

# Vaccines against papillomavirus infections and disease

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Vacunas contra el virus del papiloma humano y cáncer cervical invasor.  
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## Abstract

Squamous cell carcinoma of the uterine cervix is the second cause of cancer-related deaths in women, the higher incidence being observed in developing countries. Infection with oncogenic types of human papillomavirus (HPV) is considered the major risk factor for the development of malignancies in the uterine cervix. However, HPV is considered to be a necessary but not sufficient cause for cervical cancer and, therefore, other factors contribute to the carcinogenic process, both present in the environment and from the host. Studies performed in animals, and more recently in humans, indicate that vaccination against the capsid proteins of the virus can prevent efficiently from infection. Furthermore, therapeutic vaccines are under investigation aiming the regression of papillomavirus induced tumors. The scientific basis for the development of papillomavirus vaccines and present status of clinical trials will be addressed in this chapter. This paper is available too at: <http://www.insp.mx/salud/index.html>

Key words: *papillomavirus*; vaccine; cancer of the uterine cervix; prophylaxis

## Resumen

El cáncer de células escamosas del cérvix uterino es la segunda causa de muerte relacionada con cáncer en mujeres en el mundo; la incidencia más alta se ha observado en países en desarrollo. La infección con tipos oncogénicos de virus de papiloma humano es considerado el factor de riesgo principal para el desarrollo de malignidad en el cérvix uterino. Sin embargo, el virus es considerado una causa necesaria pero no suficiente para desarrollo de cáncer cervical y, por lo tanto, existen otros factores en el ambiente y en el huésped que contribuyen al proceso carcinogénico. Estudios desarrollados en animales, y más recientemente en humanos, indican que la vacunación en contra de la cápside de las proteínas del virus puede prevenir eficientemente la infección en forma profiláctica; además, las vacunas terapéuticas están bajo investigación con el propósito de promover regresión de los tumores inducidos por virus de papiloma humano. Las bases científicas de las vacunas desarrolladas contra este virus, y el estado actual de los ensayos clínicos que se desarrollan en el ámbito mundial, se presentan en este artículo. Este artículo también está disponible en: <http://www.insp.mx/salud/index.html>

Palabras clave: cáncer cervical; virus de papiloma humano; vacunas

## Papillomavirus biology and immune response

Papillomaviruses are epitheliotropic viruses present in the skin and mucosa of several animals. In humans, more than 100 types have been described. Mucosal and

genital HPVs, consisting of about 30 types, are divided into low-risk (HPVs 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108) and high-risk (HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68, 73, and 82), according to their presence in malignant lesions of the cervix. The latter types have been adequately evaluated as high

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risk types in relation to invasive cervical cancer. For all of them, risk estimates were greater than 30 (range 35-350) strongly suggesting that these associations are causal in nature.<sup>1,2</sup>

The genome of these viruses is a double stranded DNA molecule of about 8000 base pairs, with three identified regions: a late region (L) containing two genes -L1 and L2- which encode the viral capsid proteins; an early region (E) encoding for proteins involved in viral DNA replication and control of viral transcription, such as E1 and E2, as well as the main transforming genes E6, E7 and E5; a long control region (LCR), containing several binding sites for nuclear and viral transcriptional factors as well as promotor sequences, is found between the L and E regions. HPV genomes are found as episomes in the nucleus of infected cells of the normal cervix, where infective viral particles can be isolated. However, in some low-grade and in most of the high-grade lesions of the uterine cervix, including cancer, HPV genomes are found integrated into the host genome.<sup>3</sup> A disruption of the E1-E2 region is required for HPV genome integration. This event results in an increased expression and stabilization of the E6 and E7 transcripts. The E6 protein from high-risk HPVs binds cellular p53, promoting the degradation of p53 by the cellular ubiquitin proteolysis system. The E7 protein interacts with pRB and inactivates this cellular protein. As a consequence, E2F transcription factor is released from pRB-E2F complex, leading to transcriptional activation of several genes involved in cell proliferation. Such interactions of HPV E6 and E7 proteins interfere with several pathways involved in the control of the cell cycle and DNA repair.<sup>4</sup>

