Human papillomavirus vaccines: current status and perspectives

REVIEW

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ABSTRACT

Cervical cancer is the second cause of death from cancer among women worldwide. Long-term infection with the human papillomaviruse (HPV) can lead to malignant anogenital tumors. The HPV oncogenic types 16 and 18 account for approximately 70% of cervical cancer and have received the greatest attention. Two pharmaceutical companies, Merck and GlaxoSmithKline (GSK), have developed commercial versions of prophylactic HPV vaccine. These vaccines are based on the intrinsic ability of the L1 viral capsid protein to self-assemble into virus-like particles. Both of them target HPV16 and HPV18 with excellent safety profile and type-specific protection. However, therapeutic vaccines are designed to treat preexisting HPV infections and have been focused on the viral oncoproteins, E6 and E7. Various types of therapeutic HPV vaccines have been designed; and many of them are currently in clinical trials. So far, combined approaches appear to be more promising for treating persistent HPV infections. The aim of this review is to summarize the status of HPV vaccines and their perspectives.

Keywords: HPV, vaccines, cervical cancer

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RESUMEN

Vacunas de virus de papiloma humano: estado actual y perspectivas. El cáncer cervical es la segunda causa de muerte por cáncer para las mujeres en el mundo. La persistencia de la infección producida por el virus de papiloma humano (VPH) puede conducir a la aparición de tumores anogenitales. Los tipos oncogénicos del virus, 16 y 18, contribuyen aproximadamente al 70% de los casos de cáncer cervical y han recibido la mayor atención. Dos compañías farmacéuticas, Merck y GlaxoSmithKline (GSK), desarrollaron versiones comerciales de la vacuna VPH profiláctica basada en la capacidad intrínseca de la proteína L1 de la cápsida del virus para auto ensamblarse en partículas semejantes al virus. Ambas vacunas están dirigidas contra los tipos 16 y 18 con excelente perfil de seguridad y protección tipo-específica. Sin embargo; las vacunas terapéuticas están diseñadas para tratar las infecciones preexistentes causadas por el VPH y están basadas en las oncoproteínas E6 y E7. Varios tipos de vacunas terapéuticas de VPH han sido diseñadas y algunas de ellas se encuentran actualmente en ensayos clínicos. En el futuro, estrategias combinadas pudieran ser más promisorias para el tratamiento de infecciones persistentes causadas por el VPH. El propósito de esta revisión consiste en resumir el estado de las vacunas de VPH y sus perspectivas.

Palabras clave: VPH, vacunas, cáncer cervical

*I*ntroduction

It has been estimated that 5.5% of the worldwide incidence of cancer is attributable to human papillomavirus (HPV) infection [1]. HPV infection is the most important risk factor for cervical cancer; yet, most infections are transient and asymptomatic.

Among the cancers attributable to HPV infection, cervical cancer has received the greatest attention. Cervical cancer is the second cause of death from cancer among women worldwide; and the HPV oncogenic types 16 and 18 account for about 50 and 20%, respectively [2]. Cervical cancer is more prevalent in developing countries, where Papanicolau (Pap) screening test is not widely available, and many precancerous lesions go unnoticed and untreated. Many studies have demonstrated that most patients are unaware of HPV and its association with genital warts and cervical cancer [3-5].

HPVs infect the stratified squamous epithelia of skin and mucous membranes, where they cause benign lesions, some of which have the potential to progress to invasive cancer [6-8]. The benign lesions induced by HPVs include nongenital and anogenital skin warts, oral and laryngeal papillomas and anogenital mucosal condylomata. Long-term infection can lead to malignant anogenital tumors including cancers of the anus, penis, vulva, vagina and cervix [9].

Taking into account the worldwide prevalence of HPV and its diversity of symptoms, the design of vaccines for prophylaxis and immunotherapy is required for reducing HPV transmission and virulence.

*H*uman papillomavirus disease

After breast cancer, cervical cancer is the second cause of death from cancer among women worldwide. HPV is the most common sexually transmitted infection and a known risk factor for cervical cancer [10]. The HPV are a family of sexually transmited DNA viruses with over 100 different genotypes. They are divided into the low-risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, and 81) and high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82) categories based on the spectrum of lesions they induce. The low-risk types primarily induce benign genital condylomas and low-grade squamous intraepithelial lesions, whereas the high-risk types are most frequently associated with the development of anogenital cancers. In this regard, infection with the HPV oncogenic types 16 and 18 has received the greatest attention.

1. Parkin DM. World burden of infection-associated cancers. 22nd International Papillomavirus Conference, April 30 - May 6, Vancouver, Canada; 2005. p. 10.

2. Muñoz N, Bosch FX, Castellsagué X, Díaz M, de Sanjose S, Hammouda D *et al*. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J Cancer 2004;111:278-85.

3. Ramírez JE, Ramos DM, Clayton L, Kanowitz S, Moscicki AB. Genital human papillomavirus infections: knowledge, perception of risk, and actual risk in a non-clinic population of young women. J Women's Health 1997;6:113-21.

4. Yacobi E, Tennant C, Ferrante J, Pal N, Roetzheim R. University students' knowledge and awareness of HPV. Prev Med 1999;28:535-41.

5. Dell DL, Chen H, Ahmad F, Stewart DE. Knowledge about human papillomavirus among adolescents. Obstet Gynecol 2000;96:653-56.

6. Lowy DR, Howley PH. Papillomaviruses. In *Fields virology*. DM Knipe and PH Howley, editors. Lippincott, Williams & Wilkins. Philadelphia, Pennsylvania, USA; 2001.

