and a decrease in its stability (73). The rapid degradation of the modified protein resulted in a significantly stronger E7-specific CTL response and better tumor protection in mice. An E7-epitope DNA vaccine fused to the M. tuberculosis hsp 70 gene, using an adeno-associated virus as a delivery vehicle, protected mice from tumor challenge (74). The adeno-associated virus vector is non-pathogenic and does not express any viral genes but the gene of interest. It was found that the anti-tumor effects observed were CD4 and CD8 dependent. A vaccine comprised of a shuffled HPV16-E7 gene, with certain sequences duplicated to retain all possible T cell epitopes, conveyed protection against E7-transfected tumor cells (75). The E7 gene displayed no transforming activity in NIH3T3 cells or induction of S-phase under conditions of serum deprivation. An HPV16 E6 and E7 epitope string DNA vaccine protected mice from challenge with an HPV-containing cell line (76). Fusing the ubiquitin gene to the C-terminus of the epitope string increased the efficacy of this vaccine. Therefore, DNA vaccines encoding a mutated E7 gene or E7 epitopes offer protective and therapeutic immunity in mice without the fear of the transforming capabilities of the native E7 gene.

As with protein/peptide vaccinations, vaccines incorporating or targeting dendritic cells are also used to boost DNA vaccinations immunogenicity. Dendritic cells transfected with HPV16-E7 injected i.m. generated the greatest anti-tumor immunity in a murine HPV16 tumor model when compared to DC vaccinations administered subcutaneously or intravenously (77). High levels of E7-specific CTL and antibody responses were detected in mice vaccinated i.m. In other experiments, an adenoviral vector encoding HPV16-E7 used to target dendritic cells via CD40 interactions offered enhanced protection against HPV16 expressing tumors in a murine model (78). Targeting of the recombinant adenovirus to CD40 was achieved by conjugating an activating anti-CD40 antibody to a Fab fragment of an anti-adenovirus antibody and incubating this bispecific targeting conjugate with an adenovirus encoding HPV16-E7. This adenoviral vector induced therapeutic immunity in HPV16-expressing tumor-bearing mice. Fusion of Flt3-ligand to HPV16-E7 induced significantly higher anti-tumor effects than naked DNA encoding E7 alone. This fusion gene targeted the E7 antigen to DC, which express Flt3, thereby increasing the frequency of E7-specific CTL in vaccinated mice. These data indicate that targeting dendritic cells or directly injecting dendritic cells with protein/peptide or DNA vaccinations will enhance anti-tumor immune responses in vaccinated recipients (79). Many people undergoing treatment for cervical cancer are immunocompromised as a result of anti-cancer treatments killing the rapidly dividing cells of the immune system. To determine the effects of immunosuppressing treatments conducted prior to a vaccination, mice were vaccinated with an HPV16-E7 peptide between 0 and 16 weeks after pelvic radiation or cisplatin treatment (80). Although a reduction in peptide-specific lymphocyte precursors in the spleens of immunized mice was detected, none of the groups of mice developed tumors when challenged. These data suggest that vaccination of cervical cancer patients who have undergone prior radiation or cisplatin treatment may display decreased lymphocyte frequency, however a specific cell-mediated immune response can still be generated if the vaccine strategy is strong enough.

7. HUMAN CLINICAL TRIALS

The strategies that have proven to be successful at eradicating established lesions or protecting against virus challenge in the various animal models have been applied to preliminary human clinical trials. Many of the clinical trials have been early stage trials comprised of late stage cervical cancer patients.

