Preventive vaccines for cervical cancer

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Wheeler CM.
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Salud Publica Mex 1997;39:283-287.

Abstract

The potential use of vaccines for the human papillomavirus (HPV) in the prevention and treatment of cervical cancer is a possibility in the near future. Close to 20 genotypes of HPV, of the 75 that have been identified, infect the femine genital tract, but four subtypes (16, 18, 31 and 45) have been associated in close to 80% of cervical cancers. this article proposes that in order to design an effective prophylactic vaccine against HPV infection, an adequate immune response should be guaranteed through four goals; a) activation of antigens present in the cell; b) overcoming the host response and viral genetic variability in the T cell response; c) generation of high levels of T and B memory cells; and d) persistence of antigens.

Key words: cervix neoplasms/prevention & control; vaccines; papillomavirus, human

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Resumen

El potencial uso de vacunas de virus del papiloma humano (VPH) en la prevención y tratamiento del cáncer cervical posiblemente será implementado durante los próximos años. Cerca de los 20 genotipos de VPH de los 75 que se encuentran identificados infectan el tracto genital femenino, pero son cuatro subtipos: 16, 18, 31 y 45 los que se han asociado en cerca de 80% a cáncer cervical. En este ensayo se plantea que para poder diseñar una vacuna profiláctica contra la infección de VPH, efectiva, se debe garantizar una adecuada respuesta inmune a través de cuatro metas: a) activación de antígenos presentes en la célula; b) superar la respuesta del huésped y la variabilidad genética viral en la respuesta de células T; c) generación de altos niveles de células T y B de memoria, y d) persistencia de antígenos.

Palabras clave: neoplasmas del cuello uterino/prevención & control; vacunas; papillomavirus humano

Cervical cancers represent a leading cause of deaths in women worldwide and especially in developing countries.[1] In some countries, implementation of Papanicolaou (Pap) smear screening programs has resulted in a significant reduction in cervical cancer incidence; however, the management of cervical abnormalities remains a significant public health concern and financial burden. Vaccination efforts for the prevention of cervical cancer are currently focused on utilizing gene products of human papillomaviruses (HPVs). There are more than 75 different HPV types and approximately 35 of these types infect the genital tract.[2] HPVs are etiologic agents involved with the development of more than 90% of cervical cancers[3] and their presumed precursor lesions,[4] cervical intraepithelial neoplasia. HPVs are also associated with other anogenital carcinomas such as anal, vulvar, and penile cancers.[5] A vaccine that can prevent initial or subsequent active or persistent HPV infection could reduce the health care costs associated with abnormal Pap smear management and the morbidity and mortality attributable to HPV-associated anogenital cancers.

In both developing and developed countries, an effective prophylactic HPV vaccine represents a desirable candidate strategy for reducing cervical cancer incidence. Presently effective cervical cancer screening

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Fecha de recibido: 4 de marzo de 1997 • **Fecha de aprobado:** 6 de junio de 1997
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programs are costly and require a wide range of effective health care delivery. To be successful, these programs must provide targeted education programs to increase Pap smear screening in at-risk populations and must continually implement state-of-the-art technological advances to improve diagnostic determinations. In addition, these programs must include certified training of cytotechnologists, pathologists, and gynecologists and regulatory agencies that are responsible for maintaining ongoing procedural integrity and standardized diagnostic procedures. Even if these requirements are achieved, effective follow-up and treatment programs are necessary to impact on disease outcomes.

Control of oncogenic sequelae of HPV infection is only one reason for vaccination. HPV-associated benign genital warts or condylomas represent about 1 million cases each year requiring treatment in the United States (US).[6] Many of these cases of genital warts are resistant to conventional therapy. Initial HPV vaccine efforts will probably target the early manifestations of HPV infections that represent significant public health problems and offer more easily measured end points. Reduction in cancer incidence would then represent a future benefit. The testing of vaccines by using cancer or even severe cervical dysplasia as an endpoint may not be practical.

Vaccine Strategies

An effective prophylactic vaccine must be designed to elicit the appropriate immune response and should achieve four goals.[7] This requires activation of antigen presenting cells, overcoming host and viral genetic variability in T cell responses, generation of high levels of T and B memory cells, and persistence of antigen. Protection against viral infections can be achieved either by creating an immunological barrier at the site of viral entry or secondarily by preventing a successful infection once viral entry into the cell has occurred. The creation of an immunological barrier at the site of viral entry would be achieved presumably by IgA generated against the L1 and or the L2 HPV capsid proteins. Vaccine strategies that focus on the site of viral entry might also incorporate the targeting of immunity against the viral receptor-attachment site on the infecting virions. In any prophylactic vaccine strategy, the desired outcome would be the prevention of viral-associated lesions.