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HPVs are small, nonenveloped viruses with an approximately 8 kb circular genome. This genome encodes for 2 structural proteins, L1 and L2, which form the viral capsid. Nonstructural proteins are implicated in the virus life cycle. HPV infections usually last months or years because the viral genome successfully parasitizes the epithelial cells. Additionally, the virus evades the immune system by limiting most viral gene expression and viral replication to suprabasal cell layers.

HPV infection is highly prevalent in young population, with prevalence rates of approximately 50% in sexually active adolescent girls and young women [11]. Early age of first sexual intercourse is associated with higher susceptibility to HPV infection, possibly due to cervical immaturity [12]. In general, most genital HPV infections are benign; and a high proportion of infections related to low-grade cervical dysplasias spontaneously regress. Nevertheless, persistent cervical infection with an oncogenic HPV type is an important risk factor for progression to high-grade dysplasia, which should be treated to prevent the development of invasive cancer. The period of time between the acquisition of HPV infection and the establishment of malignancy usually takes at least 10 years [13]. The incidence of cervical cancer progressively rises for women over 25 years old; and women over 40 show the highest incidence.

Vaccines

According to the World Health Organization, HPV is associated with 500 000 new cases of cervical cancer and 250 000 associated cervical cancer deaths worldwide each year [14]. Taking into account the worldwide prevalence of HPV, the design of vaccines for prophylaxis and immunotherapy is required. The inability to grow HPV in cell cultures is one major limitation for vaccine development. Several vaccine strategies have been designed by using HPV type-specific epitopes involved in viral replication and transformation. Other strategies are based on the intrinsic capacity of the major papillomavirus virion protein (L1) to assemble into virus like particles (VLPs) [15].

Prophylactic vaccines

The goal of prophylactic vaccines would be to create an immunological barrier at the entry portal. Approved prophylactic vaccines have been directed against infective agents causing systemic disease. Neutralizing antibodies generated by prophylactic vaccination take an important role in protection. Several highly successful prophylactic viral vaccines are based on the induction of neutralizing antibodies [16]. Therefore, it is reasonable to base prophylactic vaccines against HPV on this principle. However, HPV does not have a systemic phase before colonizing the epithelium; and only after passing through the circulation it is accessible to the neutralizing antibodies present in the blood [17]. The presence of oncogenes in HPVs suggests that a subunit vaccine approach would be better than an inactivated vaccine or an attenuated live-virus vaccine.

One of the most significant advances in gynecologic oncology was the development of vaccines to prevent infection with HPV, which is found in virtually all cervical cancers. Two pharmaceutical companies, Merck and GlaxoSmithKline (GSK), were pioneers in the development of prophylactic vaccines against HPV. These are subunit vaccines based on L1, the major papillomavirus virion protein, which has an intrinsic capacity to assemble into VLP [15, 18] and contains the immunodominant neutralization epitopes of the virus. These VLP are morphologically indistinguishable from the outer shell of authentic virions. Given that only a single structural viral protein is involved in VLP production, they are noninfectious and nononcogenic particles. The formation of VLP is important for L1 vaccines because: (i) they can induce high levels of neutralizing antibodies [15, 19]; (ii) the L1 epitopes recognized by neutralizing antibodies are conformation dependent and predominantly type specific, therefore, denatured L1 does not induce neutralizing antibodies and does not protect against challenge with heterologous papillomavirus [20]; (iii) compared with structurally simple antigens, the ordered repetitive arrangement of epitopes found on VLP surface induces exceptionally potent antibody responses.

Although the vaccines developed by Merck (Gardasil) and GSK (Cervarix) are based on the same concept, there are notable differences between them. Merck vaccine is tetravalent, containing VLPs of HPV16, HPV18, HPV6 and HPV11. HPV6 and HPV11 are considered not oncogenic, but together they account for about 90% of external genital warts [21], and they also cause an estimated 90% of recurrent respiratory papillomatosis, a rare but debilitating disease that can occur in infants, as well as in adults. Merck VLPs are produced in the yeast *Saccharomyces cerevisiae* and use alum as adjuvant [22]. GSK vaccine is bivalent, containing VLPs of HPV16 and 18, the two types that account for about 70% of cervical cancer cases worldwide [21]. It is produced in insect cells, via recombinant baculovirus and uses ASO4, a proprietary adjuvant composed of alum plus monophosphoryl lipid A [23]. Both vaccines use aluminium-based adjuvants, which reduce the dose required to induce peak antibody titers and help to stabilize the vaccine during cold storage. These vaccines are administered as three intramuscular injections over a 6 month period.

Two years ago, the Food and Drug Administration (FDA) approved Gardasil, as the first vaccine to prevent HPV infection in girls and women aged between 9 and 26 years [24]. This vaccine was shown to be 100% effective in preventing precancerous cervical, vaginal and vulvar lesions caused by HPV16 and HPV18 in women who were not previously exposed to these strains of the virus [25]. Additionally, it proved to be 99% effective in preventing genital warts caused by HPV6 and HPV11. Although Gardasil protects against infection with HPV16, HPV18, HPV6 and HPV11, it will not prevent individuals that have been previously infected with either HPV16 or HPV18 from developing cervical cancer, which can occur up to 10 years after infection. This vaccine does not prevent individuals infected with other oncogenic strains of HPV from developing cancer either. These results suggest that cervical cancer screening will keep essential to detect cancer and precancerous changes caused by other HPV types. Gardasil has remained effective

7. Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002;2:342-50.

8. Bosch FX, Schiffman M, Solomon D, editors. Future directions in epidemiologic and preventive research on human papillomaviruses and cancer. J Natl Cancer Inst Monogr 2003.

9. Schiffman M, Kjaer SK. Chapter 2: natural history of anogenital human papillomavirus infection and neoplasia. J Natl Cancer Inst Monogr 2003. p.14-9.