7.1. Clinical Trials using Proteins/Peptides

The identification of immunogenic HPV-peptide epitopes in mice that protected against challenge from HPV-expressing tumors and provided therapy of HPV-expressing tumors (48), and also the identification of immunogenic human HLA-A2-binding HPV16 (51) and HPV18 (81-83) epitopes, formed the basis of preliminary peptide clinical trials49. HLA-A2-binding peptides were chosen as this HLA type is one of the most common MHC class I molecule among caucasians. HLA-B18 binding epitopes of HPV16-E6 and E7 have also been identified (84). Antigen-specific CTL can be generated using autologous DC pulsed with an HPV16-E6/E7 fusion protein (85) or pulsed with the C-terminus of the E2 protein (86). Also, memory CTL responses against HLA-A2 restricted E6 and E7 peptides are detectable in some CIN and cervical carcinoma patients, but not in normal subjects (2). Taken together, these data indicate that a protein/peptide vaccine to boost a patient's immune response against HPV may be efficacious.

A L2E7 fusion protein of HPV6 with Alhydrogel was administered to 27 subjects that had genital warts (87).

All subjects made serum IgG antibodies against L2E7 and five subjects completely cleared their warts within 8 weeks and had no recurrences. A phase I/II clinical trail involving the vaccination of terminal patients harboring HPV16 positive cervical carcinomas with HPV16-E7 peptides was conducted. Two HPV16-E7 peptides and a universal T helper peptide emulsified in the adjuvant Montanide ISA 51 were administered in increasing doses to patients. At the highest dose, the vaccine was well tolerated and did not induce side effects (88). The patients in the previous study were analyzed for their cellular immune responses against the vaccine (89). In 4 of 15 patients with advanced cervical carcinoma, helper peptide-specific proliferative responses were induced. However, no E7-specific responses were seen. A lipidated E7 peptide was used as a vaccine in a phase I clinical trial and the authors found that 7 of 12 patients exhibited E7-specific CTL, but no clinical improvement was seen (90). The previous two trials used late stage cervical cancer patients that already may have been beyond benefit of a therapeutic vaccine. Therefore, to determine the effect of peptide vaccination in patients with lower grade lesions, a phase I trial was conducted using 18 women with CIN III or VIN III lesions (91). The patients were vaccinated with either one or two E7 peptides in incomplete Freund's adjuvant. Ten of 16 patients showed significant increases in CTL activity and cytokine release and 9 patients had partial or complete regression of their CIN lesions. Therefore, peptide vaccinations may be more efficacious in women with low-grade lesions.

A therapeutic, multiple HLA-A2 binding, HPV16-E7 epitope DNA vaccine was administered to 12 HLA-A2 positive subjects with HPV16 positive anal infections (92). All doses of the vaccine were well tolerated and three subjects experienced partial histological responses. 10 of 12 subjects demonstrated increased immune responses to the peptide epitopes in the vaccine 6 months after the initiation of therapy. In another ongoing trial, cancer patients were given immature DC or mature DC pulsed with HPV tumor cell lysates (93). The unpublished results of this trial will determine if antigen-loaded immature or mature DC are more effective at stimulating an immune response in vaccinated patients.

In a phase I/II clinical trial, eight patients with late stage cervical cancer were vaccinated with a single dose of TA-HPV, a live recombinant vaccinia virus expressing the E6 and E7 proteins of HPV 16 and 18 (94). No significant clinical side effects were observed, but each patient did mount an anti-vaccinia antibody response. Three of the eight evaluable patients developed an HPV-specific antibody response and HPV-specific CTL were detected in one of three evaluable patients. Once again, as the patients were late stage, further testing is necessary to investigate the use of TA-HPV for immunotherapy of earlier stage cervical cancers. In another phase I trial, patients with pre-invasive and invasive cancer were vaccinated with a vaccinia virus encoding a modified form of the HPV16 and 18 E6 and E7 proteins. HPV-specific CTL responses were seen in one of three patients with advanced cervical cancer, three of 12 CIN III patients, and in four of 29 patients with early invasive cancer (93). Future trials will attempt to optimize this vaccine strategy.

