

Immune Mechanism of Human Papillomavirus and Vaccine Protection

a report by

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Human papillomavirus (HPV) is the most common sexually transmitted infection in the US.¹ Current trends show that approximately 80% of women will become infected before 50 years of age.¹ Persistent infection with HPV is strongly associated with cervical cancer, which is the second most prevalent cancer in women worldwide. Other HPV-associated conditions include genital warts, recurrent papillomatosis, and other HPV-related cancers, including anal, vulvar, and vaginal cancer.² Cervical cancer affects an estimated 510,000 women each year.¹⁻⁴ In spite of advances in Papanicolaou testing, cervical cancer remains the major cause of death in women of reproductive age.¹⁻³ According to the World Health Organization (WHO), an estimated 288,000 HPV-related cervical cancer deaths occur each year, 80% of which are in women living in low-income countries.⁴ The highest estimated incidence rates have been shown for women living in sub-Saharan Africa, Melanesia, Latin America and the Caribbean, south-central Asia, and south-east Asia.²

Approximately 30–40 genotypes of HPV that infect the human genital tract have been identified.¹ These can be grouped into high-risk (oncogenic) or low-risk (non-oncogenic) types. High-risk types are firmly established as causative agents in cervical, vulvar, vaginal, anal, and penile cancers.¹ The high-risk genotypes HPV-16 and HPV-18 together account for 70% of all cervical cancers.¹ Low-risk or non-oncogenic genotypes cause anogenital warts, low-grade cervical dysplasia, and recurrent respiratory papillomatosis.¹ HPV-6 and HPV-11 are low-risk types, and account for 90% of genital warts.¹ In the US alone, an estimated 1 million new cases of genital warts are diagnosed per annum.¹ While most individuals with persistent HPV infections are generally healthy, impairment in immunological responses appears to be a risk factor for persistent HPV infections.⁵ Primary prevention measures appear to have the best hope for long-term effects on cancer incidence.

In June 2006, the first prophylactic HPV vaccine, Gardasil®, targeting four major disease-causing HPV types (HPV-6, 11, 16, and 18), was approved by the US Food and Drug Administration (FDA);¹ it has since been licensed for public use in more than 60 countries.⁵ A second vaccine, Cervix®, which protects against the two main high-risk HPV types (HPV-16 and 18), has also been developed.

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This article reviews the HPV growth cycle and highlights how prophylactic (and possibly future therapeutic) vaccines against HPV can prevent infection and reduce the incidence and worldwide burden of cervical cancer.

Overview of Human Papillomavirus

Viral Structure and Transmission

HPV is a circular, double-stranded DNA virus with a small genome approximately 8kb in length. The HPV genome is enclosed by a non-enveloped capsid and replicates its genome within the nuclei of infected host cells.^{6,7} The capsid has an outer protein coat that is made up of two different capsid proteins: major structural protein L1 and minor structural protein L2.³ More than 100 types of HPV have been identified to date.^{1,5,8} Of these, between 30 and 40 strains infect the human genital tract.¹

Infection with HPV occurs in basal keratinocytes, which become suprabasal differentiating keratinocytes. It is currently believed that abrasion or micro-trauma of cervical squamous epithelium facilitates virion access to the basal epithelium, primarily as a result of penetrative sexual intercourse.¹ HPV genomic replication and mature virion production are associated with host cell differentiation, and so are restricted to differentiating suprabasal keratinocytes.⁶ This stage in the HPV life-cycle is characterized by the expression of capsid genes L1 and L2.

The potential risk for infection from non-penetrative sexual contact remains undetermined. A prospective study by Winer et al. demonstrated that non-penetrative sexual activity was associated with HPV acquisition, albeit less frequently than penetrative sexual intercourse.⁹ However, owing to a lack of literature evidence, the possibility of viral transmission via non-penetrative contact remains a topic of debate.²

Viral Replication

It is believed that HPV gains access to cells by interacting with host cellular receptors that allow attachment and viral internalization.¹⁰ The host receptor(s) involved in mediating viral entry is/are currently unknown, although some studies have shown a putative role for heparin sulfates.¹⁰ Once inside the host cell, high-risk viral DNA may integrate into the host genome, an event that generally precedes progression to high-grade lesions or carcinomas.¹⁰ Otherwise, the genome exists in episomes and causes low-grade infections, or can be present in both integrated and episomal forms.^{6,10} However, once integration occurs, infectious virus is no longer produced. Thus, HPV does one of two things to the host: it either turns the host keratinocyte into a episome factory for more virus production, or it does not produce new virus and has the potential to kill the host. The HPV

oncogenes E6 and E7 play an important role by helping the oncogenic virus to evade the immune system and use the host cell replication machinery to replicate and survive.⁶