10. Pitts M, Clarke T. Human papillomavirus infections and risks of cervical cancer: what do women know? Health Educ Res 2002;17:706-14.

11. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. New Engl J Med 1998;338: 423-28.

12. Kahn JA, Rosenthal SL, Succop PA, Ho GY, Burk RD. Mediators of the association between age of first sexual intercourse and subsequent human papillomavirus infection. Pediatrics 2002;109:E5.

13. Snijders PJ, Steenbergen RD, Heideman DA, Meijer CJ. HPV-mediated cervical carcinogenesis: concepts and clinical implications. J Pathol 2006;208:152-64.

14. World Health Organization. Initiative for vaccine research. Human papillomavirus. 2006. Geneva, Switzerland, World Health Organization, http://www.who.int/vaccine_research/ diseases viral_cancers/en/index3.html. Accessed 6/29/06.

15. Kirnbauer R, Booy F, Cheng N, Lowy DR, Schiller JT. Papillomavirus L1major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proc Natl Acad Sci USA 1992;89: 12180-4.

16. Zinkernagel RM. On natural and artificial vaccinations. Annu Rev Immunol 2003;21:515-46.

17. JB, Schneerson R, Szu SC. Hypothesis: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. J Infect Dis 1995;171:1387-98.

18. Hagensee ME, Yaegashi N, Galloway DA. 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 1993;67: 315-22.

19. Rose RC, Reichman RC, Bonnez W. Human papillomavirus HPV type 11 recombinant virus-like particles induce the formation of neutralizing antibodies and detect HPV-specific antibodies in human sera. J Gen Virol 1994;75:2075-79.

20. Breitburd F, Kirnbauer R, Hubbert NL, Nonnenmacher B, Trin-Dinh-Desmarquet C, Orth G *et al*. Immunization with virus like particles from cottontail rabbit papillomavirus CRPV can protect against experimental CRPV infection. J Virol 1995;69: 3959-63.

21. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV *et al*. International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348:518-27.

for 2.5 to 3.5 years after three doses; and it appears to be safe. A separate study, known as FUTURE II [26], showed that Gardasil prevented 100% of HPV-related vaginal and vulvar precancers after two years. Two additional Phase III studies were initiated in 2004 with scheduled conclusive date at 2009, in order to evaluate the efficacy of this vaccine in mid-adult women 26-45 years old and in 16-23 years old males.

The GSK vaccine, Cervarix, has shown to be promising in clinical trials [23]. This vaccine induced approximately 100% protection from cervical dysplasia associated with HPV 16 and 18, and high levels of protection against persistent infections with the same types [23, 27]. Cervarix has demonstrated to be partially protective against infection by a subset of HPV types not included in the vaccine [24]. The cross-protection was strong against HPV45, which is closely related with HPV18. Merck has not reported any result regarding cross-protection, so it is difficult to know whether the cross-protection represents an advantage of GSK vaccine over Merck product.

Recent preclinical studies indicated that as for L1 VLP vaccines, the protection against experimental papillomavirus challenge by L2 vaccination can be mediated by neutralizing antibodies [28]. In contrast to L1 VLP vaccination, vaccination with L2 induces antibodies that cross-neutralize diverse mucosal HPV genotypes *in vitro* [29]. These findings suggest the possibility of a simple pan-HPV prophylactic vaccine derived from L2 sequences. More recently, a preclinical study was conducted to determine whether vaccination with HPV16 $L2_{17-36,}$ a highly conserved epitope in divergent HPV types, in the context of a synthetic lipopeptide vaccine candidate, is broadly protective against challenge with homologous and heterologous pseudovirions. The results suggest that lipopeptide approach has the advantages of direct chemical synthesis, stability, potential to be delivered without needles, and generation of cross-protection [30].

Therapeutic vaccines

After initiation of sexual activity, genital HPV infections are rapidly acquired [31] but the malignant progression with high-risk HPV types takes at least 10 years. The VLP vaccines are unlikely to induce regression of established lesions [32], therefore the development of effective therapeutic vaccines against HPV is required.

Development of antivirals or therapeutic vaccines against HPV could be the strategy to treat preexisting viral infection. Antivirals have been developed for the treatment of some viral infections [33], but not against HPV. The potential vaccines have been focused on E6 and E7, the viral oncoproteins. These proteins are required to maintain the transformed states of HPV-induced neoplastic cells; therefore they are targets for therapeutic vaccine intervention. HPV E6 is able to interact with p53, leading to its dysfunction; and also keeps the telomerase length above its critical point, protecting the cell from apoptosis. HPV E7 binds to retinoblastoma protein, leading to tissue proliferation.

Various types of HPV vaccine targeting HPV16 E6 and E7 proteins have been tested in experimental approaches such as: viral and bacterial vector vaccines, peptide vaccines, recombinant protein vaccines, DNA vaccines and dendritic cells (DC)-based vaccines. Most of the studies have focused on E7 because its sequence is more conserved than that of the E6 gene, and it is better expressed and immunologically characterized [34].

Viral and bacterial vector vaccines

Viral, as well as bacterial vector vaccines are highly immunogenic and can express proteins. Bacterial vector vaccines can deliver engineered plasmids too. Both of them have a limited clinical application because of safety concerns and pre-existing viral immunity in the recipient.

It has been reported that immunotherapy targeting E6 and/or E7 using vaccinia vectors generates strong cytotoxic T-lymphocyte (CTL) activity and antitumor responses [35-38]. These preclinical and clinical results have shown the immunogenicity and safety of therapeutic vaccines based on viral vectors, and provides the basis for further clinical efficacy studies.