There are no good data identifying immune responses to specific HPV proteins that are relevant to protective immunity. Additionally, it is unclear whether vaccines will be able to induce an effective immune response in individuals who may have ultimately become persistently infected with HPV, since persistence might indicate a constitutive inability to recognize important viral determinants. It should be noted that neutralizing antibodies can be detected using cell culture-based assays in the sera of individuals harboring HPV infections.[8] The prevalence of PV neutralizing antibody following HPV infection in humans and its relevance to disease protection remain to be established.

In vitro propagation of HPVs has been reported;[9] however, these systems will not at this time produce adequate amounts of virion for the purpose of vaccination. Even if adequate quantities of attenuated HPVs could be generated, they may not be desirable from the point of view that their genomes harbor transforming genes. The most encouraging experimental vaccine results come from PV vaccine models in animals. In these model systems, prophylaxis has been induced by L1 and L2 capsid proteins. Recombinant PV L1 and L2 capsid proteins that self-assemble into virus-like particles (VLPs) can be produced in large quantities in bacterial,[10] insect,[11] yeast[12] and other eukaryotic cell systems.[13] These VLPs have proven useful as PV vaccines in three animal models including the beagle dog,[14] the rabbit[15] and the cow.[16] VLPs mimic the conformation of native virions,[17] can produce type-specific neutralizing antibody responses in animals[18] and can be neutralized by human sera in cell culture-based assays.[8] Immunization with 0.01 μg of canine oral papillomavirus (COPV) L1 VLP protects dogs from infection after live virus challenge.[14] Immunization with denatured L1 capsid proteins does not protect against challenge with live COPV, demonstrating the importance of conformational epitopes to VLP vaccine efficacy.[14] Additionally, resistance to live virus challenge can be transferred to a native animal by transfer of sera from a rabbit immunized with cottontail rabbit papillomavirus (CRPV) VLPs.[19]

PV infections can also be prevented in the cow and rabbit models by independent immunization with the L2 capsid protein alone.[20,21] The L2 protein when incorporated into L1 VLPs does not appear to increase the overall VLP particle immunogenicity when compared to VLPs comprised of L1 alone,[19] although particle stability may be enhanced. The DNA-binding capacity of the L2 protein[22] may result in encapsidation of host DNA during VLP self-assembly and thus increased contamination of VLP vaccine preparations with host cellular DNA may argue for VLP formulations that contain only L1.

Although VLP-based HPV vaccines are promising, polynucleotide vaccines using non-replicating DNA plasmids encoding viral antigens might prove

advantageous. In animal models, DNA-based influenza vaccines have provided a means by which cell-mediated immunity may be induced.[23] Studies using CRPV have demonstrated conformationally-specific neutralizing antibodies and protective immunity following immunization with DNA coding for the L1 protein of CRPV.[24] Additionally, a DNA-based CRPV E6 vaccine has recently been shown to be effective in preventing viral-induced lesions.[25] These results suggest that DNA vaccines merit consideration as potential alternatives for the vaccination of humans against HPVs. This approach may be of particular importance given the multiplicity of HPV types capable of causing disease.

Both viral and bacterial vectors are also under evaluation for HPV-specific vaccination. Attenuated viral vectors including adenovirus and vaccinia virus and the bacterial vector Salmonella have been studied extensively for their ability to provide protective mucosal immunity. Noteworthy is the finding that intranasal application of an adenoviral vector expressing the Herpes Simplex virus (HSV) glycoprotein B protects against sexual transmission of this virus.[26] This demonstrates the potential of using live viral vectors for the induction of HPV immunity in the female genital tract.

An HPV receptor-attachment site has not yet been identified; however, experimental data indicates that many PVs utilize a common receptor.[27] Thus a conserved receptor-attachment site is possible at least for a number of HPVs. Targeted immunity against this site would overcome the likely requirement for multivalent HPV vaccinations. It is however probable that an HPV receptor-attachment site(s) is hidden from immune access and recognition. Further work is needed to define the relevant HPV neutralizing epitopes and the virus receptor-attachment site. This information may be incorporated into potential HPV vaccine designs.

If a vaccine that will erradicate virus already replicating in epithelial cells is considered, a strategy that will eliminate the infection must be able to prime for cytotoxic T cell lymphocytes (CTLs) directed against epitopes that are derived from endogenously processed viral proteins presented in the context of the major histocompatibility complex (MHC). Purified HPV antigen preparations, live viral and bacterial HPV-specific vectors and DNA-based vaccinations are all reasonable strategies to accomplish this goal. The candidate HPV proteins under consideration for this purpose are the E1 and E2 proteins needed early in infection for viral genome replication and the E6 and E7 transforming proteins. Expression of these early HPV proteins presumably occurs within the basal epithelial cells. There appears to be a minimal immune response to these proteins during natural infection presumably due to the low levels of proteins produced and presented. Although mechanisms of targeted immune down regulation have not been reported for HPVs, as seen in other persistent viral infections, such mechanisms may also play a role in the observed lack of HPV-specific immune responses.

