Vaccine (2008) **26**, 2669–2679

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journal homepage: www.elsevier.com/locate/vaccine

REVIEW

Prospects and prejudices of human papillomavirus vaccines in India

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Received 13 October 2007; received in revised form 5 March 2008; accepted 12 March 2008
Available online 14 April 2008

KEYWORDS
Human papilomavirus;
Cervical cancer;
Vaccine

Summary Cervical cancer is the most common cancer and a leading cause of cancer deaths among women in developing countries. The disease is caused due to persistent infection of one or more of about 15 high-risk human papillomaviruses (HR-HPVs), most commonly by HPV types 16/18. In India, over 98% of cervical cancer cases harbor HPV infection and HPV 16 is the type exclusively (80–90%) prevalent. Unlike the West, HPV infection is most common in women in their third decade (26–35 years) of sexual activity and invasive cancer also arises much later with a peak at about 45–55 years of age. Recently, two successful prophylactic HPV vaccines, a quadrivalent (HPV16/18/6/11) 'Gardasil' by Merck and a bivalent (HPV16/18) 'Cervarix' by GSK have been developed. Several other approaches including plant-based edible, pentameric capsomere-based intranasal and DNA-based vaccines have also been employed to develop prophylactic vaccines. Also, several therapeutic vaccines either protein/peptide based or DNA based are in clinical trials but are yet to establish their efficacy. Though there are several issues regarding implementation of the already developed vaccines in resource limited countries, efforts are being made to develop cost-effective second-generation vaccines. If cost minimized, HPV related new technologies involved in screening tests and vaccines are expected to reduce incidence of cervical cancer and deaths it causes in women from developing countries.

Contents

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doi:10.1016/j.vaccine.2008.03.056

Introduction

With the recent US Food and Drug Administration (FDA) and the European Union approval for the quadrivalent HPV vaccine 'Gardasil' and the possible licensing of the bivalent HPV vaccine 'Cervarix' in the near future, the prospects of preventing the second most common cancer among women are promising. But along with this promise, these vaccines have raised a number of important concerns and issues with specific relevance to the developing world where most of the cervical cancer cases occur. Here we discuss the socio-cultural and economic issues of implementation and effectiveness of these vaccines in developing and resource-poor regions of the world along with the future prospects of second-generation HPV vaccines and the worldwide HPV vaccination program.

Papillomaviruses and cancer

The scientific journey that started with the understanding of the infectious nature of skin warts and papillomas at the dawn of the 19th century has come a long way to well-established fact of papillomavirus as tumor virus, their association with human cancers and the recent development of vaccines against human papillomavirus.

It was in early 1980s, when it was discovered that there are novel HPV types associated with cervical cancer, other anogenital cancers and their precursors [1]. This led to enormous boost in research activities in the field. The major credit for this goes to the German scientist Harald zur Hausen who cloned two most important high-risk HPV types 16 and 18 [2] and showed for the first time the profound association between human papillomavirus infection and cervical cancer [3]. The association is well established by a large number of clinicoepidemiological, molecular and experimental studies on HPV. It has been also revealed that HPV is not only associated with cervical cancer but also with a sizable number of oral, esophageal and other anogenital cancers namely cancers of the vulva, vagina, penis and anus [4—8]. Further, research in early 1990s towards understanding immunological aspects of HPV and its pathogenesis has lead to the development of strategies towards developing vaccine against oncogenic HPVs. With this scientific journey of papillomavirus research for about a century is now started bearing fruits in the shape of possible prevention and control of cervical cancer through vaccination.

Cervical cancer and human papillomavirus infection

Cancer of the uterine cervix is the second most common cancer among women worldwide. There are estimated 493,000 new cases and 274,000 deaths due to cervical cancer in 2002 with more than 80% cases occurring in developing countries [9]. In India, cervical cancer is a leading cancer among women with annual incidence of about 130,000 cases and 70—75,000 deaths [10]. Thus India shares about one fourth of the global cervical cancer burden. A large number of risk factors are known to contribute to high incidence of this disease but most important of them are early age of marriage (<18 years), multiple sexual partners, multiple pregnancies, poor genital hygiene, smoking, use of oral contraceptives, religion, ethnicity, etc. [11]. But the most important factor has been considered to be the infection of human papillomaviruses (HPVs). The availability of organized screening by cytological Pap test has substantially reduced the cervical cancer burden by about 70—75% in developed countries during the past four decades [12]. In contrast, there is inadequate or complete non-existence of screening in major parts of India and majority of cases are often detected at late stages. Interestingly, before introduction of screening programs in 70s, the incidence of cervical cancer in most of the developed nations was almost similar to that of developing countries.