Viral Prevalence and Immune Response

Prevalence rates of HPV peak in the first few years following the initiation of sexual activity.¹¹ However, studies have shown that newly acquired infection is short-lived and ‘clears,’ i.e. becomes undetectable with standard methods, within one to two years in more than 90% of cases.¹² ‘Clearance’ suggests complete elimination of the infection, but it is not known whether the virus is actually cleared from all persons infected or rather becomes latent and persists at levels below the limit of detection; this latter theory is consistent with my personal view.^{11,12} Consistently, it has been postulated that latent or low-persistent HPV infections can re-emerge in older women, for example due to age-related immunological decline or in immunosuppressed women, such as AIDS patients and transplant recipients.¹¹

What is known is that HPV infections are typically transient by detection with standard methods except for a small subset of women who develop persistent infection.¹¹ Inadequate immunological control of high-risk HPV infections resulting in high viral persistence and older age are believed to be key determinants of risk of progression to cervical cancer.^{13,14} Moreover, women who are immunosuppressed by infection with HIV are at increased risk for infection with multiple types of HPV.^{11,15} Furthermore, an effective host immune response and the genetic make-up of the individual may be important determinants for the persistence and progression of HPV-induced cervical cancer.⁵

The majority of genital HPV infections are asymptomatic.² There is no viremia, no viral-induced cytolysis, and little inflammatory response to trigger innate or adaptive immunity.¹⁶ As indicated above, in the majority of infected women disease does not occur, indicating that in spite of the modest inflammatory response to infection, a successful cell-mediated (humoral) response likely occurs in most individuals.

Antibodies to the HPV major capsid (L1) protein occurs in some infected individuals within a few months of infection.^{12,16} Most HPV-infected individuals, however, do not develop antibodies, and those who do have low serum antibody levels: fewer than 40% of HPV-16 infected individuals tested are shown to be seropositive.¹⁷ It is noteworthy that current serological testing methods are relatively insensitive.¹⁶

A subset of infected women experience chronic persistent infection, which is consistent with an inadequate immune response.¹¹ There is, however, a lack of direct evidence associating host immunological response to risk for HPV persistence, and a few studies failed to demonstrate any link.^{11,18-20} There is also no convincing evidence that natural immunity provides complete or partial protection against other HPV types.

Introduction to Vaccines

Two HPV vaccines have been developed using recombinant DNA technology: Gardasil and Cervarix.² These vaccines are prophylactic and for maximum population effectiveness must be administered before sexual activity commences, i.e. to pre-pubescent females.²¹ The American College of Obstetricians and Gynecologists, as well as other advisory

groups, recommends vaccination to girls 11–12 years of age as part of a routine immunization schedule.¹

The vaccines are prepared using the L1 protein of the viral capsid, which can self-assemble into empty protein shells called virus-like particles (VLPs) when expressed in cells such as brewer's yeast.² VLPs act as immunogens and are able to induce high titers of neutralizing antibodies; they are not infectious because they do not contain any viral DNA.²⁵ Cervarix, a bivalent vaccine developed against HPV genotypes 16 and 18, is created using the L1 protein of the viral capsid by recombinant expression in Sf9 insect cells using a baculovirus vector, whereas Gardasil is a quadravalent vaccine against HPV-6, 11, 16, and 18 prepared from yeast (*Saccharomyces cerevisiae*).² Administering the vaccines involves three intramuscular injections over six months. This regimen is a challenge in developing countries. Developments of prophylactic L2 protein vaccines as well as therapeutic vaccines are currently being explored.

