Prophylactic and Therapeutic Vaccines for Genital Papillomavirus Infection

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The development of potential therapeutic and prophylactic vaccines for human papillomavirus (HPV) infection is a very exciting area of HPV research. There are a number of features of HPV biology that makes the development of a vaccine particularly difficult, although there are several examples of vaccines that have had spectacular success in the prevention of other viral diseases. Our poor understanding of the immune response to HPV infection is the first problem. We do not understand the mechanism by which spontaneous clearing of warts is generated and therefore cannot particularly target this pathway in the development of a vaccine. Furthermore, there is no in vitro culture system nor an animal model for HPV. Another problem is that there is no ready source of live virus that might be exploited for a live attenuated viral vaccine, such as was used with poliovirus. Although most other viruses spend a portion of their life cycle in the systemic circulation where they are vulnerable to neutralizing antibodies, HPV remain exclusively in the epithelium and thus antibodies must transverse the basement membrane and reach the other layers of the skin or mucosa to be effective in preventing infection. Significant progress is being made in the development of potential vaccine candidates despite these and other confounding factors. Key words: antibodies/virus-like particles. Journal of Investigative Dermatology Symposium Proceedings 6:238–243, 2001

MICROBIOLOGY

Human papillomaviruses (HPV) are small, nonenvelopedicosahedral viruses with a diameter of about 55 nm containing 8–10 genes on circular double-stranded DNA. Three functional regions are contained in the HPV genome. Transcription enhancer and promoter elements are found in the long-control region. Open reading frames are found in the early region, the products of which control viral replication, transcription, and cellular transformation, and encode for E6 and E7 oncoproteins. Two capsid structural proteins (the L1 major and the L2 minor) are encoded in the late region. There is immunogenic, and thus vaccine potential, in each of these proteins. Humans can be infected by over 80 types of papillomavirus. High-risk HPV types include HPV 16, 18, 31, 35, 45, 51, 52, 56, 58 and 66 as they may cause squamous cell carcinoma and premalignant cervical lesions (Lorinez et al., 1992; Kiviat and Koutska, 1993; Schiffman et al., 1993; Bosch et al., 1995). Over 90% of cervical carcinomas (Gissmann et al., 1983; Brown et al., 1993; Walboomers et al., 1994) are associated with HPV types 16 and 18. At least 80% of anal carcinomas contain HPV DNA, type 16 being the most common (Zaki et al., 1993). Over 90% of condyloma acuminata are caused by low-risk HPV types 6 and 11.

IMMUNOLOGY/IMMUNOBIOLOGY OF INFECTION

Stratified squamous epithelial cells are infected by HPV. E1 helicase and E2 transactivator are responsible for viral DNA replication and RNA transcription. The viral capsid proteins (L1 and L2) are produced in the upper layers of differentiating epithelium. Human papillomavirus-containing epithelial cells are shed from the surface of the skin. A relatively large area of skin, probably in the order of 2–3 mm² (Steele and Gallimore, 1990), can be infected by the progeny of a single HPV-infected stem cell. As there is no cell lysis, there is no release of viral proteins and poor antigen presentation to the immune system. Antibody to both capsid proteins of the virus are produced, however, during the course of HPV infection (Christensen et al., 1992). After an average of 6 mo following infection this acquired immunity occurs and may be protective against reinfection, but is not likely to be therapeutic.

The progression of HPV infection to clinical disease appears to be regulated by host immune responses to HPV. Both humoral and cell-mediated immune responses are elicited by HPV. A crucial role in modulating the effects of HPV, such as lesion persistence and spontaneous regression, is played by cellular immunity, particularly the T cell system. The fact that immunosuppressed individuals (transplant, lymphoma, HIV disease) have enhanced HPV proliferation and an increased frequency of HPV infection and associated disease (Kast et al., 1996) illustrates the importance of cellular immunity. In addition, reduced numbers of Langerhans cells are found in CIN (Sherman et al., 1998), and dense infiltrates of T lymphocytes and macrophages are found in regressing warts.

In benign lesions viral DNA remains extrachromosomal. The viral DNA is incorporated into the host chromosome in most cervical cancers. Two of the viral genes, E6 and E7, are consistently retained and expressed in cervical cancers. These same HPV types show transforming activity (Schlegel et al., 1988) in cultured cells. Continued growth requires these
transformation proteins that may also act as tumor rejection antigens.