Several large epidemiological studies carried out world over have showed presence of HPV in as high as 99% of cervical tumors and established association of certain specific HPV types as the necessary cause of progression to cervical cancer and are termed as high-risk HPV types [13]. In addition, there are several cofactors that may be necessary for progression of HPV infected cervical lesions to cancer. Besides, the role of host cellular and genetic factors, immunodeficiency, HPV variants, viral load and viral integration are also considered to be important during progression and development of cervical cancer. Natural history and biological behavior of HPV infection and cervical lesions along with the possible role of other factors are presented in Fig. 1.

Till date, more than 111 genotypes of HPV have been described, but only about 30 of them are associated with anogenital cancer. Mainly HPV types 16 and 18 are considered as most prevalent ''high risk'' types for cervical cancer while HPV types 6 and 11 are considered to be the most prevalent low-risk types associated with benign lesions and genital warts. There are at least thirteen more high-risk HPV types (31,33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82) and three probable high-risk types (26, 53, 66) [14]. HPV

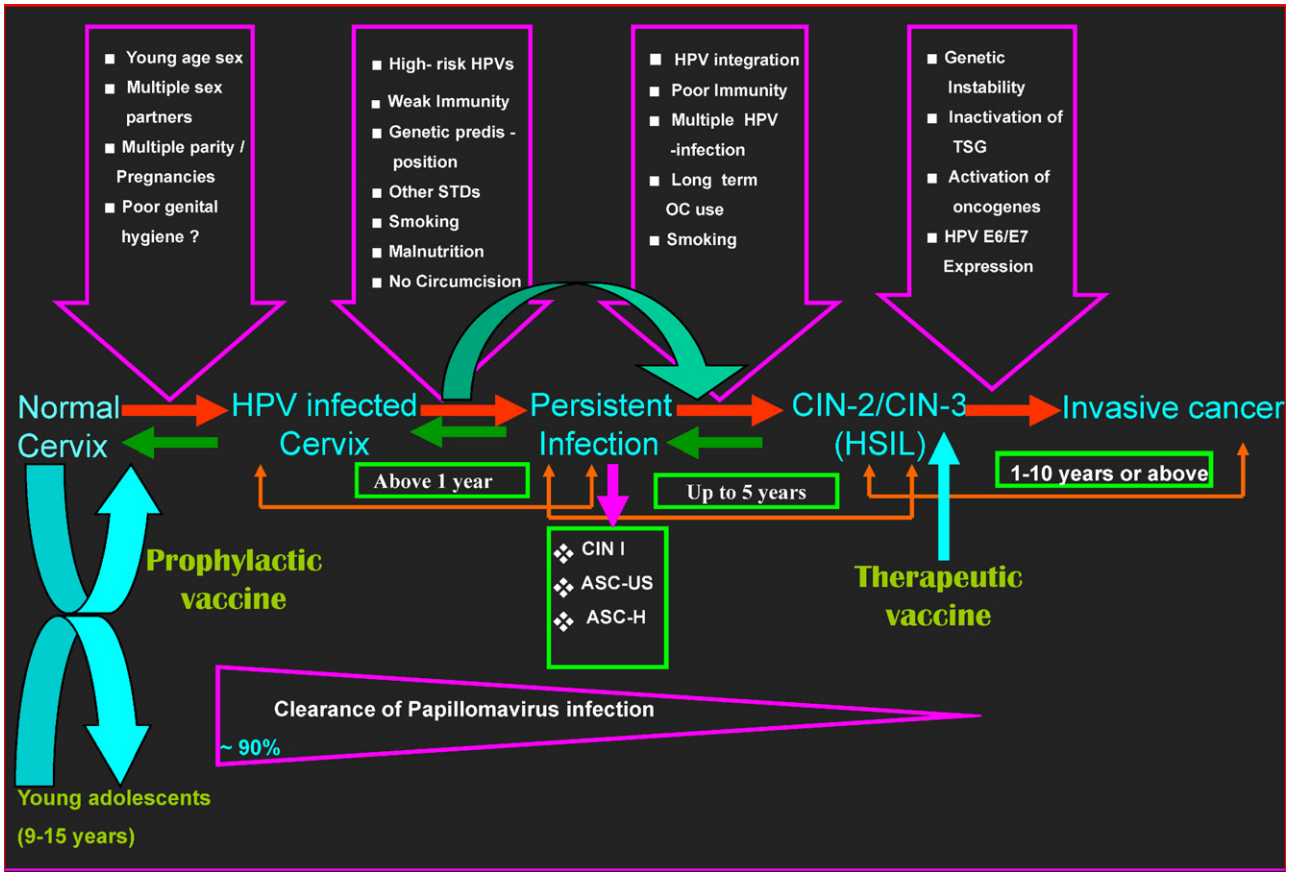

Figure 1 Biological behavior of HPV infection and development of cervical cancer.