Other studies have been performed using modified adenovirus-expressing HPV16 E6 or E7 [39]. A recent study described an adenovirus based delivery system and showed that immunization with an adenoviral vector expressing calreticulin-HPV16 E7 fusion protein eradicates E7-expressing established tumors in mice, and induces long-term immunological memory [40]. In another study, the use of adeno-associated virus encoding HPV16 E7 fused to heat-shock protein (Hsp) was found to induce cellular immune responses and antitumor activity [41].

Another viral vector vaccine strategy based on the use of alphaviruses such as Semliki Forest Virus (SFV) and Venezuelan Equine Encephalitis Virus (VEE), was conducted to induce antitumor immunity. The advantage of this strategy is that these viruses express the RNA of the E6 and E7 oncogenes into host cell chromosomes. Moreover, there is no preexisting immunity against these viruses in the majority of the population. Two studies using SFV demonstrated the effects of the immunization route on the induction of specific CTL response [42, 43]. Interestingly, the VEE replicon particles have DC tropism and are more attractive for delivering HPV antigens and inducing antitumor immunity [44].

Attenuated bacterial vectors (*e.g.*, *Salmonella*, *Listeria monocytogenes*, *Lactobacillus lactis*) can be used as bacterial carriers to deliver proteins or plasmids of interest to antigen-presenting cells. *Salmonell*a has been used as bacterial vector to develop vaccines encoding HPV16 E6 and E7 [45] or HPV16 L1 and E7 [46], and it has been found to elicit humoral and cytotoxic immune responses. Gunn and colleagues [47] found that intraperitoneal or oral vaccination with recombinant *Listeria monocytogenes* secreting HPV16 E7 can induce regression of established tumors immortalized by HPV16. Similar findings were reported for *Lactobacillus lactis* expressing E7 and IL-12 [48]. A recent study with *Listeria* as bacterial vector vaccine showed eradication of solid implanted tumors in a transgenic mouse model of cancer [49].

Peptide vaccines

The concept of HPV peptide vaccines is based on identification and chemical synthesis of immunodominant T-cell epitopes which can induce specific immune responses. The relative ease of construction

22. Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR *et al*. Prophylac-tic quadrivalent human papillomavirus types 6, 11, 16, and 18 L1 virus-like particle vaccine in young women: a randomized double-blind placebo-controlled multi-centre phase II efficacy trial. Lancet Oncol 2005;6:271-8.

23. Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A *et al*. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet 2004;364:1757-65.

24. FDA licenses new vaccine for prevention of cervical cancer and other diseases in females caused by human papillomavirus. Available from: http://www.fda.gov/ bbs/topics/NEWS/2006/NEW01385.htm

25. National Cancer Institute: Cancer Vaccine Fact Sheet. Available at: http://www.nci.nih.gov/ cancertopics/factsheet/cancervaccine

26. Olsson SE, Villa LL, Costa RL, Petta CA, Andrade RP, Malm C *et al*. Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine. Vaccine 2007;25:4931-9.

27. Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM *et al*. Sustained efficacy up to 4.5 years of a bivalent L1virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet 2006;367:1247-55.

28. Embers ME, Budgeon LR, Pickel M, Christensen ND. Protective immunity to rabbit oral and cutaneous papillomaviruses by immunization with short peptides of L2, the minor capsid protein. J Virol 2002;76:9798-805.

29. Pastrana DV, Gambhira R, Buck CB, Pang YY, Thompson CD, Culp TD *et al*. Cross-neutralization of cutaneous and mucosal Papillomavirus types with antisera to the amino terminus of L2. Virology 2005;337:365-72.

30. Alphs HH, Gambhira R, Karanam B, Roberts JN, Jagu S, Schiller JT *et al*. Protection against heterologous human papillomavirus challenge by a synthetic lipopeptide vaccine containing a broadly cross-neutralizing epitope of L2. Proc Natl Acad Sci USA 2008;105:5850-5.

31. Taira AV, Neukermans CP, Sanders GD. Evaluating human papillomavirus vaccination programs. Emerg Infect Dis 2004; 10:1915-23.

32. Schiller JT, Lowy DR. Papilloma virus-like particles and HPV vaccine development. Semin Cancer Biol 1996;7:373-82.

33. Littler E, Oberg B. Achievements and challenges in antiviral drug discovery. Antivir Chem Chemother 2005;16:155-68.

34. Ahn WS, Bae SM, Kim TY, Kim TG, Lee JM, Namkoong SE *et al*. A therapy modality using recombinant IL-12 adenovirus plus E7 protein in a human papillomavirus 16 E6/E7-associated cervical cancer animal model. Hum Gene Ther 2003;14: 1389-99.

and production, chemical stability, and lack of oncogenic or infectious potential have made peptides attractive vaccine candidates. However, several obstacles limit the widespread usefulness of peptide vaccines, including their low immunogenicity and need for a better adjuvant. Nonetheless, current efforts are defying these limitations and many promising discoveries are making their way to improve this approach.

Approximately 40% of Caucasians carry HLA-A2 alleles; therefore the HPV16 E7 peptides presented by this allele were employed as immunogens in clinical trials [50]. In a Phase I study [51] two HPV16 E7 peptides known to be recognized by CTLs were used in escalating doses with Incomplete Freund's Adjuvant to treat patients with cervical intraepithelial neoplasia (CIN)/vulvar intraepithelial neoplasia. Three out of 10 patients controlled dysplasia after peptide vaccination suggesting clinical benefits of peptide vaccines in CIN patients. The significant finding of this study was that the majority of patients had a detectable immune response in peripheral blood cells after four injections of the $E7_{12-20}$ peptide vaccine. The augmented immune responses observed in this trial are similar to those observed in a peptide trial in vaginal and cervical cancer [50].