Infection with high-risk HPV types is frequent among sexually active women, with incidence ranging from 15 to 40%.<sup>5</sup> When additional cervical specimens are taken from these women in follow-up surveys, the majority of the infections are found to be transient. However, a small proportion of infected women has persistent infection with high-risk HPV types. Previous reports have demonstrated that women persistently infected with oncogenic HPV types are more likely to develop malignant cervical lesions. Persistence of HPV is due mostly to their ability to evade the immune system.<sup>6</sup>

Infections with HPV are accompanied by an immune response that is both humoral and cellular.<sup>7</sup> Several studies have shown that serological diagnosis of HPV infection using genetically engineered HPV capsids (also known as Virus-Like Particles or VLP) correlate well with HPV DNA presence in cervical smears. The antibodies invoked recognize type-specific conformational epitopes present on VLPs, particularly against

the viral capsid protein L1, and the humoral response (IgG) against HPVs is stable over time. Neutralizing antibodies are generated, although frequently in low titers, and are considered to be host protective against further infection with that virus type. Moreover, HPV VLP ELISAs show sensitivities between 50 and 60%, very high specificities (>90%) and show good interlaboratory agreement. Therefore, VLP serology has been used as a marker of cumulative exposure to HPV, and sexual behavior, despite the observation that seroconversion may be delayed or never occur in a subset of women testing positive for HPV DNA. Overall, frequency and titer of several types of antibodies generated against HPV show a great variability that is dependent on the HPV type specificity, on the recognized epitopes, on the type of samples, and sensitivity of the assay. The mere definition of cutoff values can hinder comparison between different epidemiological surveys. Despite the fact that integration of HPV genomes impairs the expression of capsid antigens, stronger positive associations have been described for tumors of the anogenital region when compared to seropositivity in patients with epithelial cancers in other anatomical locations, consistent with the HPV DNA evidence.<sup>8</sup> Another source of misclassification could originate from different viral loads present in lesions or a differential immune response according to anatomical site. Concerning type specificity, it has been demonstrated that VLPs of each HPV type induce serum antibody response that are genotype-specific with the exception of HPV types 6 and 11 which are considered to be cross-reactive and HPV 31 and 45 who show low levels of cross-reactive antibodies against HPV 33 and 18, respectively.<sup>9,10</sup> Likewise, variants of HPV 16 are considered to belong to the same serotype.<sup>11</sup>

#### Development of papillomavirus vaccines. Animal models

Since the early 1990s, several groups have succeeded in generating recombinant L1 only or L1 and L2 virus-like particles (VLPs) from any papillomavirus known,<sup>12</sup> that resemble very closely the native virions but are devoided of the viral genome. VLPs produced in both prokaryotic (bacteria) and eukaryotic cells (yeast, insect, mammalian) reproduce the antigenicity of native virions by presenting conformational epitopes that have been shown to induce type specific virus neutralizing antibodies.<sup>12</sup> Moreover, VLPs have been shown to bind directly to dendritic cells leading to their activation and ultimately a potent T and B cell immune responses.<sup>13,14</sup> Although VLPs are the natural candida-

tes for immunogens, several other antigens, including structural and non-structural papillomavirus proteins have been tested in different protocols.<sup>15</sup> In addition, HPV early proteins such as E7, E6 and E2 have been fused to VLPs.<sup>16,17</sup> These chimeric VLP vaccines have been shown to induce neutralizing antibodies and to elicit CTL responses specific to the early viral protein in murine models, as mentioned below. Finally, recombinant virus, in different vectors, have been used as DNA vaccines, either alone or in combination with adjuvants.<sup>18</sup>