16/18 is estimated to account for more than 80% of invasive cervical cancer including CIN 3, vulvar intraepithelial neoplasia (VIN) 2/3 and for 50% of CIN 2 lesions [15]. In India, 85—90% cervical cancer cases are squamous cell caricinoma but only 10—15% cases are adenocarcinoma. Interestingly, in India HPV 16 is the most prevalent type both in squamous cell carcinoma as well as adenocarcinoma while global reports indicate preferential occurrence of HPV 18 in adenocarcinoma [16,17]. Altogether HPV types 16, 18, 45, 31, 33, 35, 52, 58 are responsible for about 90% of all cervical cancers worldwide [18,19]. Of low-risk HPV types, HPV 6 and 11 are most prevalent, but occurrence of other low-risk types such as HPVs 40, 42, 43, 44, 54, 61, 70, 72 and 81 has also been reported. Though Bosch et al. [20] demonstrated that HPV types 16 and 18 are present in more than 70% in cervical cancer cases after studying cervical cancer patients from 25 countries, the prevalence of HPV type 16 in India is found to be exclusively very high (~90%) [21—23] while occurrence of HPV type 18 varies from 3 to 20%, followed by other high-risk types such as HPV 45, 33, 35, 52, 58, 59 and 73 [24,25].

The distribution of HPV type has also been found to vary depending on geographical locations and the most consistent variation has been observed in the prevalence of HPV 16 rather than other types. Worldwide analysis in an IARC report, it was shown in cytologically normal women that a large proportion of HPV-positive women found to be infected with HPV 16 in Europe rather than sub-Saharan Africa [26]. An intermediate prevalence of HPV 16 was found in South America whereas a significant heterogeneity was seen across the Asian population. Not only the geographical locations but also the cultural variations influence the sexual behavior of women and their male partners leading to differential acquisition of new HPVs [27]. In a National HPV mapping study in India [28], prevalence of HPV type 16 was found to be highest in Chennai (88%), whereas it was least in Jammu & Kashmir (14.2%) (Fig. 2) [29,30]. This extreme low prevalence of HPV in Jammu and Kashmir may be because of circumcision in Muslim population.

Occurrence of an exclusive high prevalence of HPV16 puts India in an advantageous position because both the new vaccines are against HPV 16 and 18. These will have maximum impact in India, as they would be able to take care of about 90% of cases. However, before introduction of HPV vaccines in India it is also essential to know the age-related incidence of high-risk HPV infection from pre-adolescent girls to older women. Fig. 3 shows the age-wise prevalence of HPV and HR-HPV types 16/18 infections among healthy female subjects [Das et al., unpublished; [25,31—33]]. Interestingly, the peak of HPV infection in Indian women appears to reach at later stage in the third decade of their sexual life at 26—35 year in contrast to 18—25 year reported in western countries [34].

Prophylactic HPV vaccines

Currently, two successful prophylactic HPV vaccines—quadrivalent 'Gardasil' (HPV 16/18/6/11) developed by

Table 1 Summary of the current status of the selective human papillomavirus vaccines

Antigen used	Type of vaccine	Nature of vaccine	Current status	Mode of administration	Reference
Quadrivalent HPV types (HPV 16/18/6/11) L1 (Gardasil)	Prophylactic	Virus like particle (VLP), protein	US-FDA approved	Injectable	[35,36,39]
HPV 16/18 L1 (Cervarix)	Prophylactic	Virus like particle (VLP), protein	Applied for US-FDA approval	Injectable	[37,38]
HPV16 E6/E7	Therapeutic	Fusion protein	Phase I Clinical trial	Injectable	[49]
HPV 16 E7	Therapeutic	Peptide	Phase I Clinical trial	Injectable	[50]
HPV 16/18 E6/E7	Therapeutic	Recombinant Vaccinia virus	Late stage cervical cancer	Injectable	[53]
Second-generation vaccine					
Other high-risk (HPV 31, 45, 33) L1	Prophylactic	Virus like particle (VLP), protein		Injectable	[54]
HPV 16 L1	Prophylactic	Capsomeres (pentameric), protein	Animal Model	Intranasal delivery	[57]
HPV 16 L1 in plant	Prophylactic	Virus like particle (VLP), protein	Animal Model	Oral delivery	[58]
HPV 16 L1 as recombinant bacteria	Prophylactic	VLP produced in recombinant Lacto bacillus, protein	Animal Model	Mucosal	[59]
HPV 16 L1	Prophylactic	DNA based	Animal Model	Parentaral/oral	[75]
HPV 16 E7	Therapeutic	DNA based	Clinical trial	Injectable with Micro particles (ZYC101)	[77]
HPV 16 E7 detox (Sig/E7detox/Hsp70)	Therapeutic	DNA based	Phase I	Injectable	[78]
HPV 16 L1/L2-E7	Chimeric (Prophylactic/Therapeutic)	Fusion protein	Animal Model	Injectable	[79]
HPV 16 L2E7E6	Chimeric (Prophylactic/Therapeutic)	Fusion protein	Phase I/II clinical trial	Injectable	[80]

