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God's Gift to Women: The Human Papillomavirus Vaccine

Commentary

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Summary

An Australian newspaper recently bestowed Ian Frazer the title of "God's gift to women" for his research team's part in developing a vaccine to help control cervical cancer. Here Frazer discusses this work and the science behind the vaccine.

Designed to prevent infection with some types of human papillomavirus (HPV), a vaccine (Gardasil) recently approved by the Food and Drug Administration for use in 9- to 26-year-old women in the USA is the first pharmaceutical compound specifically developed to prevent the common human malignancy cervical cancer. Another similar product (Cervarix) should become available next year. These are conventional protein-adjuvant vaccines, comprising alum adjuvanted viral capsids of multiple HPV serotypes assembled from recombinant viral capsid protein. The vaccines, delivered systemically, induce neutralizing antibody, protecting against infection with the incorporated HPV serotypes in skin and at mucosal surfaces. The nature of HPV infection, the consequent health problems, and the host response to infection have been defined during vaccine development, which has proven a considerable exercise in epidemiology and public health research. The considerable time lag between vaccine deployment and public health benefit, together with perceived effects of vaccine availability on human sexual behavior, is currently influencing vaccine introduction. Nevertheless, HPV vaccines should eventually eliminate a number of epithelial cancers and reduce the annual burden of cancer deaths globally by 5%–10%.

The Natural History of Human Papillomavirus Infection

Papillomaviruses (PV) were among the first defined "filtrable agents" (McFadyean and Hobday, 1898), and in the 1930s Peyton Rous showed that the Shope (cottontail rabbit) PV causes epithelial cancer (Kidd et al., 1936). However, an association of PV with human epithelial cancer was not suggested until the pioneering work of Zur Hausen and colleagues in the late 1970s (Zur Hausen, 1977). PV uses epithelial-cell differentiation to undergo its vegetative life cycle, which has proven difficult to replicate in vitro. Thus, detection of PV in clinical samples had to await molecular techniques. Furthermore, without viral antigens, classification of PV types by serology was delayed, and the diversity of PVs, and of their associated diseases, was unrecognized. PV infections in cows (bovine PV), dogs (canine oral PV), and rabbits (cottontail rabbit PV) are mostly self-limiting and are considered to be relatively trivial. Association with epithelial cancer was observed for bovine PV-1 and for cottontail rabbit PV but was not considered to have a human counterpart because human warts do not turn malignant.

In the 1850s, an Italian pathologist, Rigoni Stern, suggested that cancer of the womb might have an infectious etiology, after observations on the greater incidence of this cancer in prostitutes than in nuns. Various agents, including herpes viruses, were subsequently considered as candidates. Human PV had been thought likely to be a single agent, responsible for skin warts. Molecular cloning techniques, however, demonstrated a variety of PVs, falling into what we now recognized as four broad families (Table 1). Zur Hausen and colleagues proposed (Zur Hausen, 1977) that some PVs might be responsible for human cervical cancer. They showed that, distinct from the PVs associated with genital warts, PVs infecting anogenital skin could be isolated from cervical cancers and cervical-cancer-derived cell lines. Improved methods for detecting PVs via DNA hybridization allowed extensive epidemiological studies by the International Agency for Cancer Research (IARC) and others, and these studies established that approximately 100% of cervical cancer, and a percentage of other anogenital and head and neck cancers, can be attributed to "high-risk" human PVs.

PV infections resolve more slowly than most viral infections, as anyone who has watched a favorite wart is aware. "High-risk" HPV infections persist in the cervix in 2% of those infected and convey a risk of cancer, and the fact that they persist more frequently in immunosuppressed patients suggests a role for specific immunity in viral elimination. Two nonstructural PV proteins, E6 and E7, which substantially alter epithelial-cell replication and differentiation, continue to be expressed in HPV-associated cancers. PV infection alters epithelial-cell growth and differentiation and does not induce cell death or local inflammation because the mature virion is shed by epithelial desquamation. Immunological studies of PV infection subsequent to the recognition of multiple PV types have shown that natural infection produces only weak specific serological responses to the viral capsid and, with the onset of invasive cancer, to the E7 nonstructural protein (Frazer, 2004). Cell-mediated immune responses to viral nonstructural proteins, particularly E2, E6, and E7, are associated with regression of infection. The mechanism of regression remains uncertain, although there may be clues in the ability of topically applied imiquimod, an activator of Toll-like receptor 7 (TLR7) and TLR8 and a promoter of local inflammation, to induce regression of only those HPV-associated lesions to which it is applied. Of course, none of the above information was known when I started