In two separated studies, the use of CpG oligodeoxynucleotide [52] and dsRNA (poly I:C) [53] as adjuvants mixed with CTL peptides demonstrated antitumor therapeutic efficacy.

The potency of HPV16 E7 peptide-based vaccines can be enhanced by using a new adjuvant, in which gangliosides are incorporated into the outer membrane protein complex of *Neisseria meningitidis* to form very small size proteoliposomes (VSSP) [54]. We have demonstrated that a vaccine composed of a minimal CTL peptide mixed with VSSP adjuvant can generate strong E7-specific antitumor immunity. This preparation might be successfully used to enhance tumor-specific antigen presentation, thereby increasing antigen-specific antitumor immune response. These results suggest that VSSP markedly enhance the number of tumor specific CTLs [55]. Daftarian and colleagues [56, 57] have performed two separated studies using VacciMax, a proprietary combination of encapsulated CTL epitopes fused to the T-helper epitope. Their results suggest that a single administration of VacciMax induces eradication of established HPV16-expressing tumors in young mice [56] and rejection of large HPV16-expressing tumors in aged mice [57].

In contrast, the use of long E6 and E7 overlapping peptide vaccines containing all potential CTL and T-helper epitopes, may elicit an effective HPV16-specific response able to eradicate preexisting infections. The long peptide vaccine concept was tested in animal models and further confirmed in human beings. In mice, an HPV16 E7 long peptide vaccine induced strong HPV16-specific $CD4^+$ and $CD8^+$ T-cell immunity, capable of eradication of established HPV16-positive tumors [52]. Subsequently, this concept was tested in the cottontail rabbit papillomavirus outbred rabbit model [58]. In this regard, a clinical grade synthetic long peptide vaccine was defined. In a Phase I trial, four subcutaneous injections with a pool of peptides in Montanide ISA-51 in patients with advanced cervical cancer revealed that this vaccine was safe and immunogenic [59]. In order to delineate the T-cell response induced by the HPV16 synthetic long peptide vaccine in human beings, Welters and colleagues [60] assessed the magnitude, breadth, type and polarization of vaccine-induced HPV16-specific T cells in a group of six patients, who were vaccinated after surgical removal of their HPV16-positive cervical tumor. They concluded that the HPV16 E6 and E7 synthetic long peptides are able to vigorously enhance the number and activity of HPV16-specific $CD4^+$ and $CD8^+$ T cells to a broad array of epitopes in all vaccinated patients.

Recombinant protein vaccines

Protein vaccines have many advantages compared with peptide vaccines. Protein based-vaccines bypass major histocompatibility complexe (MHC) restrictions because they contain whole MHC epitopes for inducing humoral and cellular responses. Although protein vaccines are appropriate to elicit humoral responses, using an adjuvant can change and direct the type of response elicited by the vaccine.

Preclinical studies with Quil-A and CpG-oligodeoxynucleotide as adjuvants mixed with E7 recombinant proteins showed E7-specific $CD4^+$ T helper lymphocytes and $CD8^+$ CTL responses against challenge with HPV16 E7-expressing tumors [61, 62]. In other preclinical study, immunization of C57BL/6 mice with fusion protein comprising *Mycobacterium bovis* BCG Hsp 65 linked to HPV16 E7 (Hsp-E7) protected the animals against challenge and rechallenge with a murine tumor cell line (TC-1) expressing E7 protein [63].

In a clinical trial based on Hsp-E7 for the treatment of CIN3 no patient had progressive disease and 48% had complete responses [64]. More recently the use of Hsp-E7 fusion protein has resulted in some clinical responses when administered to patients with cervical and anal precancerous lesions [65-67].

Other fusion protein strategies have been used. In a Phase I clinical trial it was observed that the immunization with HPV16 L2/E6/E7 fusion protein induced humoral and T-cell-mediated immune responses [68]. Lacey and colleagues conducted a Phase II clinical trial using TA-GW fusion protein (HPV6 L2 fused to E7 protein) for the treatment of genital warts [69].

DNA vaccines

Compared with protein or peptide vaccines, DNA vaccines allow sustained expression of antigen on MHC-peptide complexes and the vaccine components are produced within the host cells. Several strategies have been designed in order to enhance the immunogenicity of naked DNA vaccines due to their weak intrinsic potency. DNA vaccine potency has been improved by: (i) co-administration with immune modulatory molecules that modify intra or intercellular movement of antigen, or other DC properties; (ii) encapsulation; (iii) delivery routes for plasmid DNA.

Many reports have supported E7-targeting strategies. Conjugating E7 genes into the sequence of liposome-associated membrane proteins-1 enhanced E7-specific protective immunity against challenge with TC-1 tumor cells in mice [70]. Kim and colleagues

35. Borysiewicz LK, Fiander A, Nimako M, Man S, Wilkinson GW, Westmoreland D *et al*. A recombinant vaccinia virus encoding human papillomavirus types 16 and 18, E6 and E7 proteins as immunotherapy for cervical cancer. Lancet 1996;347: 1523-27.

36. Lamikanra A, Pan ZK, Isaacs SN, Wu TC, Paterson Y. Regression of established human papillomavirus type 16 HPV-16 immortalized tumors in vivo by vaccinia viruses expressing different forms of HPV-16 E7 correlates with enhanced CD8+ T-cell responses that home to the tumor site. J Virol 2001;75:9654-64.

37. Kaufmann AM, Stern PL, Rankin EM, Sommer H, Nuessler V, Schneider A *et al*. Safety and immunogenicity of TA-HPV, a recombinant vaccinia virus expressing modified human papillomavirus HPV-16 and HPV-18 E6 and E7 genes, in women with progressive cervical cancer. Clin Cancer Res 2002;8:3676-85.