Efficacy Trials

Both prophylactic vaccines have been evaluated in randomized, placebo-controlled clinical trials, and both showed efficacy against persistent infection due to HPV types 16 and 18 in women.^{2,22} For the quadrivalent vaccine, four phase II and III double-blind, placebo-controlled studies have been conducted, which together randomized 20,887 women 16–26 years of age.²³⁻²⁸ A combined analysis of the four clinical trials was conducted by Ault et al.²⁵ The primary end-points measured were external genital warts, cervical intraepithelial neoplasia (CIN) of any grade, CIN grade 2/3, and adenocarcinoma *in situ* (AIS). At enrollment, 73% of women were HPV-type-naïve, but the remaining 27% were HPV-positive for at least one type (HPV-6, 11, 16, or 18), 7% of whom had evidence of exposure to more than one type; thus, 93% of subjects were naïve to at least three of the four types. Ault et al. reported 99% efficacy against HPV-16/18-related CIN 2/3 or AIS.²⁵ In this report, a further analysis of intention-to-treat populations also showed a significant reduction in CIN 2/3 and AIS lesions, even in women previously infected with at least one HPV type.²⁵ Of this subset of women, which included women with previous infections with one or more vaccine type, the quadrivalent vaccine showed 44% efficacy for HPV-16/18-related CIN 2/3 or AIS.²⁵ Similar efficacy was demonstrated against external genital warts. The authors concluded that although the quadrivalent vaccine strategy is of highest advantage in HPV-naïve young women, many women in the general population who have been exposed to one to three HPV types are likely to benefit from quadrivalent vaccination.^{1,25,29}

For the bivalent vaccine, phase III trials of 9,258 vaccinated women between 15 and 25 years of age showed a combined efficacy of 90.4% against HPV-16 and 18-associated CIN >2, and 93.3 and 83.3% efficacy, respectively, against HPV-16- and 18-related lesions.²² It should be noted, however, that the bivalent vaccine is not designed to protect against genital warts.¹

The protection mechanism exerted by the VLP vaccines remains undetermined; however, it is possible that both sustain protective antibody levels and that memory B cells play a role.³⁰

Cross-protection and Immunogenicity

In addition to efficacy against their formulated HPV subtypes, the two vaccines demonstrate some partial protection (cross-protection) against

other HPV types, including types related to HPV-16, such as HPV-31, 52, and others, and types related to HPV-18, such as HPV-45, 59, and others.² Multiple-type vaccines may therefore provide additional protection to young women.¹ Recent first-analysis data on cross-protection have shown that Gardasil offers some additional protection against 10 oncogenic HPV types (HPV-31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) not included in the vaccine.³¹ Efficacy of the 10 virus types against CIN 1–3 or AIS was 27%, and 38% against CIN 2/3 and AIS.³¹ Similarly, the bivalent vaccine has demonstrated six-month protection against persistent infection with HPV-45 and HPV-31, with 59.9 and 31.6% efficacy, respectively.^{1,22} The bivalent vaccine also offers 12-month partial protection against 12 combined infections with non-16 or non-18 HPV types (27% efficacy).^{1,22}

It is possible that additional HPV types in one vaccine will offer broader coverage, but too many types could lead to diminished returns, with insufficient antibody of any one type to be effective.^{16,32} Trials of polyvalent vaccines are under way to assess the optimal number of HPV types for one vaccine.^{16,32}

The maximum duration for disease protection has not been determined.² In a report by Villa et al., the quadrivalent vaccine was effective for five years.^{30,33} According to a study by Olsson et al., a challenge dose of quadrivalent vaccine after five years in a subset of 241 previously immunized women resulted in a marked increase in antibody titer levels.³⁰ At present, it is unclear whether a booster vaccination would also be

needed; in my opinion, a booster vaccine would not be required for at least 10–15 years after the initial vaccination schedule.

Conclusion

Worldwide, cervical cancer is the second most common cancer in women. Women living in developing countries bear the greatest burden in terms of morbidity and mortality, because these women do not have access to the cervical screening programs that are available in more affluent countries. Cervical cancer is one of the first cancers shown to be solely caused by a virological agent, namely oncogenic genotypes of HPV. Molecular biological tools have helped to define the epidemiology, natural history, and causative link of HPV with cervical cancer, and have also helped in the establishment of effective and safe prophylactic vaccines. The recently developed cervical cancer (or HPV) vaccines based on VLP technology have the capacity to reduce a great proportion of the burden from cervical cancer worldwide. The vaccines have a very high efficacy (almost 100%) against the related HPV-16 and HPV-18 vaccine types in people who have not yet been infected with these types. In addition, the quadrivalent vaccine provides a very high degree of protection against anogenital warts. However, cervical cancer screening will still be necessary in vaccinated women, as current vaccines cannot provide complete protection against all oncogenic types. Challenges to success include the high cost of the HPV vaccines, endorsement by governments and policy-makers, and educational efforts. Cervical cancer should now be considered a vaccine-preventable disease. ■

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