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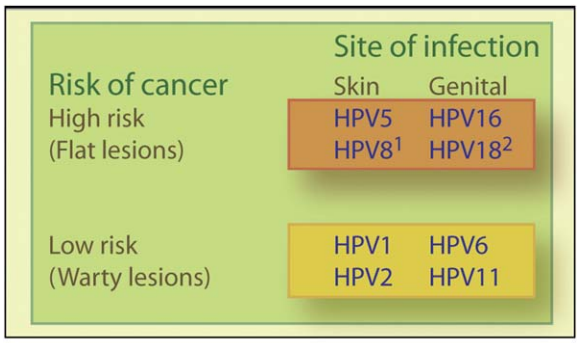
Table 1. Broad Classification of Human Papillomavirus Infection and Associated Cancer Risk



[1] Cancer risk only in immunosuppressed subjects or in subjects with the genetic mutation (Ramoz et al., 2002) associated with epidermodysplasia verruciformis.
[2] Most commonly associated with anogenital malignancy (from about 30 "high risk" genital HPV types).

my research training in immunology after I migrated from Scotland to Australia in 1981.

Compromised Immunity and Genital Disease—My Introduction to HPV Infection

When I took up a position as a trainee clinical immunologist with Ian Mackay at the Walter and Eliza Hall institute in Melbourne Australia in 1981, I initiated a cohort study of men who had sex with men (the "Middle Park" cohort study). Undertaken with a family practitioner, Peter Meese, and his colleagues, this study of persistent hepatitis B virus infection was, before the recognition of HIV-1, somewhat unexpectedly transformed into an early study of the natural history of HIV infection in Australia (Frazer et al., 1986a). A substantial proportion of men in the Middle Park study were immunocompromised, meaning that they had reduced numbers of helper T cells, and we observed that they did not easily get rid of genital warts. This observation led me to develop an interest in the immunobiology of HPV infection, which at that time was essentially unknown territory. I discussed this topic with Gabrielle Medley, a Melbourne cytopathologist responsible for the highly effective state cervical-cancer screening program. We were aware of Zur Hausen's work demonstrating a possible association of HPV infection with cervical cancer. This led us to start screening the men in the Middle Park study for cytological evidence of HPV-associated anal precancer. The increased frequency of anal cytopathology in men with $CD4^+$ T cell deficiency, a result of what we by then knew to be HIV infection (Frazer et al., 1986b), convinced us that helper T cell responses might be important in the control of HPV infection. After a move to Brisbane, Queensland in 1985 to take up a position as director of the immunology service at the Princess Alexandra Hospital, I was fortunate to be able to recruit Robert Tindle, and together we did much of the early work defining the immunogenicity of the nonstructural proteins of HPV16, the virus type most associated with cervical cancer. Animal studies demonstrated immunogenicity of an HPV protein associated with cellular transformation (E7) (Tindle et al., 1991). It was clear then that human HPV immunobiology would be progressed by creation of HLA-matched cells expressing HPV antigens as targets for an assay of cell-mediated immunity and by better animal models of persistent epithelial infection without inflammation.