38. Davidson EJ, Boswell CM, Sehr P, Pawlita M, Tomlinson AE, McVey RJ *et al*. Immunological and clinical responses in women with vulval intraepithelial neoplasia vaccinated with a vaccinia virus encoding human papillomavirus 16/18 oncoproteins. Cancer Res 2003;63: 6032-41.

39. He Z, Wlazlo AP, Kowalczyk DW, Cheng J, Xiang ZQ, Giles-Davis W *et al*. Viral recombinant vaccines to the E6 and E7 antigens of HPV-16. Virology 2000; 270:146-61.

40. Gómez-Gutierrez JG, Elpek KG, Montes de Oca-Luna R, Shirwan H, Sam Zhou H, McMasters KM. Vaccination with an adenoviral vector expressing calreticulin-human papillomavirus 16 E7 fusion protein eradicates E7 expressing established tumors in mice. Cancer Immunol Immunother 2007;56:997-1007.

41. Liu DW, Tsao YP, Kung JT, Ding YA, Sytwu HK, Xiao X *et al*. Recombinant adeno-associated virus expressing human papillomavirus type 16 E7 peptide DNA fused with heat shock protein DNA as a potential vaccine for cervical cancer. J Virol 2000;74:2888-94.

42. Daemen T, Riezebos-Brilman A, Regts J, Dontje B, van der Zee A, Wilschut J. Superior therapeutic efficacy of alphavirus-mediated immunization against human papilloma virus type 16 antigens in a murine tumour model: effects of the route of immunization. Antivir Ther 2004; 9:733-42.

43. Riezebos-Brilman A, Regts J, Freyschmidt EJ, Dontje B, Wilschut J, Daemen T. Induction of human papilloma virus E6/E7-specific cytotoxic T-lymphocyte activity in immune-tolerant, E6/E7-transgenic mice. Gene Ther 2005;12:1410-4.

44. Nishimoto KP, Laust AK, Wang K, Kamrud KI, Hubby B, Smith JF *et al*. Restricted and selective tropism of a Venezuelan equine encephalitis virus-derived replicon vector for human dendritic cells. Viral Immunol 2007;20:88-104.

45. Krul MR, Tijhaar EJ, Kleijne JA, Van Loon AM, Nievers MG, Schipper H *et al*. Induction of an antibody response in mice against human papillomavirus HPV type 16 after immunization with HPV recombinant Salmonella strains. Cancer Immunol Immunother 1996;43:44-8.

[71] demonstrated the enhancement of E7-specific immune responses and DC survival by co-administration of E7-containing DNA with DNA encoding antiapoptotic proteins. Hsu and colleagues [72] reported the increased immunogenicity of a DNA vaccine (conjugation of Hsp 70 to HPV16 E7 genes) against tumor challenge. One of the most recent studies exploring DNA-based approaches combines a DNA vaccine with Hsp 70; and demonstrates that using autologous Hsp 70 enhances antigen specific immune responses in a therapeutic setting [73].

Plasmid DNA encoding fragments derived from E6 and E7 of HPV16 and HPV18 was encapsulated in biodegradable particles (ZYC101a), and applied to women with CIN2 or CIN3, showing 67-72% resolution of CIN2/3 in < 25-years-old age group *versus* 23% in placebo group [74]. On the other hand, delivery routes of plasmid DNA play an important role on the nature of immune responses. In this regard, previous studies have suggested gene gun delivery as the best approach for DNA vaccine delivery in animals [75, 76].

DC-based vaccines

The importance of innate responses, and specially DCs for the optimal development of specific immune responses, is well known. DCs are essential for initiating an immune response, presenting the antigen to the T cell, and determining the nature of the immune response developed by T cells. A better understanding of DC biology, the cytokines produced by DCs and their mechanisms for antigen presentation will allow better vaccine designs. The immune status of cancer patients at the time of immune treatment is another aspect to be taken into account.

In patients with cervical cancer (HPV16 and 18 positive), E7-pulsed autologous DC promote the induction of human papillomavirus-specific $CD4^+$ and $CD8^+$ lymphocytes. A strong $CD8^+$ CTL response, capable of lysing autologous HPV-infected cancer cells in these patients was demonstrated [77]. In a pilot clinical study of 15 cervical cancer patients treated with DC-based vaccine, specific cellular immune responses were detected in four out of 11 patients [78]. Although DC-based therapies are still being pursued, no significant advances have been detected in either delivery methods or combination therapies which address immune evasion strategies by HPV.

Final considerations

Undoubtedly, one of the most significant advances in gynecologic oncology was the development of vaccines to prevent HPV infection. They are expected to reduce the burden of HPV infection and its related diseases within a few decades. Several questions are directly related to the performance of the vaccine: (i) protection endurance; (ii) induction of cross-protection against genital infection by HPV types which are not included in the vaccine; (iii) protective capacity in men. On the other hand, general knowledge about HPV and HPV-associated diseases is limited and may affect vaccine acceptability. The sexual nature of HPV infection may introduce barriers to parental consent not previously encountered with other vaccines. HPV-associated cervical cancer is more prevalent in the developing world, where screening with the Pap test is not widely available. Additionally, the HPV vaccine is likely to be more expensive than many other vaccines directed against several diseases.

