Vaccines against human papillomavirus and perspectives for the prevention and control of cervical cancer

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Abstract

Today, "persistent" infections by certain types of human papillomavirus (HPV) are considered necessary for developing cervical cancer. Producing efficient vaccines against these viruses may eventually lead to a great reduction in incidence and mortality rates of this cancer. In the case of HPV, the production of traditional vaccines usually based in dead or attenuated viruses is not possible due in part to the lack of systems where large quantities of viral particles could be obtained. Fortunately, the expression of the late L1 protein alone, or in combination with L2, leads to the generation of structures resembling true virions that have been called virus-like particles (VLPs) and constitute excellent candidates as prophylactic vaccines. VLPs have shown to be very immunogenic, and have prevented development of natural or challenged infections in both animal systems and humans. Recently, HPV16 VLPs were shown to be very efficient to prevent the development of "persistent" infections, as determined by PCR assays, in a large group of vaccinated women. Therapeutic vaccines, on the other hand, are expected to have an impact on advanced lesions and residual illness, by taking advantaje of the fact that early E6 and E7 genes are thought to be constitutively expressed in cervical tumors and precursor lesions. Finally, DNA-based vaccines could represent a useful alternative for preventing infections by genital HPV. This paper is available too at: http://www.insp.mx/salud/index.html

Key words: human papillomavirus; cervical cancer; prophylactic vaccines; therapeutic vaccines; immunity; perspectives

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Resumen

Actualmente, las infecciones "persistentes" por algunos tipos del virus del papiloma humano se consideran como necesarias para desarrollar cáncer cervicouterino. Por ello, el desarrollo de vacunas eficientes contra estos virus se ha considerado de suma importancia para poder eventualmente ayudar a controlar esta enfermedad, en países donde los programas de detección oportuna no han dado aún los resultados deseados. En el caso de estos virus no es posible el desarrollo de vacunas tradicionales, las cuales están basadas generalmente en el empleo de virus atenuados o muertos. Esto debido a la falta de sistemas eficientes para producir partículas virales en cantidades suficientes para ser usadas en programas masivos. Sin embargo, de manera afortunada, la expresión de la proteína viral tardía L1, sola o en combinación con la proteína L2, lleva a la generación de estructuras similares a los viriones infectivos y que han sido denominadas "partículas semejantes a virus" o VLP. Estas preparaciones de cápsides vacías han sido probadas ya en diferentes modelos animales, incluidos los humanos. Recientemente, se ha reportado que la las VLP del virus del papiloma humano tipo 16 son capaces de prevenir el desarrollo de las infecciones "persistentes" causadas por algunos tipos del virus del papiloma humano, consideradas precursoras del cáncer cervicouterino. Este artículo también está disponible en: http://www.insp.mx/salud/index.html

Palabras clave: virus de papiloma humano; cáncer cervicouterino; vacunas profilácticas; vacunas terapeúticas; inmunidad; perspectivas para control

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Some manifestations of human papillomavirus (HPV) infections have been undoubtedly known since old times. The viral etiology of warts was revealed more than a century ago, when it was first demonstrated that canine warts could be transmitted to healthy animals by using ultra-filtered extracts from warts. Ten years later, human warts were proven to be transmitted in a similar way. Although the infectious nature of cervical cancer was also suspected more than a century ago, several decades had to elapse before scientists linked this tumors with HPV infections and then were able to identify and define the specific viral types associated with the malignant proliferation of genital epithelia. In the early 80s were identified HPV types 16 and 18, which are specifically associated with a majority of genital tumors.[1]

Today, we know the existence of approximately 200 different types of HPV, all of which are specifically associated with the development of benign and in some cases malignant lesions of epithelial cells from the skin, the anal, oral and genital mucosas in humans. The role that "persistent" infections caused by some types of these viruses play in the development of tumors from the uterine cervix has been firmly established and indeed, infections by some HPV types are now considered as necessary for the development of this type of cancer.[2] Various strategies have been designed to develop vaccines against these viruses that could eventually lead to prevent viral infections and thus, cervical cancer development.