A Trip to Cambridge and a Fortuitous Collaboration

In 1989, with the intent of learning how to optimize recombinant expression of HPV nonstructural proteins in mammalian cells, and of making a mouse transgenic for these proteins, I took a sabbatical visit with Lionel Crawford and Margaret Stanley, both acknowledged experts in HPV pathobiology, in the department of Pathology in Cambridge, England. The research program kept me busy with many late nights in the lab, but it was not as productive as I'd hoped. I found that E7, when overexpressed in cells, provoked early cell differentiation and death, without the need for immunological intervention. The E7 transgenic mouse model had to await a further sabbatical, in 1993, to the lab of Paul Lambert in Madison (Frazer et al., 1995). The result was a longstanding collaboration, which has proven extremely useful in defining the requirements for effective immunotherapy for persisting epithelial infections. However, the Cambridge visit had one very important outcome: my meeting with Jian Zhou, a Chinese clinical scientist, then working with Lionel Crawford on expression of HPV genes in mammalian cells by the use of recombinant vaccinia virus. He wanted to better understand how HPV transformed epithelial cells. Together, we came up with the idea that if PV genes when overexpressed singly were lethal to cells and natural HPV16 virus was not available, these problems might be overcome by construction of an artificial HPV16 papillomavirus, which would allow regulated expression and study of PV genes. We thought this might be achieved by packaging the PV genome in the PV capsid proteins, which we could use to infect cells in vitro. Indeed, we subsequently went on to achieve this (Zhou et al., 1993). However, at that time both of our visits to Cambridge were drawing to a close, and I persuaded Jian that we could work together if he and his wife, Xiao Yi Sun, came to Brisbane. He agreed, and this proved to be a happy choice. In late 1990 and early 1991, as part of our strategy for making synthetic HPV16, we demonstrated that expression of the two viral capsid proteins of HPV16 (L1 and L2) in monkey kidney cells, via a doubly recombinant vaccinia virus, resulted in assembly of virus-like particles (VLPs), visible with electron microscopy (Zhou et al., 1991). This was the first convincing demonstration that HPV16 could actually form a capsid because this virus had not been seen with electron microscopy in HPV16-associated clinical lesions. To achieve this, we expressed the major capsid protein of HPV16 (L1) from the second initiation codon in the L1 gene. We identified this initiation codon by comparing the gene sequences of the various PV L1 genes then sequenced. This primitive exercise in comparative genomics was undertaken with paper and pencil because our only lab computer was not up to the job. We and others (Rose et al., 1993; Kirnbauer et al., 1992) subsequently demonstrated that PV L1 genes of various HPV types, if expressed with more efficient eukaryotic expression systems, would self assemble into viral capsids (VLPs)

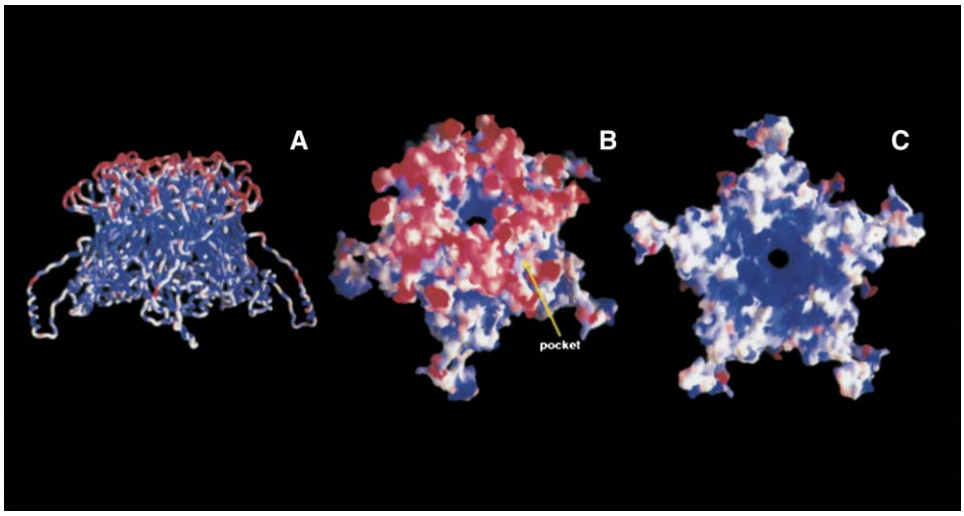


Figure 1. Distribution of L1 Sequence Variation in 49 HPV Types

The crystal structure of an L1 pentamer, 72 of which together comprise the HPV virus-like particle, is shown from various angles. Positions that are highly variable between HPV genotypes are red, fully conserved positions are blue, and positions of intermediate variation are white.
(A) Seen from the side and showing conservation of the regions of interaction between pentamers.
(B) Seen from outside the viral capsid and showing the hypervariability of the interface of the pentamer with the outside world.
(C) Seen from within the capsid and showing conservation of the interior of the pentamer.
Figure reprinted from (Chen et al., 2000).

without L2, albeit with somewhat reduced efficiency. L1 VLPs are deficient in the ability to package viral DNA, and they form the basis of the VLP-based vaccines designed to prevent HPV infection. Of course, for the production of infectious virions we needed L2, with which viral episomes could be packaged with reasonable efficiency to make infectious virus, although better techniques for producing infectious pseudovirions have subsequently been developed by others (Buck et al., 2005).

