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Development and Implementation of Papillomavirus Prophylactic Vaccines

Ian H. Frazer

Translation of basic scientific findings into practical patient outcomes is a significant exercise even when the goal is conceptually straightforward, as in the development of a vaccine for an infectious disease. Recognition of the association of cervical cancer with papillomavirus infection encouraged development of a vaccine to help with prevention of this very common cancer, causing over 250,000 deaths each year worldwide. To introduce a vaccine program, it was however necessary to develop a technology for making viral Ag, demonstrate that systemic immunization could provd mucosal surface protection in the genital tract, develop assays for vaccine potency, and understand enough about the epidemiology and natural history of the infection to plan effective intervention strategies. This process took ~25 years. The major hurdle, now that effective vaccines are available, is to ensure their deployment in the countries where they are most needed. The development and deployment of human papillomavirus vaccines demonstrate the benefits of collaborative research activity across the globe, and between academia and industry, to translate scientific discoveries into public health benefits. The Journal of Immunology, 2014, 192: 4007–4011.

A n association of viral infection with development of proliferative skin lesions in animals was recognized almost as soon as viruses were first described as filtrable agents causing disease (1). Evidence for association of viral infection with specific cancers in humans took longer, as causality could not be tested according to Koch’s postulates. It is now accepted that nearly 20% of the global burden of cancer in humans can be attributed to viral infection (2). One family of viruses, papillomaviruses (PVs), is recognized as responsible for a quarter of virus-associated human cancers and 5% of the global cancer burden. One major cancer, cervical cancer, occurs only as a consequence of human PV (HPV) infection of the cervix and is responsible for over 250,000 deaths annually (3).

From the perspective of the viral immunologist, PVs are difficult to work with, as they are species specific and can only replicate in differentiating epithelia. Unlike most viruses, PVs have not been grown in monolayer cell culture, as their life cycle requires differentiating epithelial cells, and even in organotypic culture it has proven hard to produce more virus out than was provided as the infecting inoculum (4). These issues delayed research on HPVs, as although it was recognized that the Shope (cottontail rabbit) PV was able to induce cancers in rabbits (5), it was not possible, until the advent of the molecular era, to determine that there were more than two HPVs responsible for skin and genital warts. Because wart lesions almost never turn malignant, and serology for PV infection was difficult in the absence of quality Ag material (6), the association of HPV infection with cancer had to wait for molecular cloning and hybridization technology. This allowed the hypothesis (7) and subsequent demonstration in the 1980s by zur Hausen and colleagues (8, 9) of a PV DNA in cervical cancers that was related to the PV DNA in genital warts, but was not identical. Molecular typing of many cancers worldwide has subsequently established that >99% of cervical cancer is associated with 1 of 10 genotypes of α-clade HPV (10) termed “high risk” genital PVs. The commonest association is with HPV16, as first identified by zur Hausen and colleagues, which is associated with >50% of cervical cancer worldwide. Subsequent epidemiological studies have established that genital tract infections with cervical cancer–associated PVs are extremely common, as >50% of young women and men are infected with HPV16 within 3 years of onset of sexual activity (11). Such infections are generally benign, associated with neither symptoms nor cellular abnormalities (12), and are relatively short-lived, with 50% of genital infection cleared within a year (11). However, persisting PV infection of the cervix with a high-risk HPV is associated with increasing risk of cervical dysplasia, and eventually malignancy ensues in ~30% of women with persisting dysplasia (13). Epidemiological studies have also extended the catalog of human PVs to >200 (14), of which most are β-clade viruses and appear to be benign skin saprophytes, although ~5 β-clade types are associated with skin cancer in patients with a rare genetic disorder, epidermodysplasia verruciformis (15). The α-clade infects the genital tract, with two (HPV6 and HPV11) genotypes responsible for most genital warts, and ~10 (including HPV16 and HPV18) associated with risk of cervical cancer, whereas several other genotypes produce no evident disease. It is also now accepted that ~50% of other anogenital cancers (vulvar, penile, and anal) and ~50% of oropharyngeal cancers are associated with risk of HPV infection (16).

Abbreviations used in this article: HPV, human papillomavirus; PV, papillomavirus; VLP, virus-like particle.

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The immunological challenge: translating knowledge into outcomes

Vaccines are the most effective public health measure for reducing disease burden, after safe food and water. Although it was not clear in the late 1980s whether HPV infections were rare and commonly initiated cancers or, as turned out to be the case, common with cancer a rare consequence of persisting infection, the argument for developing a prophylactic vaccine was strong. Protection of cattle against challenge with bovine PV2 by a vaccine consisting of purified bovine PV2 virus (17) and protection of dogs against canine oral PV by formalin-inactivated virus (18) suggested that vaccination against human PVs would be practical.

