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Review Article

Current and Next-generation Vaccines against Human Papillomaviruses

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Human papillomaviruses (HPVs) infect the squamous epithelium, and cause skin warts, genital warts and cancers, including uterine cervical cancer. Amongst the enormously diverse types of HPVs, HPV16 and HPV18 are most prevalent and responsible for approximately 70% of cervical cancer cases. Current preventive HPV vaccines contain virus-like particles which are composed of L1 major capsid proteins of HPV16 and HPV18. Although bivalent and quadrivalent vaccines exhibit excellent preventive efficacy and safety, they have several limitations. First, since the protection against HPV is type-restricted, the remaining 30% of cervical cancers and warts cannot be prevented. Second, due to the absence of therapeutic activity in the vaccines, people already infected by the HPVs cannot benefit from the current vaccines. Therefore, new preventive and therapeutic vaccines are required for better control of HPV-associated diseases. New developments include a novel nonavalent preventive vaccine that contains five additional cancer-associated HPV types, and it has been tested and approved in 2014. Recently, several groups reported promising clinical results with novel therapeutic HPV vaccines. This review provides an overview of the success of current preventive vaccines and perspectives on the next-generation HPV vaccines.

Key Words: Papillomavirus vaccine, Uterine cervical neoplasm, Sexually transmitted disease

I. INTRODUCTION

Human papillomaviruses (HPVs) are DNA viruses that infect and replicate in the squamous epithelium of skin and mucous membranes. HPVs are etiologically linked to benign and malignant diseases of skin and mucosal epithelia, such as skin warts, genital warts and anogenital cancers. Of the 12,700,000 new cancers that occurred in 2008 worldwide, 610,000 cases (4.8%) were attributable to HPV infection (1, 2). Notably, almost every uterine cervical cancer is caused by HPV infection, and it is the third leading cause of female cancer deaths in less developed countries (3). Vaccines targeting HPV-associated cervical cancers have been developed, and contributed to the decrease in prevalence of HPV-associated diseases in many high-income countries. However, current HPV vaccines have several limitations in the prevention of cervical cancers, and other HPV-associated diseases which are not covered by the current vaccines exist.

This review provides a brief overview of HPV-associated diseases, the successes and limitations of current first-generation preventive HPV vaccines, and perspectives on the next-generation preventive HPV vaccines and therapeutic HPV vaccines.

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II. HPV and HPV-associated Diseases

HPVs are non-enveloped capsid viruses that have double-stranded circular DNA genomes. The icosahedral capsid encloses an approximately 8 kbp genome that encodes six early proteins (E1, E2, E4, E5, E6 and E7) and two late structural proteins (L1 and L2) (4). Among them, E6, E7, L1 and L2 proteins are important with regards to carcinogenesis and vaccine development. E6 and E7 are HPV oncoproteins which inhibit activities of the tumor suppressor genes P53 and RB, respectively, and L1 and L2 are major and minor capsid proteins, respectively (4). The icosahedral capsid of HPV is composed of 72 capsomeres which consist of an L1 pentamer and an L2 monomer. These two structural proteins are major targets of vaccines for developing neutralizing antibodies.

There are more than 200 types of HPVs (5), each with distinct tissue tropisms, and they can be divided into mucosal HPVs or cutaneous HPVs (Table 1). Mucosal HPVs can be further subdivided into high-risk types and low-risk types based on their association with cancer (6). Twelve types of high-risk mucosal HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) are classified as Group 1 carcinogen by International Agency for Research on Cancer (WHO/IARC) (4). Most HPV infections are subclinical and transient, with 90% of people clearing infection within 24 months (7, 8). However, persistent infection with high-risk HPV types may cause the development of cancers. Those are uterine cervical, penile, vaginal, vulvar, anal, and oropharyngeal cancers. Especially, uterine cervical cancer is the third most common female malignancy worldwide, and is responsible for 275,000 deaths annually (1). HPV infection often causes low-grade cervical dysplasia: cervical intraepithelial neoplasia (CIN) 1, which usually clears spontaneously (60% of cases) and rarely progresses to cervical cancer (1%). However, persistent infection with high-risk HPV types can cause high-grade cervical dysplasia (CIN 2, 3), where a lower percentage of high-grade lesions spontaneously clear (40% and 33% for CIN 2 and 3, respectively), and a greater proportion progress to invasive cervical cancer if left untreated (5% and >12% for CIN 2 and 3, respectively) (9~11). Infection with HPVs is considered as a prerequisite for uterine cervical cancer (12), and almost all cases are attributable to high-risk HPV types (13). Moreover, HPV infection is etiologically associated with 88% of anal cancers, 70% of vaginal cancers, 43% of vulvar cancers, 50% of penile cancers, and 13~56% of oropharyngeal cancers (1).