The animal models of papillomavirus infection have proved extremely valuable in the search and development of anti-viral vaccines both prophylactically and therapeutically,<sup>15,19,20</sup> Immunization with purified species-specific VLPs induces neutralizing antibodies that protect against live virus challenge in rabbits, cows and dogs. Among these models, the mucosal infections caused by COPV (canine oral papillomavirus)<sup>21</sup> and ROPV (rabbit oral papillomavirus)<sup>22</sup> are those that resemble more the human genital papillomavirus infections. Interestingly, in bovines, vaccines based on the same viral target can be effective either prophylactically or therapeutically, inducing regression of early lesions, thus challenging the concept that viral structural protein vaccines would provide protection from, and non-structural protein vaccines cure of, virus-induced lesions.<sup>19</sup> The duration of protection is long lasting, at least in rabbits<sup>23,24</sup> where neutralizing antibodies have been induced upon injection of CRPV VLPs. A COPV-VLP vaccine has been shown to efficiently prevent the development of mucosal lesions in dogs.<sup>25</sup> In Rhesus macaques, vaccination with an HPV-16 L1 VLP vaccine induced strong humoral, including neutralizing antibodies, and cellular immune responses.<sup>26</sup> Interestingly, a low level of neutralizing antibodies was generated when the vaccine was delivered as a DNA vaccine or by administration of an adenoviral vector expressing HPV 16 L1, indicating that the delivery system may interfere with the nature of the immune response. Non-human primates have also been used to test a chimeric VLP vaccine consisting of both HPV L1 proteins and fragments of proteins from Simian Immunodeficiency Virus (SIV) and Human Immunodeficiency Virus (HIV).<sup>27</sup> Systemic and mucosal administration of these HPV-SHIV VLPs generated responses against HPV L1, very weak antibodies or T cells responses to SHIV VLPs were only observed in macaques that were DNA primed before receiving the HPV-SHIV vaccines.

Although no papillomavirus is known to infect the regular laboratory mice, experimental models are available based on mouse cell lines transformed with high-

risk HPV capable of inducing tumors and metastasis. These models provide a mean to test the immune responses generated by different vaccination strategies and information is rapidly accumulating.<sup>18,28,29</sup> Moreover, these animal models have been used to test for different adjuvants and administration regimens, showing variable efficacies. Combined immunotherapy strategies include the use of BCG linked to HPV 16 E7,<sup>30,31</sup> as well as administration of allogeneic tumor cells expressing both the viral oncoproteins and granulocyte-macrophage colony-stimulating factor (GM-CSF).<sup>32</sup> This mouse model has been particularly useful in testing different administration routes: Intranasal and oral administration of HPV VLPs alone or in combination with different adjuvants has been shown to be immunogenic.<sup>33-35</sup> Recently, HPV 16 L1 capsomeres, administered both sub-cutaneously and intranasally, have been shown to induce a potent CTL response, comparable to that obtained with VLPs.<sup>36</sup> Similar results have been previously described for COPV-induced lesions in dogs.<sup>37</sup> Murine models have been instrumental to show that anti-specific HPV responses can be obtained by DNA immunization, which can both control tumor and metastasis development and promote tumor regression.<sup>38-41</sup> DNA vaccines have also been tested in rabbits<sup>42,43</sup> and dogs<sup>44</sup> with similar results. Altogether, the encouraging results obtained with different animal models have warranted the development of HPV vaccines in humans.

#### Vaccines to prevent human papillomavirus infection

Vaccines to prevent HPV infection aim to induce neutralizing antibodies directed to conformational epitopes of capsid proteins which most probably will be type specific. The protection against infection depends on the amount of antibodies produced by the host, its availability at the infection site and the persistence of the neutralizing antibodies along time. Analogous to the studies conducted with animals, VLPs for the most prevalent HPV types have been generated and tested as vaccine candidates in several ongoing clinical trials. Most of these studies are randomized, placebo-controlled, blinded trials, that include young women living in different countries around the World. So far, the published studies with prophylactic HPV vaccines in humans have shown that the vaccine is well-tolerated and generates strong immune responses that are several orders of magnitude higher than those exhibited by naturally infected populations. These safety and immunogenicity trials were done with VLPs against low-risk type 11 generated in insect cells<sup>45</sup> or yeast,<sup>46</sup>