Figure 2 Prevalence of human papillomavirus 16 (HPV 16) in different geographical regions of India.

Merck while bivalent 'Cervarix' (HPV 16/18) by Glaxo SmithKline (GSK) are recommended for vaccinating young adolescent girls at or before onset of puberty. In these vaccines viral capsid proteins are present in the form of spontaneously reassembled virus-like-particles (VLPs) expressed either in yeast for 'Gardasil' or in baculovirus for 'Cervarix'. These two vaccines protect from infection with two of the most common cancer-causing HPV types 16 and 18 and more than 70% of cervical cancer cases are associated with these two HPV types [35—39]. Both the vaccines were found to be highly immunogenic, safe, well tolerated and effective in preventing incident and persistent HPV infections including developing precancerous lesions [Table 1].

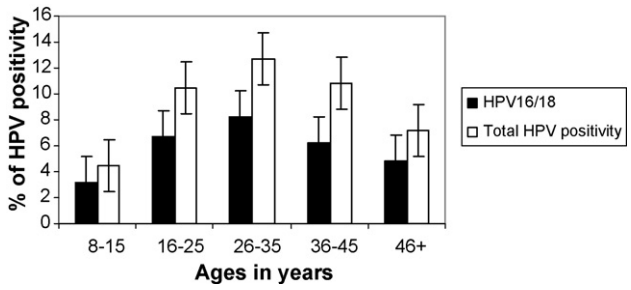

Figure 3 Age wise prevalence of HPV and HPV 16/18 in healthy women.

The quadrivalent vaccine is given in a series of three 0.5 ml intramuscular injections over six months, with 0, 2 and 6-month schedule. Each 0.5 ml dose contains 20 μg each of HPV 6 and HPV 18 and 40 μg each of HPV 11 and HPV 16 with amorphous aluminium hydroxyphosphate sulphate as adjuvant [40]. The available data from several 'Gardasil' clinical trials [35,36,39] indicated that the vaccine induced a high level of antibody formation in the three dose regime but it did fall from the initial peak to a level still significantly higher to prevent infection and is found to persist for more than 48 months post-vaccination. The overall vaccine efficacy was found to be 96% in preventing persistent infection with the HPV genotypes included in the vaccination [36]. Similar trials with the development of genital warts were also conducted and found to be 96% effective [41,42]. 'Gardasil' has also shown to offer 100% protection against vulvar and vaginal intraepithelial neoplasia (grade 2—3) [43]. Although it has been shown in efficacy trials that neutralizing antibodies persist beyond 5 years with an approximate decline of 10 folds over the first 2 years after the first peak and can prevent type-specific persistence HPV infection, certainly longer monitoring is required to defend that the antibody titre maintains the level sufficient to prevent HPV infection and subsequently will not lead to development of cervical cancer [44]. However, this vaccine is now approved by the US-FDA for human vaccination and is being introduced for adolescent vaccination in several schools in the US.

GSK's vaccine 'Cervarix' is also given intramuscularly in 0.5 ml dose at 0, 1 and 6-month schedule contains 20 μg each of HPV 16 and 18. It has a proprietary adjuvant ASO4 comprising of 500 μg of aluminium hydroxide and 50 μg of 3-deacylated monophosphoryl lipid A which induced a stronger and sustained immune response. Extended follow up studies have showed that more than 98% seropositivity was maintained for HPV 16/18 antibodies [38]. An efficacy of about 91.6% against incident infection has been found to be up to 4.5 years in a randomized control trial [38]. During long-term follow up, it was found that not only the HPV types contained in the vaccines were controlled, but infections by other HPV types were also reduced specially genetically closely related types such as HPV 45 (vaccine efficacy 60%) and to a lesser extent, HPV types 31 and 52 (efficacy 32—36%) [45].