The first translational goal (Fig. 1) on the pathway to developing protection against HPV infection and cervical cancer was to derive a source of Ag for HPVs. As PV could not be grown in culture, the standard approaches to vaccination through production of inactivated or attenuated virus were not practical. The PV virion was well understood as a non-enveloped capsid comprising 72 pentamers of a major (L1) capsid protein, and a variable amount of a minor (L2) capsid protein. Some L1 genetic material cloned from tumor cells was available in prokaryotic expression vectors, which produced an immunogenic L1 protein (19). However, this was isolated from inclusion bodies, presumably in denatured form, and it seemed likely that correct folding and assembly of L1 into virion-like structures would be necessary to produce antigenic material that would express epitopes to induce host-protective Ab responses. Several groups achieved expression of PV L1 and production of virus-like particles (VLPs), visualized by electron microscopy, in the early 1990s using a range of expression systems. This strategy had already proven practical for a recombinant hepatitis B vaccine. Initial research was undertaken with recombinant vaccinia vaccinia expressed in COS cells with L1 and L2 genes cloned from a clinical isolate (20), and with recombinant baculovirus in insect cells with L1 cloned from a tumor and, with better yields, using the L1 gene from clinical material (21–24). In each case, L1 protein expressed in eukaryotic cells, which, for HPV16, required expression from the second translation initiation codon in the L1 open reading frame, self assembles into VLPs morphologically resembling the naturally occurring virus. Self-assembly allowed for a scalable industrial process producing replicates of the PV virion sufficiently accurate in protein conformational structure to become the basis of a potential vaccine.

The second translational goal was to determine whether a vaccine based on HPV VLPs would induce virus-neutralizing Ab and host protection. Again, the inability to grow PV presented problems, and there were also the conceptual issues of whether systemic immunization would provide genital tract protection, and whether it was desirable to immunize with material from a virus associated with cancer. The natural immune response to HPV infection was not well studied. Development of Ab was slow and relatively weak after infection, with only 50% of subjects naturally infected with HPV16 producing measurable virion-specific Ab within a year of infection (25). Furthermore, past HPV infection does not appear to protect effectively against reinfection with the same genotype of the virus, as demonstrated by significant rates of infection in placebo recipients in the pivotal vaccine trials with serological evidence of past HPV infection. Therefore, utilization of human sera from infected subjects as reference standards for immunogenicity and protection was not feasible. However, a key study in dogs challenged with canine oral papillomavirus after immunization with COPV L1 VLPs (26) showed that L1 VLPs administered as a vaccine with or without adjuvant induced high-titer Ab and protective immunity against challenge with live virus. Protection required only Ab as protection and could be passively transferred by immune serum, and Ab had to be directed at conformational determinants on the VLP, as denatured VLPs did not produce protection. Studies examining the in vitro–neutralizing activity of polyclonal antisera raised against individual VLP types in animals established that HPV genotypes are distinct serotypes, and therefore that the vaccines would need to be multivalent. Development of mAbs specific for PV virions (27) and partial solution of the crystal structure of the PV VLP (28) confirmed the clinical and laboratory evidence that PV genotypes were also distinct serotypes, and that neutralizing Ab was directed against surface-conformational determinants on the external face of the L1 molecule that were nonconserved between HPV genotypes.

A series of HPV genotype-specific Ab assays were developed to monitor the development of Ab and host protection. Again, the inability to grow PV presented problems, and there were also the conceptual issues of whether systemic immunization would provide genital tract protection, and whether it was desirable to immunize with material from a virus associated with cancer. The natural immune response to HPV infection was not well studied. Development of Ab was slow and relatively weak after infection, with only 50% of subjects naturally infected with HPV16 producing measurable virion-specific Ab within a year of infection (25). Furthermore, past HPV infection does not appear to protect effectively against reinfection with the same genotype of the virus, as demonstrated by significant rates of infection in placebo recipients in the pivotal vaccine trials with serological evidence of past HPV infection. Therefore, utilization of human sera from infected subjects as reference standards for immunogenicity and protection was not feasible. However, a key study in dogs challenged with canine oral papillomavirus after immunization with COPV L1 VLPs (26) showed that L1 VLPs administered as a vaccine with or without adjuvant induced high-titer Ab and protective immunity against challenge with live virus. Protection required only Ab as protection and could be passively transferred by immune serum, and Ab had to be directed at conformational determinants on the VLP, as denatured VLPs did not produce protection. Studies examining the in vitro–neutralizing activity of polyclonal antisera raised against individual VLP types in animals established that HPV genotypes are distinct serotypes, and therefore that the vaccines would need to be multivalent. Development of mAbs specific for PV virions (27) and partial solution of the crystal structure of the PV VLP (28) confirmed the clinical and laboratory evidence that PV genotypes were also distinct serotypes, and that neutralizing Ab was directed against surface-conformational determinants on the external face of the L1 molecule that were nonconserved between HPV genotypes.