Immune Responses to Human PV and to Human PV Virus-like Particles

PVs are genetically stable double stranded DNA viruses, and comprise over 200 largely immunologically distinct genotypes. Because high-risk genital PV infections (although so common that for practical purposes they can be regarded as universal) are frequently sub clinical, it remains unclear whether natural immunity induced by infection protects against subsequent viral challenge. This supposition can, however, be inferred for the PV genotypes associated with visible skin and genital warts, as skin warts are commonly a problem of childhood, and genital warts of the first few years of sexual activity. The basis of protection remains unclear. For the common human genital PV types, humoral immune responses after natural infection are largely directed to conformational determinants of the major capsid protein L1, are PV type-specific, and are weak and slow to appear, with only 50% of subjects seroconverting, mostly by one year after infection (Carter et al., 1996). Thus, antibody naturally induced by PV infection may not be the sole means of protecting against further infection. Cows are protected by prior (resolved) bovine PV-1 infection against further viral challenge, and could also be protected against bovine PV-1 challenge by prior immunization with formalized virions prepared from cow warts (Campo, 1994). Protection was associated with antibody that could bind to bovine PV1 virions. Similar results were obtained for canine oral PV, which infects oral mucosa (Suzich et al., 1995). The canine oral PV model showed that VLPs could induce protection, which could be transferred to another animal by the immunoglobulin fraction of serum, whereas denatured VLPs induced immunity to L1 but not to conformational determinants, and no protection. These results suggested that vaccines to prevent human PV based on VLPs would likely be successful if they induced neutralizing antibody. However, the human PV infections of interest are of genital mucosal surfaces and the possibility remained that a strong local mucosal immune response would be required for protection. B cell epitopes of the native virions were mapped by monoclonal antibodies developed against VLPs or virions (Christensen et al., 1996). Despite the extensive amino acid sequence homology (>80%) between the major capsid proteins of the different PV, each PV genotype turned out to be a largely distinct serotype, at least as far as antibodies raised against the native virus structure are concerned. Resolution of the crystal structure of the L1 capsid protein assembled into a mini-VLP (Figure 1) demonstrated that the peptide domains comprising the internal structure of the correctly folded L1 protein, and which interact with other L1 proteins to form the capsomer, are highly conserved, whereas the variability between PV types maps to the domains on the external face of the virion (Chen et al., 2000; Carter et al., 2006). Some cross reactivity between closely related genotypes (6 and 11, 16 and 31, 18 and 45) could be predicted from the monoclonal antibody studies and the mapping of cross-reactive epitopes for these types to likely surface sites on the virion.

Clinical Trials—Assays, Endpoints, and Findings

Two vaccines derived from the VLP technology described above are currently being trialed worldwide.

Gardasil, produced by Merck, includes VLPs that are produced from recombinant yeast and correspond to two human PV types (HPV16 and HPV18) responsible for about 70% of cervical cancer and two types (HPV6 and HPV11) responsible for >90% of genital warts. Cervarix, produced by Glaxo Smith Kline (GSK), includes VLPs that are produced in insect cells via recombinant baculovirus and that correspond to HPV16 and HPV18. The Merck vaccine is adjuvanted with aluminum hydroxide gel ("alum"), and the GSK vaccine is adjuvanted with alum and monophosphoryl Lipid A. Antibody is likely to be the mode of protection against infection induced by vaccination, although this has yet to be formally established because the vaccines to date have proven to be 100% effective in clinical trials, and therefore no correlative marker of protection has been defined. Furthermore, VLP vaccines induce complex immune responses against multiple conformational and linear epitopes of the L1 protein. Assays for antibody fall into three categories: competitive immunoassays where serum is used to displace one or a cocktail of monoclonal antibodies of defined specificity from VLPs, conventional enzyme-linked immunoassays that use VLP substrates, and pseudovirion neutralization assays where inhibition by serum of binding and uptake of VLP capsids into reporter cells is measured. Assays for PV capsid antibody are not standardized, although the World Health Organization (WHO) is developing a reference serum to assist in this process (Ferguson et al., 2006). Results to date indicate that, to a good approximation, each of the assays similarly ranks the magnitude of the total VLP-specific immune responses induced by vaccination of human subjects with human PV16 VLPs. However, it is not clear whether particular specificities of antibody to conformational determinants on the virus are necessary for neutralization or whether the different immunoassays are similarly comparable for immune responses induced by other human PV types.