These VLP-based vaccines are obviously not therapeutic but the vaccine trial data showed that there is anamnestic response to a single dose of vaccine in previously infected subjects [36]. The basis of this immune response is not clear as 100% efficacy rates observed to date do not allow conclusions about surrogate markers of this phenomena. However, previously demonstrated animal experiment showed that it may be due to production of virus neutralizing antibody [46].

The duration of these vaccine trials is only about 5 years but there is no evidence of decreased efficiency for prevention of HPV infection. The duration of vaccine efficacy could be known better with longer follow up.

Although these vaccines are expected to provide protection against other malignancies such as vaginal, anal, vulver, oral, esophageal and laryngeal papillomatosis which are associated with these HPV types, against which vaccines are developed, they will certainly not provide protection against about 10—30% of cervical cancer which arise due

to infection of other high-risk HPV types (31, 35, 39, 45, 51, 52, 56, 58, etc.). It is suspected that some of these HPV types may take lead because of change in microenvironment. So a vaccinee still will be vulnerable to develop cervical cancer by other HR-HPV types. Since the data on neutralizing antibody titers of these vaccines are now available only up to 5 years of post-vaccination and since the interval between the infection and development of cervical cancer will take more than 10—20 years [38,39,47], there is a need for long term follow up indicating screening for other HPV infections.

Adverse effect

As of February 2007, 385 cases of adverse effects of using Gardasil were reported by Vaccine Adverse Event Reporting System (VAERS). Most of them had noticeable adverse effects including few life threatening and disabling conditions that required hospitalization. Most of these cases showed effect either immediately or next day after vaccination. The most common adverse events were pain and swelling at injection site, headache, fever and vomiting. These adverse effects were observed when 'Gardasil' was given either alone or mostly in combination with other vaccine for the adolescents. This raises the question of safety of the vaccine when given in combination with other vaccines (http://www.nvic.org/Diseases/HPV/HPVrpt.htm).

Therapeutic HPV vaccine

Although prophylactic vaccines appear to be successful, it would take decades to perceive the benefits because it takes 10—20 years to develop invasive cervical cancer. Therapeutic vaccines are to bridge the temporal deficit by attacking already persistent HPV infections and to treat cervical cancer in women. So the development of therapeutic vaccines for HPV is warranted because there is an estimated 5 million women worldwide already infected with HPV which will develop invasive cervical cancer [48]. However, major theoretical obstacle to develop such vaccines is that the immunological determinants for viral persistence or regression remain poorly defined, although it is clear that patients with impaired cellular immunity are at increased risk of persistent HPV infection and carcinogenic progression.

Several animal studies showed promising results and indicated that therapeutic HPV vaccine may regress disease progression. As a result, several therapeutic HPV vaccines are in phase I and phase II clinical trials (Table 1) [49—53]. Most efforts have been directed towards the early proteins, HPV E6 and E7 or small peptides derived from them, mainly because these are the major transforming viral proteins that are invariably retained and expressed throughout the full spectrum of HPV-related disease progression and cervical carcinogenesis. In a phase I study of HPV 16-specific immunotherapy with E7 fusion protein and ISCOMATRIX™ adjuvant in women with cervical intraepithelial neoplasia (CIN, $n = 31$), Frazer et al. [49] showed that this immunotherapy is well tolerated and the vaccinated subjects developed HPV 16 E6E7 specific immunity. The clinical trial data showed that antibody response, delayed type hyper sensitivity, *in vitro* cytokine release, and CD8 T cell responses to E6 and E7 proteins were each significantly greater in immunized subjects than in placebo recipients [49]. In another phase I clinical study with HPV16 E7 peptide vaccine for HPV positive women ($n = 18$) with high-grade cervical and vulvar intraepithelial neoplasia showed that 12 patients cleared the virus and only 3 of them cleared dysplasia after vaccination, but an increased S100+ dendritic cell infiltrate was observed in 6 of 6 patients [50]. Another phase I/II trial in late stage cervical cancer patients ($n = 8$) with a live recombinant vaccinia virus expressing the E6 and E7 proteins of HPV 16 and 18 (TA-HPV) showed that each patient mounted an antivaccinia antibody response and three of eight patients developed an HPV-specific antibody response. HPV-specific CTLs were detected in one of three evaluable patients [53].