The third translational goal was to ensure a pathway, and the necessary reagents, for commercial development of vaccines based on the PV VLPs. Extensive epidemiological studies in the 1990s had defined the widespread nature of high-risk HPV infections, their common acquisition shortly after onset of sexual activity, and the HPV genotypes most commonly associated with cancer. Having established patentable technologies for VLP production, two vaccine companies (Merck & Co. and GSK) took on the risk of developing and testing potential clinical vaccine products. Merck used yeast fermentation technology to make a quadrivalent vaccine based on HPVs 6, 11, 16, and 18 that might potentially protect against 70% of cervical cancer, while GSK used baculovirus/insect cell fermentation to make a bivalent HPV16 and HPV18 vaccine that would potentially protect against 70% of cervical cancer but not genital warts. Both vaccines use adjuvanted VLPs: Merck uses a proprietary aluminum hydroxide formulation, and GSK uses an aluminum hydroxide adjuvant supplemented with monophosphoryl lipid A, a TLR4 agonist. A series of HPV genotype-specific Ab assays were developed to assess vaccine immunogenicity, including ELISA with VLP substrate (30), competitive inhibition assays using labeled
VLP-specific mAbs (31), and neutralization assays utilizing VLP pseudovirions that could infect a reporter cell line with a reporter gene (32). These assays have subsequently been facilitated by Ab standards, which have been developed and validated through the World Health Organization (33). The efficacy endpoint of the phase 3 clinical trials of the HPV VLPs was selected as prevention of premalignancy (cervical intraepithelial neoplasia grade 2 or 3) attributable to the HPV genotypes incorporated in the vaccines, and, for the Merck vaccine, also prevention of genital warts. The vaccines proved extremely effective in preventing cervical and other premalignancy in large phase 3 clinical trials of women aged 15–26 who had no evidence of prior infection with HPV genotypes of the relevant genotypes (34). However, no protection against development of premalignant disease was seen for women already infected, but without evidence of disease at the time of recruitment. Data on comparable Ab production then allowed prediction of protection for younger women and men prior to onset of sexual activity, as these are the preferred target population for publicly funded immunization programs. Registration of the vaccines for use to assist in prevention of cervical cancer and, for the quadrivalent vaccine, for prevention of genital warts, followed in most countries of the world between 2006 and 2010. Ab data have also been compared between the two available vaccines. The GSK vaccine, adjuvanted with a stronger adjuvant, produces higher titer Ab (35). However, both vaccines proved to be essentially 100% effective in the phase 3 clinical trials (36), and there is currently no evidence to suggest that better initial Ab response translates into better long-term protection. Both vaccines induce stable long-term Ab titers, without evidence of breakthrough infection even in those subjects with low initial Ab levels following vaccination, precluding establishment of a surrogate marker for protection, and both have proven safe in use, with only the usual local and mild systemic reactions to vaccines commonly reported (37), while the frequency of severe allergic reactions (38, 39) is very low and comparable to other vaccines.

The fourth translational goal is to ensure effective deployment of the HPV vaccines. For the developed world, cervical cancer prevention prior to vaccine development was largely achievable through effective implementation of screening programs based on cervical cytology assessment and treatment of premalignancy by surgical means. Vaccines are therefore an additional measure, with the rationale for introduction based on analysis of cost-effectiveness and public wishes and acceptability. Some countries, including Australia and the United Kingdom, were early adopters of universal publicly funded immunization of young women before the onset of sexual activity, and, from 2013, the Australian program has been extended to young men. The success of the program in Australia can be measured by a >80% uptake rate of vaccination among 12- to 14-ya-old girls, and by the virtual disappearance of genital warts during the last 5 years, not only among immunized younger women, but also among unimmunized younger men (40), presumably as a result of the protective effects of herd immunity. Significant reductions in the prevalence of HPV16 and HPV18 DNA have been similarly observed in cervical smear samples from 18- to 24-ya-old women (41). Whereas herd immunity may help protect men from HPV-associated anogenital and oropharyngeal cancers, the low level of coverage of the female population in many countries (42) is likely currently insufficient to provide good herd immunity, a justification for immunization of young men as a public health measure where cost-benefit analysis permits.