In general, vaccines developed up to date against different viruses are basically of two types: i) traditional vaccines, also called prophylactic, which prevent infections by neutralizing viral particles and that generally use attenuated or dead viruses, and ii) therapeutic vaccines, aimed at eliminating existing infections. In general, effective prophylactic vaccines induce the production of important levels of neutralizing antibodies and thus protect against infections. An efficient vaccine against genital HPV should be able to prevent infections and re-infections, by generating an adequate immune response on the site and at the time of infection(s).[3]

In the case of HPV, it is not possible to prepare traditional vaccines in a conventional way, since there are still no efficient means to quantitatively produce viral particles. The specific tropism of HPV for human epithelial cells of the skin and mucosas, constitutes a first limitation in the development of systems that could enable the study of virus-host relationships under natural conditions and consequently, could lead to the development of efficient systems for producing viral particles in large quantities. The favorable field for the production of such particles is the differentiating epithelia and even there, natural infections are not very productive (usually low number of viral particles are produced). In recent years, important efforts for developing systems that could overcome such limitations have been made. Among them, the most widely used are the "raft" culture of epithelial cells in an air-liquid interface, as well as the transplant of tissues infected with HPV to immunodeficient mice. These systems, however, are not adequate for the production of viral particles in large quantities for vaccination programs.

Virus-like particles (VLPs) and prophylactic vaccines

A very efficient alternative has been found to face the problem of the low production of HPV virions in conventional systems. It is based on the observation originally carried out by Jang Zhou and colleagues more than a decade ago, who realized that the expression of the late L1 protein in combination with L2, led to the generation of structures similar to those found in virions.[4] These empty virus-like structures have been called virus-like particles, or VLPs, and constitute excellent candidates for vaccines, as they have been shown to be very immunogenic and innocuous, since they do not carry any viral genetic material. Today, the majority of prophylactic vaccines so far designed for HPV involve the use of VLPs. They are generally composed of one or two of the structural late viral proteins and based on the already mentioned fact that L1 alone, or in combination with L2, is self-assembled and forms empty capsids, that may be efficiently used to induce neutralizing antibodies that have been shown to prevent infections by several specific viral types.[5]

Antigenic properties of VLPs make them very attractive candidates for their use as prophylactic vaccines in massive programs. The protective efficiency of VLPs preparations has been tested in several animal models by different groups. Natural infections produced by different types of animal papillomaviruses, such as the cottontail rabbit papillomavirus (CRPV), the canine oral papillomavirus (COPV), and the bovine papillomavirus type 4 (BPV-4), have been efficiently prevented by using vaccination schemes with the corresponding VLPs of each virus.[6-8]

In the case of vaccines against different HPV, studies with types 6, 11, and mainly 16, had confirmed previously established concepts and validated results obtained with neutralization assays.[9] Studies have also shown that different HPV types represent unique viral

serotypes and that a succesfull vaccine would require inclusion of VLPs of each type for which protection is expected.[10] In a phase 1 trial where the safety and immunogenicity of an HPV-11 VLP vaccine was validated, it was found that the candidate vaccine was well tolerated and induced high levels of both binding and neutralizing antibodies. Significant increases in HPV-specific INF-gamma and IL-5 production was observed from peripheral blood mononuclear cells culture supernatants.[11]

Studies with HPV16 VLPs have gone through different animal models and clinical trials until showing recently a complete protection against persistent HPV16 infection and related cervical dysplasia after vaccinating women with the corresponding VLP preparation. More than two thousand women received three 40µg doses of HPV16 VLP at 0, 2 and 6 months. These woman underwent periodical examination to evaluate any adverse reaction, and to determine the presence of HPV16 DNA and neutralizing antibodies, together with women from a similar group who received placebo. Results are very encouraging since all woman in the vaccinated group were protected against persistent HPV16 infection and associated cytological abnormalities.[12]

This prophylactic vaccine against HPV type 16 should represent in the near future a useful alternative for young women starting their active sexual life, since they constitute the population that could most benefit from it. In this sense, it will be necessary to consider also vaccination of young males in order to achieve a higher efficiency, by reducing the total number of individuals that actually may propagate viral infection. Current expectations are excellent to implement in the years to come, massive programs using prophylactic vaccines against a variable combination of main HPV types involved in the development of cervical cancer. In Latin America, these combination of VLPs may include types 18, 31, 33, 45, and 58. Estimates indicate, however, that it will take time before these vaccines may have a considerable impact on invasive cancer rates, particularly in underdeveloped countries. That is why nowadays, timely detection of early lesions, together with education of the populations will undoubtedly constitute the basic means to efficiently prevent this illness.