Several attempts are also being made to deliver HPV E6 and E7 oncoproteins as vaccines using their fusion constructs with potential adjuvanting TLR agonists or cytokines [51,52]. However, these papillomavirus proteins are not expressed on the cell surface, there is little potential for antibody-dependent cytotoxicity to mediate regression. Instead, potentially effective cytotoxic responses will probably require a vaccine that induces the presentation of small virally encoded peptides to antigen presenting cells. In cells that possess class I molecules, the normal process of partial intracellular degradation of cytoplasmic or nuclear viral proteins can, following the binding of small viral peptides to the class I molecules, lead to the induction of antigen-specific reactivity of CD8-positive cytotoxic T lymphocytes (CTLs).

Second-generation vaccine

The main goal of the second-generation vaccine (Table 1) is to develop vaccines that will be more suitable to resource-limited countries, that is to reduce the cost of production, to have a longer shelf-life, single dose delivery, long lasting immunity, should be stable at room temperature and it could incorporate other oncogenic HPVs [54—59]. Recently, HPV 16 L1 pentameric capsomeres produced in *E. coli* showed to induce neutralizing antibodies in animal model. Capsomeres represent a highly stable alternative to VLPs and as it can be easily expressed in *E. Coli* and would be cheaper to produce [57]. Several strategies including production in bacteria or plants have been reported to reduce the cost of production [58]. It was also reported that HPV16 L1 VLP could be produced in transgenic plants or transiently expressed in edible plants/yeast that can be delivered orally as it is easily digestable and immunogenic. However, it will require much more VLPs to induce a strong antibody response compared to parenteral injection [54,55,58]. Efforts are underway for industrial scale production of L1 VLPs in transgenic plants. Several other attempts are being made to reduce the cost of delivery of VLP vaccine by making needle-free mucosal spray or oral delivery [58,59]. Nardelli et al. showed that needle free mucosal vaccination, an alternative of VLP vaccine delivery could reduce substantial amount of cost by minimizing the dose and maximizing the response [60]. It was also suggested that lyophilized VLPs in a powder formulation might be an alternative to aqueous droplets for mucosal delivery.

One of the second-generation vaccines that can address the majority of limitations of the current VLP vaccines could be ''Genetic vaccines'' or ''DNA vaccines''. In the last few years, several DNA vaccines have been developed against variety of viral, bacterial as well as parasitic infections in animal models showing long lasting immunity and protection [61—65]. Clinical trials of DNA vaccines have been done or are underway for various diseases, including cancer, influenza, hepatitis B, HIV, and malaria [66—73]. Recently, first DNA vaccine against West Nile virus has been approved by the Department of Agriculture, United States (USDA) for commercial use in horse [74].

Since genetic vaccine is a plasmid-based vaccine, it is cost-effective and simple to produce in large quantities. Robustness, single dose administration and stability of DNA vaccine even in higher temperature provide an edge over the other vaccines, particularly for distribution in the remote areas. Recently, Rocha-Zavaleta et al. have shown that parenteral and oral immunization with plasmid DNA expressing HPV 16 L1 (3 doses of 100 μg each) could induce systemic and mucosal antibody production together with cytotoxic T lymphocyte responses in animal models [75]. Twelve months after immunization, antibodies were still detected in mouse vaccinated subcutaneously. The responses are increased if codon-modified genes are used as it increases expression [76].

Encouraging data from animal models have led to employ several therapeutic HPV DNA vaccines in clinical trials. Clinical trial of HPV 16 E7 plasmid DNA delivered with microparticle (ZYC101) to HPV 16 positive women with cervical high-grade intraepithelial neoplasia (CIN II/III) demonstrated 33% with complete histologic response, 73% with immunologic response but without serious adverse events [77]. In another phase I clinical trial with attenuated HPV 16 E7 DNA (a mutation that abolishes the Rb binding site, E7 detox) fused with heat shock protein 70, signal sequence (Sig/E7detox/Hsp 70) was carried out in HPV16 positive patients with high grade CIN lesions at John Hopkins [78]. Three doses of 500, 1000 and 3000 μg DNA were delivered intramuscularly at one-month intervals. So far, no adverse or dose-limiting side effects were observed at any dose level of the DNA vaccine [78]. Since HPV 16 is the type exclusively prevalent in India (~90%) and the other aggressive oncogenic type is HPV 18 (3—19%), the development of HPV DNA vaccine efforts is being focused on these two high-risk genotypes only.

In order to incorporate the therapeutic intervention along with prophylactic vaccination, several attempts were made to develop chimeric vaccines [79,80]. These vaccines elicited both humoral immune response as well as cell-mediated immune response. Clinical trial of chimeric VLPs (L2E7E6 fusion protein vaccine) had shown to enhance HPV type 16, E6 and E7-specific T-cell immunity in healthy volunteers through vaccination with TA-CIN [80]. These vaccines may be relevant for a population who do not go for routine screening but already have HPV related cervical disease.