8. CONCLUSIONS

This review has summarized the developments in protein/peptide vaccinology against HPV. As a result of the species-specific nature of papillomaviruses, animal models have served as an invaluable resource for testing various vaccine strategies. From the work completed, it is clear that the adaptive arm of the immune system is required for protection from viral infection and is required for clearance of established lesions. The ideal vaccine will have to initiate B and T cell responses against multiple different HPV epitopes. Once an appropriate antigen is chosen as the target for the vaccine, the appropriate delivery system has to be chosen. There is a collection of adjuvants and immunomodulators available for the dispersal of particulate antigen. Dosage and vaccination schedule are other issues that will be encountered and will have to be addressed.

The high-risk HPV-E6 and E7 proteins are transforming. As a result, DNA vaccinations may not be the most safe vaccination strategy. Mutations to render the proteins encoded in DNA vaccines non-functional may reduce the immunogenicity of the protein encoded (56). Also, DNA vaccines have not generated robust immune responses in humans thus far. Vaccinations with purified proteins can induce both humoral and cellular immune responses against multiple epitopes present within the proteins (65). Proteins are readily produced and purified in large amounts, yet are unstable for long periods at room temperature making them difficult to dispense to remote regions of the world. Protein vaccinations sometimes require the use of immune stimulating adjuvants and multiple immunizations to generate robust immune responses. Peptides are also readily produced, but in contrast to proteins, are very stable. Peptide vaccines administered in adjuvants induce robust cellular immune responses because they do not require further processing by antigen-presenting cells (48, 49). However, peptide vaccines require the ability of the peptide to bind in a haplotype-specific Major Histocompatibitity Complex (MHC) binding groove. Therefore, the patient being vaccinated must be Human Leukocyte Antigen (HLA)-typed. Vaccination with intact proteins or long, overlapping peptides alleviates the need for HLA-typing, as all peptide epitopes are present. Peptide vaccinations can induce either T cell activation or tolerance based on the peptide concentration over time and peptide persistence in the vaccine recipient. (95, 96). Peptide tolerization has been shown to result in an enhanced tumor outgrowth (97). With regard to vaccine development, a few considerations have to be taken into account. A vaccine will have to overcome HLA-restriction and may have to initiate immune responses against multiple HPV genotypes. This is the result of the fact that an individual can be infected with any or more than one of the distinct high-risk HPV genotypes. Some of the antigenic B cell epitopes of one HPV type have been found to cross-react with other HPV types (98-101) indicating that vaccination with one epitope may offer protection against multiple types. Also, it has been found that two HPV16 epitopes bind to five different HLA-A2 alleles (102). The tumor may also escape the immune system by losing

expression of a specific surface antigen, thus allowing the tumor to hide from immune system detection (103, 104). Therefore, a multi-valent vaccine strategy, which is able to induce immune responses restricted through multiple HLA molecules against multiple viral gene products and viral types, may be the most advantageous.

Currently the field is switching to virus-like particle (VLP) vaccines, a derivative of a protein vaccine. VLP vaccines, comprised of the L1 or both the L1 and L2 papillomavirus capsid proteins, induce both humoral and cell-mediated immune responses in the absence of adjuvant (105-108). The strong cell-mediated response is a consequence of the VLP interacting, activating, and getting processed by antigen-presenting cells such as dendritic cells (109-111). Chimeric VLP, which have a protein/peptide fusion to either the L1 or L2 virus capsid protein are capable of initiating CTL responses against the fusion protein/peptide. Poly-epitope L1 or L2 fusions can be constructed that initiate CTL responses against each of the individual epitopes strung together in the fusion (112). Therefore, chimeric VLP vaccines are an attractive strategy for the generation of both a prophylactic and therapeutic immune response. However, it is still up for debate that pure protein/peptide vaccines are efficacious in the therapeutic setting as the E6 and E7 proteins do not generate a good immune response by themselves. Further work in human clinical trials with the vaccines found to be efficacious in animal models may elucidate a vaccine strategy that can be used in the future to prevent HPV infection and provide therapy for eradicating established papillomavirus lesions in infected patients.

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