Therapeutic vaccines

Several therapeutic vaccines have been developed, mainly against HPV type 16, and some of them have been already tested in clinical trials. These vaccines are designed to eliminate the residual illness, after the treatment of high grade intra-epithelial lesions or invasive cancer. A relevant aspects of cervical cancer is the fact that in the vast majority of the tumors, viral E6 and E7 oncogenes are retained and expressed in a constitutive way. Moreover, continuous expression of E6 and E7 seems necessary to prevent HPV-transformed cells from entering apoptosis and/or senescence.[13] The situation is very attractive to stimulate a response against this very specific tumor antigens. That is why in this case, the majority of therapeutic vaccines already developed are intended to stimulate the immune system against E6 and/or E7 early viral proteins. This is expected to produce an immune response that could eventually eliminate infected cells and even destroy tumor cells that constitutively express these two non-structural early viral antigens. One of the major limitations of this approach is the fact that the existing alterations in most tumors will possibly prevent the efficient use of these vaccines. In addition, the continuous presence of E6 and E7 proteins in infected and transformed cells, possibly during decades, could create an unfavorable environment to stimulate a response against them that could eventually destroy HPV-containing cells.

Therapeutic vaccines for HPV have been based on peptides, proteins, chimeric proteins (containing fragments of two or more proteins), DNA, viral vectors, bacterial vectors, dendritic cells, and modified tumor cells. As mentioned, several prophylactic vaccines against HPV have been developed and tested in phase I and II clinical trials. These studies include vaccines based on recombinant vaccinia virus expressing E6 and E7 from both HPV types 16 and 18,[14] peptides, or a lipidated peptide from E7,[15,16] fusion proteins with HPV 16 E6 and E7,[17,18] and dendritic cells.[19]

Chimeric VLPs (cVLPs) have been constructed by replacing up to 60 carboxy-terminal aminoacids of the HPV16 L1 protein with fragments of the HPV16 E7 protein.[20] Immunization with cVLPs containing a tumor-specific antigen induced a protective response, indicating that cVLPs could be used in clinical trials for therapeutic purposes.[21] Recently, it was shown that the use of cVLPs from different HPV types in prime/boost regimens should increase the efficacy and usefulness of cVLP vaccines for treating cervical neoplasia.[22]

The enormous problem posed by cervical cancer has led several groups to develop alternative strategies aimed at reducing tumors' growth. One of such alternatives is based on the fact that tumor growth may be controlled by reducing the expression of E6 and E7 oncogenes, that are finally responsible for the abnormal growth of tumor cells. In addition to the fact that E6 and E7 are retained in the majority of the tumors, in

most cases, the E2 gene is usually lost or not expressed.[23] The product of the E2 gene is a regulator of viral early transcription and replication, and in the case of genital HPV, suppresses transcription of E6 and E7 oncogenes. Thus, it has been suggested that the E2 gene may be used to inhibit the expression of viral oncogenes and thus stop tumor growth. It is known that expression of the E2 protein in HeLa cells causes cell-cycle arrest, apoptosis and senescence.[13,24] Based on this and other observations, we believe that it would be useful to assess the effect of the E2 gene on the growth of tumors from the uterine cervix since pre-clinical trials have shown a significant inhibition of tumor growth of HPV-containing cells, by E2. Recently, recombinant adenoviruses expressing E2 have been shown to induce cell cycle arrest and apoptosis in a variety of tumor cells.[25]

DNA vaccines

Unlike traditional vaccines, or VLPs which are still difficult to produce on a large-scale basis and are thus expensive for developing countries, DNA-based vaccines could represent an alternative for expressing HPV antigens in animal tissues and thus generate a protective immune response. DNA vaccines, referred to by many authors as third-generation vaccines, have already shown their efficiency in several animal trials, where they have been able to induce a protective immune response.[26] The DNA vaccines already tested have included genes that code for antigens from various pathogens or tumors, instead of using the corresponding proteins. Initial observations in this field showed the efficiency of a DNA "vaccine" to protect injected animals against a challenge of influenza virus. The animals were immunized by injecting DNA that encodes for the Nucleoprotein (NP), an internal and well-preserved protein of influenza A virus. Animals developed specific antibodies against NP, as well as a strong CTL response. This fact showed the enormous potential of this technology for inducing a restricted CTL response for class I MHC molecules in a simple way.[26]