Low-risk mucosal HPVs induce genital warts (condyloma acuminatum), which are benign hyperproliferative lesions. Anogenital warts are one of the most common sexually transmitted diseases. In a systematic review, the worldwide annual incidence of anogenital warts ranged from 160 to 289 per 100,000 persons (14). More than 90% of genital warts are caused by HPV6 and HPV11 (Table 1). Genital warts are generally benign and resolve within 6 months. However, about 30% of genital warts recur, which can result in lengthy, expensive, painful treatments, and substantial psychological burden (9).

Cutaneous HPVs induce skin warts, benign tumors of the cutaneous epithelia. Nineteen HPV types are frequently found in skin warts, with HPV1 being most prevalent, followed by HPV27, HPV57 and HPV2 (15) (Table 1). Skin warts are common in children, but they may also constitute

Table 1. Common HPV types and HPV-associated diseases

	Most common types	Less common types
Mucosal HPVs		
High-risk (cervical cancer)	16, 18	31, 33, 35, 39, 45, 51, 52, 56, 58, 59
Low-risk (genital warts)	6, 11	30, 42, 43, 44, 45, 54, 61, 70, 72, 81
Cutaneous HPVs (skin warts)	1, 2, 27, 57	3, 6, 7, 10, 11, 28, 29, 40, 41, 43, 63,77, 91, 94, 117

Table 2. Overiview of approved preventive vaccines

	Cervarix	Gardasil	Gardasil 9
HPV types	16, 18	6, 11, 16, 18	6, 11, 16, 18, 31, 33, 45, 52, 58
Producer cells	*Trichoplusia ni* insect cells	*Saccharomyces cerevisiae* yeasts	*Saccharomyces cerevisiae*
Adjuvant	500 μg aluminum hydroxide + 50 μg monophosphoryl lipid A	225 μg aluminum hydroxyl-phosphate sulfate	500 μg aluminum hydroxyl-phosphate sulfate

a significant burden for immunocompromised adults. For example, organ transplantation recipients often suffer from confluent skin warts occurring at multiple body sites. There are numerous treatment options for skin warts such as cryotherapy and electrodesiccation, however, no single treatment has been proven as completely curative (16, 17). Similar to genital warts, treatment for skin warts can be lengthy, expensive, and painful, and recurrence is common.

III. Current Preventive Vaccines Containing of L1 Virus-like Particles

1. Success of current preventive HPV vaccines

The most common high-risk HPV types associated with cancers are HPV16 and HPV18, which are responsible for approximately 70% of uterine cervical cancers. Two first-generation preventive HPV vaccines targeted these two most oncogenic virus types. Gardasil (Merck & Co) and Cervarix (GlaxoSmithKline) were licensed in 2006 and 2007 respectively, and have been widely used primarily in high-income countries (18). The development of these vaccines was made possible with the observation that recombinant L1 (the major capsid protein), when overexpressed, spontaneously self-assembles into virus-like particles (VLPs) that structurally resemble the papillomavirus virions but lack genomic material (19). Cervarix is a bivalent vaccine which contains L1 VLPs of HPV16 and HPV18 produced in *Trichoplusia ni* insect cell lines infected with recombinant baculovirus vectors, and can prevent 70% of uterine cervical cancers caused by HPV16 and HPV18. Gardasil is a quadrivalent vaccine which contains L1 VLPs of HPV6, HPV11, HPV16 and HPV18 produced in yeasts (*Saccharomyces cerevisiae*), and can prevent more than 90% of genital warts caused by HPV6 and HPV11 in addition to the prevention of cervical cancers induced by HPV16 and HPV18. Both vaccines contain an aluminum salt adjuvant, and Cervarix additionally contains monophosphoryl lipid A, which activates the Toll-like receptor 4 (20) (Table 2).