VLP vaccines are unlikely to induce regression of established lesions; therefore, a therapeutic vaccine against HPV infection would be highly desirable to prevent the cancer-associated complications of HPV infections. Various types of therapeutic HPV vaccines have been described in experimental systems, and many of them are being tested in clinical trials. There are notable differences among therapeutic vaccine strategies (Table 1). Peptide, protein, DNA and viral/bacterial vector based vaccines are promising approaches for human use. In contrast, DC-based vaccines are patient-specific; thus, their commercialization is limited. Current treatment of cervical dysplasia is restricted to excisional or ablative procedures that remove or destroy cervical tissue, with efficacy rates of approximately 90%. Conventional procedures such as chemotherapy, radiation and surgery have some limitations in treating most malignancies. Therapeutic vaccines have certain advantages over conventional approaches because they are well tolerated and very specific, and they have long-term effects. Taking these reasons into account, combined approaches may also be useful for treating persistent HPV infections. Using therapeutic vaccines as adjuvant modality would be expected to increase clinical benefits in cervical cancer patients under conventional therapies. Challenges of therapeutic vaccines include the immunocompromised state of cancer patients, difficulties in stimulating the immune system, the immune evasion mechanisms used by tumor cells, and safety concerns.

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Table 1. Advantages and disadvantages among therapeutic HPV vaccine types

Vaccine type	Advantages	Disadvantages
Viral/Bacterial vector	-highly immunogenic -CTL epitopes can be naturally processed and presented, and can be more efficiently delivered to target cells	-generation of anti-vector humoral immunity -safety concerns
Peptide	-lower costs -safe -relatively easy to produce	-poorly immunogenic -HLA restriction -labile *in vivo*
Protein	-circumvention of the need for HLA matching	-appropriate to elicit humoral responses, when administered alone
DNA	-overcoming the limitation of HLA restriction -plasmid DNA contains unmethylated CpG motifs that may act as potent immunological adjuvants -relatively inexpensive, easy to purify, and very stable -no preexisting immunity -safe	-weak intrinsic potency of naked DNA vaccines
DC	-*ex vivo* maturation and stimulation of DC bypassing the viral negative regulation -directly targeting DC to induce vigorous immune responses	-high costs -patient-specific -limited commercialization

46. Jabbar IA, Fernando GJ, Saunders N, Aldovini A, Young R, Malcolm K *et al*. Immune responses induced by BCG recombinant for human papilloma virus L1 and E7 proteins. Vaccine 2000;18:2444-53.

47. Gunn GR, Zubair A, Peters C, Pan ZK, Wu TC, Paterson Y. Two Listeria monocytogenes vaccine vectors that express different molecular forms of human papilloma virus-16 HPV-16 E7 induce qualitatively different T cell immunity that correlates with their ability to induce re-gression of established tumors immortalized by HPV-16. J Immunol 2001;167:6471-79.

48. Bermúdez-Humarán LG, Cortes-Pérez NG, Lefèvre F, Guimarães V, Rabot S, Alcocer-González JM *et al*. A novel mucosal vaccine based on live Lactococci expressing E7 antigen and IL-12 induces systemic and mucosal immune responses and protects mice against human papillomavirus type 16-induced tumors. J Immunol 2005;175:7297-302.

49. Souders NC, Sewell DA, Pan ZK, Hussain SF, Rodriguez A, Wallecha A *et al*. Listeria-based vaccines can overcome tolerance by expanding low avidity CD8+ T cells capable of eradicating a solid tumor in a transgenic mouse model of cancer. Cancer Immun 2007;7:2.

50. Steller MA, Gurski KJ, Murakami M, Daniel RW, Shah KV, Celis E *et al*. Cell-mediated immunological responses in cervical and vaginal cancer patients immunized with a lipidated epitope of human papillomavirus type 16 E7. Clin Cancer Res 1998;4:2103-09.

51. Muderspach L, Wilczynski S, Roman L, Bade L, Felix J, Small LA *et al*. A phase I trial of a human papillomavirus HPV peptide vaccine for women with high-grade cervical and vulvar intraepithelial neoplasia who are HPV 16 positive. Clin Cancer Res 2000;6:3406-16.

52. Zwaveling S, Ferreira Mota SC, Nou-ta J, Johnson M, Lipford GB, Offringa R *et al*. Established human papillomavirus type 16-expressing tumors are effectively eradicated following vaccination with long peptides. J Immunol 2002;169:350-8.

53. Cui Z, Qiu F. Synthetic double-stranded RNA polyI:C as a potent peptide vaccine adjuvant: therapeutic activity against human cervical cancer in a rodent model. Cancer Immunol Immunother 2005;54:1180-90.

54. Mesa C, de Leon J, Rigley K, Fernández LE. Very small size proteoliposomes derived from *Neisseria meningitidis*: an effective adjuvant for Th1 induction and dendritic cell activation. Vaccine 2004;22:3045-52.

55. Torrens I, Mendoza O, Batte A, Reyes O, Fernández LE, Mesa C *et al*. Immunotherapy with CTL peptide and VSSP eradicated established human papillomavirus HPV type 16 E7-expressing tumors. Vaccine 2005;23: 5768-74.

56. Daftarian P, Mansour M, Benoit AC, Pohajdak B, Hoskin DW, Brown RG *et al*. Eradication of established HPV 16-expressing tumors by a single administration of a vaccine composed of a liposome-encapsulated CTL-T helper fusion peptide in a water-in-oil emulsion. Vaccine 2006;24:5235-44.

57. Daftarian PM, Mansour M, Pohajdak B, Fuentes-Ortega A, Korets-Smith E, Macdonald L *et al*. Rejection of large HPV-16 expressing tumors in aged mice by a single immunization of VacciMax encapsulated CTL/T helper peptides. J Transl Med 2007;5:26.

58. Vambutas A, DeVoti J, Nouri M, Drijfhout JW, Lipford GB, Bonagura VR *et al*. Therapeutic vaccination with papillomavirus E6 and E7 long peptides results in the control of both established virus-induced lesions and latently infected sites in a pre-clinical cottontail rabbit papillomavirus model. Vaccine 2005;23: 5271-80.

59. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP *et al*. Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high risk HPV16 in end stage cervical cancer patients shows low toxicity and robust immunogenicity. Clin Cancer Res 2008;14: 169-77.

60. Welters MJ, Kenter GG, Piersma SJ, Vloon AP, Löwik MJ, Berends-van der Meer DM *et al*. Induction of tumor-specific CD4+ and CD8+ T-cell immunity in cervical cancer patients by a human papillomavirus type 16 E6 and E7 long peptides vaccine. Clin Cancer Res 2008; 14:178-87.

61. Fernando GJ, Murray B, Zhou J, Frazer IH. Expression, purification and immunological characterization of the transforming protein E7, from cervical cancer-associated human papillomavirus type 16. Clin Exp Immunol 1999;115:397-403.

62. Kim TY, Myoung HJ, Kim JH, Moon IS, Kim TG, Ahn WS *et al*. Both E7 and CpG-oligodeoxynucleotide are required for protective immunity against challenge with human papillomavirus 16 E6/E7 immortalized tumor cells: involvement of CD4+ and CD8+ T cells in protection. Cancer Res 2002; 62:7234-40.

63. Chu NR, Wu HB, Wu TC, Boux LJ, Mizzen LA, Siegel MI. Immunotherapy of a human papillomavirus HPV type 16 E7-expressing tumour by administration of fusion protein comprising *Mycobacterium bovis* bacilli Calmette-Guerin BCG hsp65 and HPV16 E7. Clin Exp Immunol 2000;121:216-25.

64. Einstein MH, Kadish AS, Burk RD. HSP-based immunotherapy HspE7 for treatment of CIN 3. Gynecol Oncol 2005;96:912a-913a.

65. Palefsky JM, Berry JM, Jay N, Krogstad M, Da Costa M, Darragh TM *et al*. A trial of SGN-00101 HspE7 to treat high-grade anal intraepithelial neoplasia in HIV-positive individuals. AIDS 2006;20:1151-5.

66. Roman LD, Wilczynski S, Muderspach LI, Burnett AF, O'Meara A, Brinkman JA,*et al*. A phase II study of Hsp-7 SGN-00101 in women with high-grade cervical intraepithelial neoplasia. Gynecol Oncol 2007;106:558-66.

67. Einstein MH, Kadish AS, Burk RD, Kim MY, Wadler S, Streicher H *et al*. Heat shock fusion protein-based immunotherapy for treatment of cervical intraepithelial neoplasia III. Gynecol Oncol 2007;106:453-60.

68. de Jong A, O'Neill T, Khan AY, Kwappenberg KM, Chisholm SE, Whittle NR *et al*. Enhancement of human papillomavirus HPV type 16 E6 and E7-specific T-cell immunity in healthy volunteers through vaccination with TA-CIN, an HPV16 L2E7E6 fusion protein vaccine. Vaccine 2002;20:3456-64.

69. Lacey CJ, Thompson HS, Monteiro EF, O'Neill T, Davies ML, Holding FP *et al*. Phase IIa safety and immunogenicity of a therapeutic vaccine, TA-GW, in persons with genital warts. J Infect Dis 1999;179:612-8.

70. Ji H, Wang TL, Chen CH, Pai SI, Hung CF, Lin KY *et al*. Targeting human papillomavirus type 16 E7 to the endosomal/lysosomal compartment enhances the antitumor immunity of DNA vaccines against murine human papillomavirus type 16 E7-expressing tumors. Hum Gene Ther 1999;10:2727-40.

71. Kim TW, Hung CF, Boyd D, Juang J, He L, Kim JW *et al*. Enhancing DNA vaccine potency by coadministration of DNA encoding antiapoptotic proteins. J Clin Invest 2003;112: 109-17.

72. Hsu KF, Hung CF, Cheng WF, He L, Slater LA, Ling M *et al*. Enhancement of suicidal DNA vaccine potency by linking Mycobacterium tuberculosis heat shock protein 70 to an antigen. Gene Ther 2001;8:376-83.

73. Li Y, Subjeck J, Yang G, Repasky E, Wang XY. Generation of anti-tumor immunity using mammalian heat shock protein 70 DNA vaccines for cancer immunotherapy. Vaccine 2006;24:5360-70.

74. Garcia F, Petry KU, Muderspach L, Gold MA, Braly P, Crum CP *et al*. ZYC101a for treatment of highgrade cervical intraepithelial neoplasia: a randomized controlled trial. Obstet Gynecol 2004;103:317-26.

75. Ray NB, Ewalt LC, Lodmell DL. Nanogram quantities of plasmid DNA encoding the rabies virus glycoprotein protect mice against lethal rabies virus infection. Vaccine 1997; 15:892-5.

76. Trimble C, Lin CT, Hung CF, Pai S, Juang J, He L *et al*. Comparison of the CD8+ T cell responses and antitumor effects generated by DNA vaccine administered through gene gun, biojector, and syringe. Vaccine 2003;21: 4036-42.

77. Santin AD, Hermonat PL, Ravaggi A, Chiriva-Internati M, Zhan D, Pecorelli S *et al*. Induction of human papillomavirus-specific CD4+ and CD8+ lymphocytes by E7-pulsed autologous dendritic cells in patients with human papillomavirus type 16- and 18-positive cervical cancer. J Virol 1999;73: 5402-10.

78. Ferrara A, Nonn M, Sehr P, Schreckenberger C, Pawlita M, Dürst M *et al*. Dendritic cell-based tumor vaccine for cervical cancer II: results of a clinical pilot study in 15 individual patients. J Cancer Res Clin Oncol 2003; 129:521-30.

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