DNA vaccines are generally made by amplifying the gene of interest in bacterial plasmids, usually containing a strong promoter that induces expression of the relevant gene. These plasmids are generally grown in bacteria, purified by conventional methods, and used to inject them directly. Usually DNA is "taken" by the cells at the site of injection and the protein of interest produced. Although plasmids generally carry a replication origin that is not functional in human cells, there has been some concern regarding the possible consequences of injecting bacterial DNA in humans.[27]

Recently, we have assesed in the laboratory the capacity of a plasmid DNA that expreses the L1 gene of HPV type 16, to induce a protective immune response. After injection of "naked" DNA we found the appearance of antibodies that were detectable twelve months after immunization. Specific IgA antibodies were also found in vaginal washes from immunized mice. Both systemic and local antibodies proved effective in surrogate neutralization assays. This results indicate that an L1-based DNA vaccine could be useful for preventing infections by genital HPV.[28]

Perspectives

Uterine cervix cancer is among those neoplasias that may be cured in the majority of cases if detected in early stages. Likewise, it constitutes a neoplasia that may be prevented if infections by certain types of HPV that colonize genital mucosal could be eliminated. That is one of the reasons why enormous efforts have been undertaken worldwide to develop vaccines against specific HPV types. These are basically of two types, those aimed at preventing viral infections throughout development of neutralizing antibodies (prophylactic vaccines) and those that are intended to control growth of transformed cells and induce the regression of pre-existing lesions or even tumors (therapeutic vaccines).

Modern strategies for the development of vaccines against HPV include mainly the use of the so-called VLPs, which in fact are made up by empty capsids, generated by the expression of L1 or L1/L2 genes in various systems. For HPV vaccines to prevent infections in genital tissues, they should be able to generate the production of neutralizing antibodies against capsid proteins, in the surface of the mucosa affected by the virus. Although results in the development of vaccines against HPV are very promising, in the opinion of some experts however, it will still be necessary some 10 -15 years more to have prophylactic vaccines against HPV available worldwide. Vaccines that will confer a high certainty level to anticipate that women immunized will be protected against "high risk" type infections and, thus, they will neither develop pre-malignant lesions nor cervical cancer in adulthood. This means that trials will not be conclusive until they have proven that effectively, immunization of individuals leads to total protection, not only of clinical infections, but also of sub-clinical infections that in the end could lead, in some cases, to the development of uterine cervix tumors.

In addition, in the case of prophylactic vaccines some estimates have been already made concerning the possible impact of massive immunization programs against HPV. These estimates were done basically in two ways: first,

estimating the number of vaccines required to prevent one single case of cervical cancer; and second, estimating the time required for the immunization program to have an impact on the total number of cases in a given population. Such calculations were made both for populations from developing countries and populations from developed countries, using incidence rates adjusted by age. For these calculations, cumulative risk values and vaccine efficiency rates have been used. The efficiency of a vaccine was determined on one hand by the efficiency of the vaccine itself against a given type of HPV and, on the other, by the rate of cancer cases attributed to that specific viral type. For example, if the most common HPV types (16, 18, 31, and 45) are responsible for approximately 80% of all cancer cases and we assume 90% as an efficiency rate for a combined vaccine against these viral types, then the efficiency of this vaccine to prevent cancer would be 72%. This means that it would be necessary to perform 200 immunizations in a developing country and up to 350 in a developed country, in order to prevent a single case of cancer among them.[29]

If we assume that a massive immunization campaign against HPV could start in 2010 and that the vaccine will be applied to all women below 15 years, differences in the total number of cancer cases would not be observed until immunized women enter the high risk cancer age group, i.e., 40-45 years old. These estimates clearly indicate that a massive immunization program against HPV, even if it is very efficient, would take many years before having a real impact in the total number of cancer cases in vaccinated populations.[29] For such reasons, it is clear that massive programs for the timely detection of cervical cancer should be improved in developing countries in order to reduce the incidence of this type of cancer.