Furthermore, the recognition of dendritic cells (DC) as powerful antigen-presenting cells capable of inducing primary T cell responses *in vitro* and *in vivo*, has recently generated widespread interest in DC-based immunotherapy of several human malignancies including cervical cancer [81]. Various therapeutic HPV vaccines are being developed and implemented in human clinical trials, with a particular emphasis on the use of autologous DC pulsed with full-length HPV 16 or 18 E7 oncoproteins as a novel strategy to induce HPV E7-specific and tumor specific T cell responses in cervical cancer patients [81].

Immunology of human papillomavirus infection

As HPV is an epitheliotropic virus, the presentation of viral antigens to the host immune system is very limited leading to generation of very weak immune response. Therefore, natural HPV infection gives rise to a slow and modest but measurable serum antibody response in most but not all infected individuals [8]. Though intensity of the serum antibody is long lasting, it depends on viral load and persistence as 70—90% of HPV-infected individuals can clear the virus naturally while small percentage of patients cannot [8,82]. Several studies have shown that L1 VLPs are highly immunogenic and protective, and that protection is mediated by neutralizing serum antibodies [46,83,84]. The measurement of HPV-neutralizing antibodies in serum has been found to be strongly correlated with L1 VLP-specific indirect ELISA test because the neutralizing epitopes are immunodominant and conformational [84]. Clinical trial data of both 'Gardasil' and 'Cervarix' showed 99% seroconversion in the individuals after one month of completion of vaccine doses against targeted HPV types. Vaccine efficacy due to virus neutralizing antibodies for precancerous lesions (cervical intraepithelial neoplasia, grade 2 or higher) caused by HPV types 16 and 18 was found to be 98% for both 'Gardasil' and 'Cervarix' [45,85].

Several studies indicated that cell-mediated immune response is important for control of established HPV-associated disease as lower rate of clearance of HPV occured in HPV-associated lesions of immunodeficient compared with immunocompetent patients [86]. Persistent infection of HPV and development of cervical precursors and their progression to invasive cancer require continued intracellular expression of viral oncoproteins E6 and E7 [82]. Therefore, therapeutic vaccines generally target these two non-structural early viral antigens and aimed to stimulate the T cell responses against these two oncogenes. Currently, several strategies have employed such as administration of peptide antigens or recombinant proteins, plasmid DNA vaccines, viral vector vaccines and administration of E7-pulsed dendritic cells to increase the cytotoxic T cell response (CTL) against HPV E6 and E7 proteins [87].

As HPV can produce both humoral and cellular immune response, a vaccine that combines both prophylactic and therapeutic activity by providing antigens for humoral and cellular immune response might have broader and better applicability. Clinical trial of chimeric VLPs (HPV16 L2-E6-E7 fusion protein vaccine) had, however, shown to produce neutralizing antibodies to seronegative vaccinees against both HPV 16 and 18 with low therapeutic efficacy [80].

HPV vaccination associated issues in developing countries

Despite successful clinical trials of two newly developed VLP-based prophylactic HPV vaccines in the West, there are number of limitations and issues associated with the vaccine itself and its implementation prospects and prejudices in developing countries like India where cervical cancer is the major cancer in women. Most importantly, since ethnicity can bring about significant difference in immune system genes involved in antibody production, it is mandatory to carry out studies on immunogenicity testing first before these vaccines are introduced in India. Another important debatable issue in developing countries is selection of target population for vaccination. Although there are reports showing strong immunological response with higher antibody titre during pre-puberty than that in post-puberty [88] and since HPV infection is acquired through sexual contact particularly those with promiscuous sex life, vaccination of adolescents in developing countries with a variety of cultural, religious, social and ethnic groups will raise many moral, social, religious and ethical issues. Preliminary survey (Das et al., unpublished) of 9—16 year old school girls and their parents suggests that majority of them are unaware of HPV and parents perceive that their children are not at risk of acquiring HPV as they come from good family. Some parents think that this vaccine will make sex safe leading to freedom to promiscuity and teenage sex, which is not very common in this region of the globe. They think this will cause social stigma and turnish their family prestige. They suspect that the vaccine itself may also cause infection to the children and are not very interested in vaccinating their daughters even if it is made available free of cost. It is therefore most important to raise general awareness about HPV, its mode of transmission, cervical cancer and HPV vaccine before it is introduced in India. It is suggested that childhood HPV vaccination might be an alternative strategy but there is no study to show data on the safety, immunogenicity and dose tolerance of childhood vaccination. Furthermore, in developing countries like India there is no established set up/system in place for childhood vaccination. Recently, many states of US have seriously considered this vaccination as pre-requisite for pre-teen girls for admission to middle school [89]. However, it appears to be a most expensive pediatric vaccine being employed for mass vaccination without knowing its ultimate efficacy in preventing cervical cancer.