as well as with high-risk type 16 VLPs produced in insect cells<sup>47</sup> or yeast.<sup>48</sup> The interim analysis of one of the trials has been recently published showing that women injected with HPV type 16 virus-like particles (VLPs) have been protected against infection when compared to the placebo group.<sup>48</sup> Moreover, during the 17-month period of follow-up, the few cases of cervical lesions attributed to this HPV type developed only in women that did not receive the vaccine. These results are very encouraging and constitute the proof-of-principle of the feasibility of this monovalent HPV vaccine developed by Merck, Sharp and Dohme. However, HPV-16 accounts for about 50% of all cervical cancers, and as mentioned earlier, the immune responses against HPV are type specific. Therefore, efforts are being made to develop multivalent vaccines that could control for most HPV infections associated with cervical disease namely HPV-18, -31, -33, -45, among other types. One of these studies is testing the safety and efficacy of a quadrivalent (HPV 6, 11, 16, 18) VLP vaccine. The study conducted in Brazil, USA, and several Nordic countries, followed more than 1 100 women for at least eight months.<sup>49</sup> Preliminary data indicate that women who received the vaccine had significantly higher amounts of HPV neutralizing antibodies than women who developed the infection naturally. It has also confirmed that the vaccine is well tolerated. Other trials are being conducted in different countries around the World and should enroll thousands of young women in the next few years. They include several phase III protocols aiming to demonstrate this vaccine's efficacy against HPV infections and cervical neoplasia. Although there are several questions to be answered, particularly concerning the efficacy and durability of the elicited immune response, it is reasonable to anticipate that the trials will succeed in demonstrating the value of VLP vaccines in the prophylaxis of HPV infections.

### Therapeutic vaccines

New therapeutic approaches to cervical cancer include immunization with early proteins and derived peptides of high-risk HPV, aiming to eliminate epithelial cells in the anogenital tract that are already infected with papillomaviruses. One modality of treatment that might achieve this would be immunotherapy, either alone or in conjunction with specific antiviral drugs.<sup>50-52</sup>

Natural immune responses to papillomavirus encoded antigens are weak, with the exception of the E7 protein, to which a humoral immune response is observed in most cases of invasive cervical carcinoma.

Immune response to the E2 and E6 proteins may also predict the regression of papillomavirus-associated disease. Thus, targeting immunotherapy to some or all of the HPV early proteins, particularly E6 and E7, is being considered for treatment of papillomavirus-induced neoplasia.<sup>53,54</sup> Immunogens include synthetic peptides, recombinant proteins, chimeric VLPs and live viral expression vectors. Pre-clinical studies indicate that these vaccines can eradicate HPV positive tumors.<sup>55,56</sup>

Although it has been possible to demonstrate that several papillomavirus early proteins are immunogenic in patients with HPV-associated cervical cancer, clinical trials of therapeutic vaccines have been conducted in patients with advanced stage of disease in whom a poor immune response is expected. Nevertheless, some positive results are available and partial responses have been obtained in patients with high-grade intraepithelial lesions.<sup>57,58</sup> Promising results have been obtained in patients with genital warts.<sup>59,60</sup> Clinical trials are underway to demonstrate the immunogenicity of a therapeutic DNA vaccine for anal dysplasia, consisting of an encapsulated plasmid bearing the sequences of HPV-16 E7 and multiple HLA-A2-restricted epitopes.<sup>61</sup> The potential use of dendritic cells expressing HPV antigens is under study.<sup>62-64</sup>

### Final considerations

Despite the encouraging results from several ongoing vaccine trials showing good safety profiles and induction of high titers of virus specific antibody by VLP-based papillomavirus vaccines, there are several open issues that require attention. Even if a vaccine is shown to be effective against a few HPV types, it is not known how long the protection will last and what will be the impact of this type of prevention in the natural history of cervical cancer. It is expected that a multivalent HPV vaccine will reduce the number of interventions such as colposcopies, biopsies and treatment of precursor lesions. However, without full population coverage, Papanicolaou-based screening procedures, in conjunction with the recently approved HPV DNA test for primary screening of cervical cancer in the USA, are likely to continue to play an essential role. Furthermore, we do not know if effective coverage against some genotypes could favor the emergence of more pathogenic types. The implications for HPV evolution as a large and diverse family of viruses ought to be considered.<sup>65</sup> Finally, the impact of various vaccination strategies in the prevention of cervical cancer, with a particular attention to its application in developing countries where an HPV vaccine is mostly

needed, has been a matter of discussion in several recent articles.<sup>66-68</sup> Concomitantly, there is much interest in the development of alternative papillomavirus vaccines, such as transgenic plants expressing papillomavirus proteins.<sup>69</sup>

Existence of tumor immune escape mechanisms puts therapeutic vaccines for HPV associated cancers in the same road as therapeutic vaccines for other cancers: so far, none have been shown to be completely effective. Development of multimodality treatments to induce strong immune responses and avoid escape from individual treatments are likely to contribute to the control of the precursors of cervical neoplasia of the uterine cervix and ultimately to invasive carcinoma.

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