Several multicenter trials established efficacy and safety of the bivalent and quadrivalent HPV vaccines (21~23). Pooled end-of-study analysis of phase III clinical trials for 3-doses program of both vaccines revealed a 100% efficacy in preventing HPV16/18-related CIN 3 high-grade precancerous cervical lesion in young women who were negative to those HPV types at the time of vaccination (21, 24). Additionally, the quadrivalent vaccine exhibited 96.4% efficacy in preventing HPV6/11-related genital warts (24). The main effectors of protection are understood vaccine-induced IgG antibodies. The administered L1 VLPs induce high titers of HPV-specific serum antibodies in vaccinated individuals, and the *in vitro* virion neutralization ability of those antibodies was demonstrated by using the sera from vaccinated people (25, 26). The observation that passively transferred sera from L1 VLP-vaccinated animals protect naïve animals from experimental infection with corresponding HPV further substantiated the role of L1-specific antibodies in the protection (20, 27, 28). In clinical trials of the two vaccines, the vaccine-induced antibody levels are substantially higher than the antibody levels induced by natural HPV infection (29, 30). Furthermore, the protection efficacy did not wane for several years. In a long-term follow-up study for 113 months (9.4 years), all bivalent-vaccinated people sustained HPV16/18-specific serum antibody levels several-fold higher than natural infection levels

(31), and no HPV16/18-associated infections and diseases occurred during this follow-up period in the vaccine group. Additionally, the safety of the two preventive vaccines has been proven in various clinical trials (21, 32~37). In a pooled analysis of completed/ongoing clinical studies and post-licensure surveillance, no differences were seen in the incidence of the serious adverse events between vaccine and control groups for either vaccine (38, 39). Initial concerns about potential effects on pregnancy outcomes were dispelled by the results of pre-licensure and post-licensure studies. The analysis showed no evidence of a rise in miscarriages in vaccinated women. In addition, post-licensure pregnancy registries have not identified any adverse signals related to either vaccine (38, 40, 41).

Based on the excellent results in clinical trials, the quadrivalent and the bivalent vaccines were licensed in 2006 and 2007 respectively. Although the efficacy trials were conducted in 15~26 years old women, both vaccines were licensed for women aged from 9 years to 25 or 26 years. Bridging immunogenicity studies in girls and boys aged 9~14 years contributed to the licensure for this extended range of subjects. In the bridging immunogenicity studies, the antibody responses in subjects aged 9~14 years were found not to be inferior, but superior to those of young women aged 15~26 years (42, 43). The quadrivalent vaccine was later also licensed for boys and young men, and the USA and Australia have included boys and young men in their national immunization programs in 2011 and 2013, respectively. By 2014, more than 57 countries had introduced the HPV vaccine into their national immunization programs (41). In South Korea, HPV is one of the optional immunization pathogens along with rotavirus (44). Within a few years of the introduction of the vaccines, evident positive effects of HPV-associated outcomes can be observed by reduced cases of cervical lesions, genital warts, and HPV prevalence. Australia is one of two countries that introduced HPV vaccination shortly after the licensure and achieved high coverage. In Australia, the incidence of high-grade cervical dysplasia (CIN 2 and 3) decreased by 38% in girls younger than 18 years old (45), and the incidence of genital warts diminished by 59% in women aged 12~26 years (46). Interestingly, genital warts in heterosexual Australian resident men aged 12~26 years also declined by 39%. This unexpected decrease may be a result from the indirect protective effects provided by high coverage of HPV vaccination in young Australian women to heterosexual men, thereby providing herd immunity (46). On the other hand, the incidence of HPV-associated disease did not decrease in older women or homosexual men whom are not affected by the immunization program (20). HPV prevalence was also affected by high-coverage vaccination; the vaccine-type HPV prevalence declined from 28.7% to 6.7% in Australia (47).

2. Limitations of current preventive HPV vaccines

As reviewed above, the current quadrivalent and bivalent preventive HPV vaccines are highly immunogenic and effective in preventing HPV-associated diseases. However, these vaccines have several limitations. Firstly, protection from HPV infection and disease is type-restricted. As mentioned earlier, there are more than 200 HPV types, and these various HPVs express distinct L1 major capsid proteins. Therefore, VLPs composed of a certain type of HPV L1 proteins elicit antibody response only to the specific HPV type. Although partial cross-reactivity against non-vaccine-targeted, but closely related HPV types was observed (26, 48~50), the antibody titers were significantly lower than type-specific titers, and cross-protection seems to wane over time (51, 52). Current HPV vaccines targeted two most common HPV types which cause uterine cervical cancer, which means approximately 70% of uterine cervical cancers can be prevented by L1 VLPs of HPV16 and HPV18 contained in the bivalent and quadrivalent vaccines, and more than 90% of genital warts can be prevented by L1 VLPs of HPV6 and HPV11 contained in the quadrivalent vaccine. However, the other 30% of cervical cancers which are induced by other high-risk HPV types cannot be prevented by the current vaccines. Likewise, up to 10% of HPV-associated genital warts remain a possibility. Additionally, current vaccines only deal with mucosal HPV types, and did not consider the cutaneous HPVs which cause recurrent skin warts. The second limitation is that the current HPV vaccines do not have therapeutic activity. Therefore, people

who have already been infected by HPV cannot benefit from the current vaccines. Another limitation is that high costs of current vaccines prevent their widespread use in less developed countries. More than 85% burden of uterine cervical cancer occurs in less developed countries. However, current HPV vaccines are the most expensive vaccines to have been developed, and, low-income countries have difficulty supporting the high cost of the HPV vaccines (53).