Because HPV 16 represents the most common HPV type found in cytologically normal women and invasive cervical cancers,[5] it will represent the primary candidate for early targeting in vaccine efforts. It appears that HPV type-specific vaccination may not provide cross protection against even closely related HPVs. It is unlikely that HPV vaccine strategies will be able to accommodate the more than 20 HPV types found associated with invasive cervical cancers.[5]

Worldwide geographic variation in type-specific HPV prevalence may represent an additional strategical issue since vaccines that are designed for one region of the world may not be applicable to other regions. Similarly, intratypic HPV variant prevalences may represent an additional complexity to consider in appropriate HPV vaccine strategies. More than twenty distinct HPV 16 variants have been identified[28] and amino acids that vary between these intratype HPV 16 variants may be important to the generation of appropriate humoral or cellular immune responses. Worldwide, HPV 16 variants appear to be characterized by distinct continental distributions.[28,29] If amino acid variations within intratype variants are critical to the generation of appropriate immune responses, vaccines developed against reference strain HPVs that are predominantly of European origin may have reduced efficacy in countries where these HPV 16 variants are rarely found.

In the US and Europe, HPV vaccine strategies that incorporate either HPV 6, 11, 16, and 18 or HPV 16, 18, 31, and 45 in combination may be evaluated in early multivalent vaccine trials. In the first case, HPV 6 and 11 vaccination would be justified for the prevention and treatment of HPV-associated genital warts and HPV 16 and 18 for the prevention and treatment of HPV-associated cervical infections. Genital wart patients will presumably represent one population in which vaccine safety and efficacy will be initially evaluated. Targeting of genital wart patients during initial vaccine efforts provides a population in which treatment efficacy and characterizations of local immune responses are more easily measurable and additionally will provide opportunities for examining males within vaccine trails. In the latter case, HPV 16, 18, 31, and

45 multivalent vaccines will target those HPV types responsible for more than 80% of all invasive cervical cancer cases worldwide.[3]

No matter which HPV types and viral proteins are chosen as HPV vaccine targets, the challenge will be to develop antigen delivery systems that can invoke the appropriate type of immune response resulting in clinical efficacy. Enhancement of immune responses may utilize chimeric molecules or double recombinants that incorporate cytokines such as IL-2 and GM-CSF. Choices of adjuvants, selected antigen dose and immunization schedule will certainly be critical and may be chosen to elicit either T helper cell type 1 (Th1) or 2 (Th2) responses or both. Route of antigen administration is likely to be important and potentially, parenteral administration of antigen may circumvent the constraints on immunogenicity seen in natural HPV infection. It is not known whether mucosal IgA will be required for protection against HPV infection.

An even greater challenge will be to identify appropriate endpoint measurements and target populations that will be used to establish vaccine efficacy. Are polymerase chain reaction-based HPV measurements required in vaccinees and at what frequency and duration would this testing be appropriate? Are HPV negative cervices the only acceptable vaccine outcome or would an acceptable outcome be obtained if low-level transient HPV infections and the absence of cervical cytological abnormalities were observed? How should cervical cytology be incorporated into monitoring vaccine efficacy? Similarly, will HPV-specific serology be a robust marker for monitoring HPV vaccine outcomes? During natural infection, even transient humoral immunity is not detected with currently available serological methods in all individuals with presumed incident HPV infection. Furthermore, little support is available to suggest that neutralizing antibody is in fact protective. What will be the long term efficacy of vaccination in preventing HPV infection and what monitoring interval is needed to ultimately predict a potential significant impact on cervical cancer incidence?

Even if a safe and effective HPV vaccine were available, a number of additional issues will remain. At what age should vaccination occur, in childhood or in adolescence? Should only high-risk populations or the general population be vaccinated? Will both males and females be vaccinated? If yes, then what are the best sampling methods for assessing HPV infection in males? What impact on the population dynamics of HPV prevalences, if any, will result from vaccination against only a few predominant HPVs? Maybe most importantly, will vaccine costs be affordable in developing countries where vaccination is most needed?

During the past decade we have watched the natural history of HPV infections unfold before us. The next decade may see the beginning of the eradication of HPV infections and certainly will see many of the questions that were posed above answered.

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