References

1. zur Hausen H. Papillomaviruses and cancer: From basic studies to clinical application. Nat Rev Cancer. 2002. 2:342-350.
2. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999. 189:12-19.
3. Frazer IH. Vaccines for papillomavirus infection. Virus Res. 2002. 89:271-274.
4. Zhou J, Sun XY, Stenzel DJ, Frazer IH. Expression of vaccinia recombinant HPV 16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles . Virology. 1991. 185:251-257.
5. Ault KA. Virus-like particles as a potential vaccine for human papillomavirus. Papillomavirus Rep. 2003;14:47-51.
6. Suzich JA, Ghim SJ, Palmer-Hill FJ, White WI, Tamura JK, Bell JA. Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. Proc Natl Acad Sci U S A. 1995. 92:11553-11557.
7. Breitburd F, Kirnbauer R, Hubbert NL, Nonnenmacher B, Trin-Dinh-Desmarquet C *et al*. Immunization with virus-like particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. J Virol 1995;69:3959-3963.
8. Kirnbauer R, Chandrachud LM, O'Neil BW, Wagner ER, Grindlay GJ, Armstrong A *et al*. Virus-like particles of bovine papillomavirus type 4 in prophylactic and therapeutic immunization. Virology. 1996. 219:37-44.
9. Harro CD, Pang YY, Roden RB, Hildesheim A, Wang Z, Reynolds MJ *et al*. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particule vaccine. J Natl Cancer Inst 2001; 93:284-292.
10. Giroglou T, Sapp M, Lane C, Fligge C, Christensen ND, Streeck RE, Rose RC. Immunological analyses of human papillomavirus capsids. Vaccine 2001;19:1783-93.
11. Evans TG, Bonnez W, Rose RC, Koenig S, Demeter L, Suzich JA *et al*. A Phase 1 study of a recombinant viruslike particule vaccine against human papillomavirus type 11 in healthy adult volunteers. J Infect Dis 2001; 183:1485-1493.
12. Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB *et al*. A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 2002;347:1645-1651.
13. DeFilippis RA, Goodwin EC, Wu L, DiMaio D. Endogenous human papillomavirus E6 and E7 proteins differentially regulate proliferation, senescence, and apoptosis in HeLa cervical carcinoma cells. J Virol 2003; 77:1551-1563.
14. 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;347:1523-1527.
15. 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;6:3406-3416.
16. 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. 4:2103-2109.
17. 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;20:3456-3464 .
18. van der Burg SH, Kwappenberg KM, O'Neill T, Brandt RM, Melief CJ, Hickling JK *et al*. Pre-clinical safety and efficacy of TA-CIN, a recombinant HPV16 L2E6E7 fusion protein vaccine, in homologous and heterologous prime-boost regimens. Vaccine 2001;19:3652-3660.
19. Mendoza L, BubenikJ, Simova J, Jandlova T, Vonka V, Mikyskova R. Prophylactic, therapeutic and anti-metastatic effects of BMDC and DC lines in mice carrying HPV 16-associated tumours. Int J Oncol 2003;23:243-247.
20. Muller M, Zhou J, Reed TD, Rittmuller C, Burger A, Gabelsberger J *et al*. Chimeric papillomavirus-like particles. Virology 1997;234:93-111.
21. Nieland JD, Da Silva DM, Velders MP, de Visser KE, Schiller JT, Muller M *et al*. Chimeric papillomavirus virus-like particles induce a murine self-antigen-specific protective and therapeutic antitumor immune response. J Cell Biochem 1999;73:145-152.
22. Da Silva DM, Schiller JT, Kast WM. Heterologous boosting increases immunogenicity of chimeric papillomavirus virus-like particle vaccines. Vaccine 2003;21:3219-3227.
23. Berumen J, Casas L, Segura E, Amezcua JL, García-Carrancá A. Genome amplification of human papillomavirus types 16 and 18 in cervical carcinomas is related to the retention of E1/E2 genes. Int J Cancer 1994; 56:640-645.

24. Desaintes C, Demeret C, Goyat S, Yaniv M, Thierry F. Expression of the papillomavirus E2 protein in HeLa cells leads to apoptosis. EMBO J. 1997. 16:504-514.
25. Demeret C, García-Carrancá A, Thierry F. Transcription-independent triggering of the extrinsic pathway of apoptosis by human papillomavirus 18 E2 protein. Oncogene 2003;22:168-175.
26. Donnelly JJ, Ulmer JB, Shiver JW, Liu MA. DNA vaccines. Annu Rev Immunol 1997;15:617-648.
27. Cichutek K. DNA vaccines: development, standardization and regulation. Intervirology 2000;43:331-338.
28. Rocha-Zavaleta L, Alejandre J, García-Carrancá A. Parenteral and oral immunization with a plasmid DNA expressing the human papillomavirus 16-L1 gene induces systemic and mucosal antibodies and cytotoxic T lymphocyte responses. J Med Virol 2002;66:86-95.
29. Plummer M, Franceschi S. Strategies for HPV prevention. Virus Res 2002;89:285-293.