IV. Next-generation Preventive Vaccines with Extended Coverage of HPV Types

As mentioned previously, the current bivalent and quadrivalent vaccines contain the VLPs yielded by self-assembly of recombinant L1 major capsid proteins. The bivalent vaccine (Cervarix, GlaxoSmithKline) contains L1 VLPs of HPV16 and HPV18, and the quadrivalent vaccine (Gardasil, Merck & Co.) contains L1 VLPs of HPV6, HPV11, HPV16 and HPV18. Although both vaccines can specifically prevent 70% of uterine cervical cancers associated with HPV16 and HPV18, extended coverage of the other 30% of cervical cancers is required. Recently, Merck & Co. developed a nonavalent HPV vaccine (Gardasil 9) with the L1 VLP technology which is utilized in the production of current HPV vaccines. In addition to L1 VLPs contained in their quadrivalent vaccine (Gardasil), the nonavalent vaccine contains L1 VLPs of five additional high-risk HPV types (31, 33, 45, 52, and 58) (Table 2). In phase III clinical trial, the nonavalent vaccine elicited non-inferior antibody response to the four shared VLPs compared to that of the quadrivalent vaccine, and the nonavalent vaccine prevented 97% of high-grade cervical dysplasia caused by the five additional HPV types (52, 54). Therefore, Gardasil 9 was licensed for use by the FDA in December 2014 (52). Although Gardasil 9 may prevent 90% of cervical cancers theoretically, up to 10% of cervical cancers associated with other high-risk HPV types are still unpreventable by the new vaccine.

An alternative strategy to increase the coverage of prevention is to target epitopes which are more conserved between HPV types (55). In contrast to the type-restricted protection provoked by L1 VLPs, the minor capsid protein L2 immunogen can induce broad cross-neutralizing antibodies (56~58). Though the induced type-specific antibody titers are lower than are those induced by L1 VLPs (59), L2 immunogen can induce immunity to type-common shared epitopes (60). The immunogenic N-terminal region of L2 has considerably conserved amino acid sequences between the high-risk oncogenic HPV types (61). Especially, the L2 domain encompassing amino acid 17~36 was identified as a highly conserved epitope, RG-1 epitope, named after a neutralizing monoclonal antibody (RG-1) which binds to this region (62), and this RG-1 epitope has been used in a majority of experimental L2 vaccine designs (55). For the development of L2-based HPV vaccines with high immunogenicity, several strategies were used. One is the use of L1 VLPs as a display scaffold (63~65). For example, Schellenbacher *et al*. (66) generated HPV16 L1 VLPs in which a surface loop of L1 is genetically replaced by the RG1 epitope of L2 protein. When administered with adjuvants, the chimeric VLPs induced robust L2-specific antibodies, which cross-neutralized numerous types of high-risk mucosal HPVs, low-risk mucosal HPVs, and cutaneous HPVs *in vitro*. *In vivo*, mice were efficiently and endurably protected against experimental vaginal challenge with the mucosal HPV types (60). A similar strategy used to raise the immunogenicity of L2 epitopes is to display them on non-HPV VLPs ranging from plant viruses to bacteriophages (67~70). Since the bacteriophage VLPs can be produced in bacterial expression systems, such as *E. coli*, it has advantage of reducing the production cost (55). Another method is genetic engineering and producing L2-based fusion proteins. Jagu *et al*. genetically ligated L2 epitopes (amino acids 11~88) from multiple HPV types to make a multimeric fusion protein (concatemer). These concatenated L2 immunogens induced antibodies that neutralize a broad range of HPV types and provide cross-protection against cervicovaginal HPV challenges in mice (71). Other methods include fusing L2 epitopes to a variety of adjuvants, such as synthetic Toll-like receptor 2 agonistic lipopeptide (57), bacterial thioredoxin (72), bacterial flagellin (a Toll-like receptor 5 agonist) (73), or a surface protein of *Lactobacillus casei* (74).