Natural immunity to PV infection in humans is complex, with a role for innate immune responses, as well as cell-mediated immune responses to viral nonstructural proteins. Protection against reinfection with PV seems solid in those with congenital antibody deficiency, suggesting that cell-mediated immunity as well as antibodies may play a role in protecting against reinfection, and VLPs induce cellular as well as humoral immune responses. Both commercially produced vaccines produce antibody responses 10- to 50-fold greater than those that follow natural infection. Use of monophosphoryl lipid A adjuvant increases the titer of total VLP-specific antibody, but even VLPs delivered without adjuvant are potent immunogens in humans, presumably because of the crystalline arrays of B cell determinants presented by the VLPs. Perhaps surprisingly, VLP-specific antibody in genital secretions in humans and in animals after vaccination is predominantly of the IgG class, and this together with the protection at mucosal surfaces conveyed by passively transferred serum immunoglobulin in the canine oral papillomavirus model, which would not include IgA with secretory piece, suggests that development of mucosal IgA responses may not be particularly important for protection against mucosal PV infection after vaccination.

Several clinical trials of the two commercial vaccines have been undertaken in 18- to 26-year-old sexually active women. These trials were reviewed recently (Lowy and Schiller, 2006) and have shown that the vaccines are 100% effective at preventing not only infection with the high-risk human PVs incorporated in the vaccines but also at preventing the resulting cervical precancer lesions and external anogenital lesions, including genital warts attributable to the vaccine incorporated human PV strains. For both the Merck (Villa et al., 2006) and the GSK (Harper et al., 2006) vaccines, antibody responses are almost universal among immunized subjects previously naïve to the relevant PV type. Peak antibody responses 2–6 months after three immunizations gradually fall over the first two years and then plateau at an amount about 10–20 times the average observed in response to natural infection, with constant amounts observed at least over the next three years. The reason for the observed persistence of antibody is unclear. VLPs are highly immunogenic repetitive arrays of capsomers, immunogenic without adjuvant, and relatively stable, and they may persist and present antigen for many years after immunization. Alternatively, natural boosting after repeated exposure to infection may maintain antibody titer. Data from a cohort of younger women show that the initial titer of antibody after immunization of prepubertal women is about three times higher than that observed in postpubertal subjects (http://www.fda.gov/ohrms/dockets/ac/06/briefing/2006-4222b-index.htm). Follow-up on this cohort, presumably mostly not exposed to virus, should clarify whether the maintained response reflects boosting through natural exposure. Subjects with prior exposure as measured by antibody or PV DNA at recruitment have substantially higher responses to the first vaccine than those naïve at recruitment.

We and others have established that L1 proteins from different PV genotypes are sufficiently closely related in sequence that there are genotype-crossreactive, cell-mediated immune responses as demonstrated by cross-sensitization of delayed type-hypersensitivity responses. The question therefore arises as to whether the phenomenon of "original antigenic sin," or the inability to respond to novel epitopes on heterotypic virus observed after immunization with a homotypic strain, might impair immune responses to newly encountered PV types in those previously immunized with or exposed to PVs of other types. The almost universal exposure to the common skin PV types does not prevent a substantial and host-protective antibody response to the B cell epitopes specific to the vaccine types, suggesting that the lack of shared B cell epitopes between types may prevent such immune diversion. Certainly, prior immunity to T cell epitopes of VLPs prevents cell-mediated immune responses to new epitopes incorporated into the VLP, which may explain the relative failure of natural immune responses to genital HPV types to rapidly clear these infections if prior T helper cell immunity to common epitopes prevents generation of new cytotoxic T cell responses.