Introduction of the HPV vaccines on a global basis have encountered some significant issues (43), including an anti-vaccine lobby that has tried to use misinterpreted adverse events in vaccine recipients unrelated to vaccination, as well as adverse events in placebo recipients, to suggest that the vaccines are not safe. This has had significant consequences in India where vaccine programs were halted (44), and in the United States where uptake has been slow (~33% for all three doses in girls in 2012) (42). Vaccine costs have also impacted on uptake. The initial programs sponsored by GSK and Merck for the developing world, and the recent announcement that the vaccines will be made available to the Global Alliance for Vaccines Initiative eligible countries at $5 U.S. per dose, and that the Gates Foundation will meet this cost for a period for approved programs (45), should enhance more rapid deployment in the countries where cervical cancer screening has not been implemented and most deaths from cervical cancer occur. One clear lesson to date is that education about the vaccine is key to its successful deployment, and that education needs to be directed at health care professionals, governments, recipients, parents, and schools to ensure effective delivery programs (46). A recent program in Vanuatu, a developing Pacific island nation, established the high prevalence of high-risk HPV infection (47) and of cervical dysplasia (48) in that country. This has enabled a joint program with GSK to deliver vaccines through schools, which achieved a 93% uptake rate of the full vaccine program across eligible children on Efate Island. This program also established that although there was a wide variation in nutritional status across the immunized 10- to 12-ya-old girls, the neutralizing Ab responses after three immunizations in the quintile with the lowest body mass index and skinfold thickness were not significantly different from those in the other quintiles (Fig. 2).

The fifth translational goal is to improve the vaccine technology to achieve better adoption and better outcomes. The recent announcement that a nine-valent vaccine based on the current VLP technology and covering five further high-risk HPV types has given good protection against each of these in HPV naive recipients, without compromising the immune response to the initial four types in previously unimmunized subjects (49), suggests that vaccination should eventually replace screening for cervical cancer as the primary means of cancer prevention. Further studies will be needed to determine whether similar broad-spectrum responses across all nine vaccine genotypes can equally be obtained in individuals already immune to one

![FIGURE 2.](http://www.jimmunol.org/)

Ab to HPV VLPs as assessed by pseudovirion neutralization in randomly selected HPV vaccine recipients in Vanuatu, stratified into quintiles by body mass index.
or more types through vaccination, or whether prior immunity may divert immune responses toward already recognized genotypes as predicted by the concept of "original antigenic sin" (50). Although prior infection with one HPV genotype does not appear to have influenced immunity to the others in the pivotal clinical trials of the current vaccines, prior immunity generated by vaccination may be a more rigorous test of this concept. This will possibly accrue increased significance if the current debate about the role of β-PV in development of squamous skin cancer (51) is resolved with the conclusion that these viruses do play a causative role, and therefore that vaccination against β-PV is desirable.

A recent study has shown that two immunizations with the GSK vaccine gave as good a durable Ab response as three immunizations, with equal efficacy for disease prevention over 4 years, whereas a single dose gave somewhat reduced long-term Ab response but nevertheless with protection (52). This observation may pave the way for cheaper and more practical vaccine delivery strategies for the developing world, although, as there are no surrogate markers of protection following vaccination based on Ab titer, this decision should be based on clinical testing. Cheaper and more practical vaccine delivery strategies may also allow programs aimed at preventing other HPV-related cancers, including oropharyngeal cancer, where men and women are equally affected, and where clinical trials to provide an evidence base for protection are unfeasible given the lengthy lag time between acquisition of infection and clinical manifestation of disease.

This review has been focused on translation of knowledge of the virology and immunology of HPV infection into practical prophylactic vaccines, and has acknowledged that these vaccines have no efficacy as immunotherapeutics for existing infection. Future research may enable development of effective immunotherapy for HPV-associated cancers based on our current knowledge of the potential target Ags and necessary delivery strategies for such vaccines (53). This research is justified by the estimated 25 million people already infected with a high-risk HPV who will in consequence develop an HPV-related cancer of the anogenital tract or oropharynx, most of whom are in developing world countries where effective treatment is not widely available.

Conclusions
A concerted effort from basic science and industry has enabled the discovery of a connection between infection with HPV and development of cervical cancer to be translated into a practical means to prevent cervical cancer through immunization in ~25 years. This success story demonstrates the benefits of collaborative research activity between academia and industry to translate scientific discoveries into public health benefits.

Disclosures
The University of Queensland derives royalties from HPV vaccines referred to in this article. The author has accepted speaking engagements and expert advisory panel consultancy payments from GSK and from Merck.

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