It is also important to note that the prevalence of cervical cancer and HPV infection in India indicate that the initiation as well as peak of HPV infection occurs at a slightly higher age group (26—35 years) women mostly in their third decade of sexual activity (Fig. 3) than that of global scenario (peak in 18—25 years) [34]. And, most cervical cancers in India arise much later by 45—60 years of age. Therefore, it will be important to test the efficacy of the vaccines in different age groups beyond 26 years as both Merck and GSK suggest that the vaccine will be equally effective in women between the age 26 and 55 years [36,38]. There is, however, no data at present to show effectiveness of these vaccines in the older age group women. Bridging studies involving younger and older women need to be carried out to establish similar level of immunogenicity and efficacy of the vaccines.

Though several randomized clinical trials have demonstrated that both the vaccines are highly immunogenic and short time effective in preventing incident and persistent type-specific HPV infection, yet several key issues are unanswered and will require further research [36,38]. The major issues are—Is there any correlation between antibody titer and protection? If yes, how long this antibody titer will persist to protect infection and whether any booster shots will be needed to be administered at regular intervals to maintain the immunity? Are three doses essential or it could be possible to achieve protection in less than three doses? Is it safe to give HPV vaccine along with other adolescent vaccines such as hepatitis vaccines and polio vaccine? Do the vaccines safe in pregnant women and children aged 4—5 years?

The biological end-point of protection against persistent HPV infection and cervical dysplasia from the specific genotypes targeted by vaccines appears to be a good surrogate marker for cancer protection, but need to wait for a long-term follow up, in order of 15—20 years, to see vaccine-induced reduction of cervical cancer including the reliability of HPV serology that is being used presently for monitoring HPV vaccination.

Because of the high cost of the present vaccines, the affordability and accessibility of these vaccines is a major concern for mass vaccination program in developing countries like India where cervical cancer is the major cancer among women mainly from low socio-economic status. Relatively high cost of VLP vaccine production and distribution, the type specificity of their protection, and the unlikely prospects of therapeutic efficacy are the impediments of particular concern for implementation of these vaccines in a resource poor setting. Though some prophylactic vaccine (Cervarix) possesses some cross-reaction to other related HPVs such as types 31, 45 and 52, it does not address other high-risk HPVs. This demands need for continued screening of vaccinated women, as there is every chance of developing CIN and cervical cancer by other HR-HPV types. Therefore, introduction of present HPV vaccination will certainly not make women free from the anxiety of contracting other HPV infection thereby, developing cervical cancer. At the same time, it will cause substantial burden to the public health budgets in India. In private hospitals in India, the cost of cervical cancer treatment is certainly higher (~$4000—5000), than the cost of present prophylactic vaccines but in government hospitals, the treatment is done almost free of cost ($100—200). Therefore, it will be almost impossible to introduce the present vaccine in India with this high cost. Though both Merck and GSK have proposed to offer vaccine at an affordable price to developing countries, it may take several years to implement and still the costs may be much higher than that of present infant and other vaccines available in India. Therefore, to bring down the vaccine price, it is important to produce these vaccines in India. John Schiller recently expressed his opinion in an 'International Symposium on HPV' that India could make their own version of vaccine, which will be cost-effective and may reach to women in India much faster than any other way [90].

Therefore, to address some of the important issues discussed above there is a need to develop a second-generation HPV vaccine which should be cost-effective and well suited to India and other developing countries for implementa-

tion and addressing regional issues. Nonetheless, it is most essential to disseminate correct information to improve understanding of both HPV and cervical cancer among medical and paramedical personnel, social workers, policy makers, parents, young adolescents and the public at large.

Conclusion

A cost-effective second-generation HPV vaccine is needed for developing countries to address various issues specific to the region. It is most important to make it easy to produce and distribute, should bypass the cold chain and also can take care of those already infected by HPV. Because HPV 16 is the only oncogeneic HPV type highly (∼80%) prevalent in India, it should be the major focus for vaccine development. It would be highly beneficial if a DNA based chimeric vaccine having both prophylactic as well as therapeutic efficacy is produced. However, till such time, an organized cervical cancer-screening program should be at place to use the existing infrastructure and coat-effective screening methods such as VIA, VILI, Pap-smear and HPV DNA tests which have been since proved to be highly effective in reducing incidence of cervical cancer.

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