Table 3. Platforms of therapeutic HPV vaccines

Platforms	Strength	Weakness
Live vector-based	High immunogenictiy	Potential infection risk
Peptide/Protein-based	Safe, stable and easy to produce	Low immunogenicity
DNA-based	Safe, stable and easy to produce	Low immunogenicity
Whole cell-based	High immunogenictiy	Labor-intensive and costly

V. Therapeutic HPV Vaccines

Current commercial preventive vaccines are effective only in HPV-naïve individuals, and do not act therapeutically in recipients who already are positive for HPV infection at the time of vaccination (21, 24, 52, 75). Development of therapeutic HPV vaccine is required for the non-invasive and non-surgical control of established HPV infections, either by inducing regression of neoplastic lesions or inhibiting progression of precancerous lesions to cancers. In contrast to the preventive vaccines which act by eliciting antibody responses, therapeutic HPV vaccines have to generate cell-mediated immune responses utilizing $CD8^+$ cytotoxic T lymphocytes which actively destroy HPV-infected cells (76). Since the late capsid proteins (L1 and L2) are expressed only in terminally differentiated epithelial cells (77), late protein-targeted immune responses cannot be expected to attack the basal epithelial cells in which the HPV infection is maintained (20). Therefore, therapeutic HPV vaccines should target HPV-encoded early proteins that are expressed throughout the layers of epithelium. Particularly, the E6 and E7 oncoproteins are the major targets of research for development of therapeutic HPV vaccines.

Several forms of experimental therapeutic HPV vaccines have been developed (76, 78, 79) (Table 3). 1) Live vector-based vaccines utilize viruses or bacteria to deliver the HPV antigen to antigen-presenting cells, including dendritic cells. 2) Peptide/Protein-based vaccines directly administer peptides or proteins derived from HPV antigens to the recipients. Then antigen-presenting cells uptake the peptides or proteins for antigen presentation to T cells. 3) DNA-based vaccines deliver plasmid DNA that contain HPV protein-coding gene into host cells. The transfected cells then express the HPV antigen. 4) Whole cell-based vaccines primarily utilize dendritic cells. These cells are pulsed with HPV antigenic peptides, proteins, or DNA *ex vivo*. Then HPV epitope-loaded dendritic cells are re-administered into the body.

Although numerous experimental therapeutic HPV vaccines have been tested in clinical trials, few of them exhibited significant clinical success (80). However, several recent therapeutic vaccines have provided promising results. Kenter *et al*. (81) tested a peptide-based HPV vaccine in women with HPV16-positive, grade 3 vulvar intraepithelial neoplasia (VIN 3). Patients were vaccinated with a mixture of synthetic long peptides from the HPV16 proteins E6 and E7 in incomplete Freund's adjuvant. Complete regression of lesions was observed in 9 of 19 patients (47.4%). Recently, Kim *et al*. (82) reported successful clinical results with a DNA-based vaccine (GX-188E) in CIN 3 patients. Through the co-expression of Fms-like tyrosine kinase-3 ligand (Flt3L) with HPV E6/E7 antigens, GX-188E facilitated the processing and presentation of HPV epitopes by dendritic cells. Seven out of nine patients (77.8%) achieved complete regression of their lesion and HPV clearance. Rosales *et al*. (83) reported successful phase III clinical trial results with a live vector-based HPV vaccine (MVA E2). MVA E2 is a recombinant vaccinia virus which contains the HPV E2 antigen gene. MVA E2 virus particles were delivered directly into the uterus of patients with high-grade CIN, and 220 of 300 patients (73.3%) exhibited complete regression of their lesion.

VI. CONCLUSION

Bivalent and quadrivalent HPV vaccines were developed and widely used primarily in high-income countries from 2006. Through the excellent preventive efficacy, current vaccines significantly diminished the incidence of vaccine type-related uterine cervical lesions and genital warts. They also decreased the prevalence of vaccine-type HPVs. Therefore, the eradication of specific high-risk HPV types from the general population is not impossible in a high coverage both-sex vaccination scenario (32). However, due to the type-restricted protection, current vaccines cannot prevent the remaining 30% of cervical cancers caused by minor high-risk HPV types other than HPV16 and HPV18. The recently approved nonavalent vaccine (Gardasil 9) can prevent 90% HPV-associated cervical cancers through the addition of five VLPs of high-risk HPV types. Experimental L2-based vaccines may further extent the coverage by inducing immunity to type-common L2 epitopes. The absence of therapeutic activity is another deficit of the current commercial preventive vaccines. Although numerous experimental therapeutic vaccines failed to exhibit clinical success, recent promising clinical results reported by several groups may realize the use of therapeutic HPV vaccines in near future. Lastly, a novel production technology is required to lower the cost of HPV vaccines, thereby widely providing the vaccines to low-income countries where the control of HPV-associated diseases is highly required.

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