There are several unresolved issues concerning vaccine efficacy. Two of particular importance are the consequence for existing PV infection of immunization with VLPs of the corresponding type and the extent to which

the serological crossreactivity between the most closely related PV types, as defined by monoclonal antibodies, might result in clinical protection. We observed that VLPs delivered without adjuvant were immunogenic in human PV6-infected patients with genital warts and, in an open label study, that regression of wart lesions in persistently infected individuals was more common than might be expected from historic controls (Zhang et al., 2000). This observation suggested that HPV VLPs delivered without adjuvant might induce a therapeutic immune response; this hypothesis is now being tested in a randomized placebo-controlled blinded study in China and Australia. The data from the prophylactic vaccine studies using alum adjuvanted VLPs suggest that there are no marked adverse consequences from immunizing currently infected subjects. There may be some therapeutic effect of VLP administration for infected subjects that have not yet made an immune response but none for those with persisting infection associated with measurable humoral immunity. The crossreactivity of the closely related human PV types suggested by epitope-mapping studies has been confirmed clinically for human PV18 and human PV45 in that the PV16 and PV 18 vaccine gives good short-term protection against PV45 but not against other, less-related types (Harper et al., 2006).

Controversies, Intellectual Property, and Access in the Developing World

Medical interventions impacting human sexuality have always been controversial, and there has been substantial "evidence-free" public debate about whether administration of a vaccine designed to prevent one sexually transmitted infection might encourage earlier onset of, or more frequent, sexual intercourse. Fortunately, the optimal age to immunize with the PV vaccine has turned out to be an immunological rather than a moral issue, as discussed above. The FDA recommendation for the Merck vaccine is for administration to 9- to 26-year-old women to prevent anogenital precancer and genital warts. The two commercial vaccines incorporate intellectual property of varied nature and diverse ownership, and despite several granted patents and a four-way patent interference in the USA, intellectual-property issues remain unresolved in the USA and several other jurisdictions. However, the companies producing vaccines have entered into crosslicensing agreements designed to ensure that their respective products will not infringe on the patents to which the other has license.

Cervical cancer is predominantly a disease of the developing world, with >250,000 deaths per annum. Deployment of a vaccine to prevent cervical cancer in the developing world poses logistic, financial, and immunological challenges. Delivery of a vaccine outside of the usual schedules of the expanded vaccine initiative (from 0–2 years) will require specific infrastructure and will attempt to reach a population not currently accessed by any public health initiatives. The price of the Merck vaccine in the USA is $360. However, both vaccine companies have indicated that they will introduce differential pricing for the developing world, as is currently the case for hepatitis B vaccines. Additionally, the Program for Appropriate Technology in Health (PATH) and the WHO are developing recommendations for possible vaccine-delivery programs for the developing world, and alternate vaccines based on bacterial recombinant L1 pentamers, possibly easier to manufacture in the developing world, are being developed (Yuan et al., 2001). However, it will be necessary to evaluate field effectiveness of the vaccines in the developing world, particularly in view of the malnutrition, endemic malaria, and adolescent iron deficiency, each of which impact the development of new immune responses and are concerns in many countries with a high prevalence of HPV infection and cervical cancer. A large vaccine project underway in Guanacaste, Puerto Rico as a joint initiative between GSK and the National Institutes of Health may help to address these issues, and similar demonstration projects are being planned elsewhere, including Vanuatu.

Conclusion

The HPV and immunology research communities can take credit for the development of the world's first vaccine to prevent a specific cancer. It would be a most satisfying outcome from my involvement in HPV immunology research to see the vaccines effectively deployed in the developing world within our lifetimes. Successful delivery to the developing world should show the way for introduction of further vaccines to prevent sexually transmitted infections, as well as common infections rarely responsible for cancer. The PV vaccine is only the second licensed vaccine to use recombinant-DNA technology to produce the antigenic ingredient, but it will surely not be the last.

Acknowledgments

This article is dedicated to Andy Zhou, the son of the late Jian Zhou. Jian Zhou tragically passed away in 2000 at the early age of 40. I acknowledge the many students and scientists who along the way have guided my career, helped with my research, and generally inspired me, as well as the very many more colleagues worldwide who have played significant roles in the development, testing, and bringing to market of the PV vaccines.

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