**CLINICAL DISEASE**

Most clinically apparent genital HPV infections are benign. Subclinical genital infections with HPV are extremely common. A spectrum of disease results from genital HPV infection ranging from asymptomatic infection to invasive cervical cancer. Based on their association with cervical cancer, HPV types have been divided into low and high risks. External genital warts are the most common clinical expression of infection with low-risk HPV (Beutner et al., 1998a). The giant condyloma of Buschke-Lowenstein caused by HPV type 6 (and sometimes HPV 11) is a rare verrucous carcinoma of the external genital area. Weeks, months, or years may be required from the time of HPV infection to development of warts. Warts, once clinically apparent, may persist, spread, grow, spontaneously regress, and/or recur. A rare but devastating condition caused by perinatally acquired laryngeal low-risk HPV infection is termed juvenile laryngeal papillomatosis (JLP) and is usually due to infection with HPV 6 or 11.

The clinical manifestations of high-risk HPV types include abnormal pap smears, low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), atypical squamous cells of uncertain significance (ASCUS), and atypical glandular cells of uncertain significance (AGUS) or, on biopsy, cervical intraepithelial neoplasia, carcinoma in situ, and invasive cervical cancer. Some anogenital cancers are strongly associated with infection with certain HPV types. Almost all invasive cervical cancers and cervical intraepithelial neoplasia (CIN) contain HPV DNA. Weeks to decades may pass between infection to the appearance of cervical dysplasia and cervical cancer. As the vast majority of women infected with high-risk HPV never develop cervical cancer, these HPV types are considered necessary but not sufficient for the development of cancer. Certain cofactors are necessary but are currently not well understood. Thus, it is believed that if the acquisition of HPV could be prevented, cervical cancer could be greatly reduced, if not eliminated.

The clinical manifestations of the high-risk HPV types on the external genital area are papular lesions (Bowenoid papulosis) or erythematous scaly patches (Bowen’s disease), which histologically have been classified as squamous cell carcinoma in situ or vulvar intraepithelial neoplasia, or penile intraepithelial neoplasia; or, in the vagina, as vaginal intraepithelial neoplasia. Rarely, invasive squamous cell cancer of the penis, vulva, or vagina results.

The oncogenic potential of the high-risk HPV types is frequently found in the anus as well as the cervix, because both structures histologically have transformation zones where columnar epithelium changes to stratified squamous epithelium. Anal HPV infection has best been characterized in men, and its natural history is the subject of ongoing studies.

**EPIEIDOLOGY/EXPECTED IMPACT OF AN EFFECTIVE VACCINE**

It has been estimated that 1% of sexually active persons in the U.S.A. have visible genital warts (Koutskey et al., 1988). Because HPV 6 and 11 cause the majority (≥90%) of genital warts, it is feasible to develop an effective bivalent vaccine for these low-risk infections. Genital warts consume healthcare resources and are emotionally troubling for the patients, although they are rarely associated with significant mortality or morbidity. Thus, immunization is an important goal. HPV infects about 50% of young sexually active women (Bauer et al., 1991; Koutskey et al., 1992; Ho, 1998). The leading cause of cancer death in women under the age of 50 worldwide is cervical cancer (Rowen and Lacey, 1998). In countries where screening is unavailable or underused the impact of HPV infection is greatest. Vaccination against HPV is an attractive goal due to this strong association between sexually acquired HPV and cervical cancer. The majority of cervical carcinomas is associated with relatively few HPV types and animal models have consistently shown that both prophylactic and therapeutic vaccination is feasible. The worldwide public health implication of both preventive and therapeutic vaccines not only includes saving lives, but also decreasing the cost of screening and treating premalignant cervical disease. The annual cost of HPV-related disease has recently been estimated by the Institute of Medicine to be $10 billion (Eng and Butler, 1996).

**HISTORY/BACKGROUND OF VACCINE RESEARCH AND OTHER STRATEGIES FOR CONTROL**

The inability to culture HPV and host tropism of the virus has hampered vaccine development. Cottontail rabbit papillomavirus (CRPV) studies established over 60 years ago that antibodies elicited by the injection of intact virions protect against experimental challenge by the homologous viral type (Shope, 1935). Intact virions displaying immunodominant epitopes were found to be needed to induce protective antibodies (Shope, 1935; Kidd, 1938; Pilacinski et al., 1986; Jin et al., 1990; Ghim et al., 1991). It was demonstrated that the L1 major capsid protein of HPV expressed in eukaryotic cells self-assembles into virus-like particles (VLP), which resemble authentic virions without the viral genome and its transforming genes. Neutralizing antibodies generated against confirmational epitopes found on the surface of native virions and VLP are sufficient to prevent infection both in vivo and in animal models.

Immunotherapy is another strategy for HPV control. Cells involved in the host defense can be collected from patients with cervical cancer or a histocompatible donor and then grown and activated ex vivo by cytokines such as recombinant IL-2 and transferred back to the cancer patient as therapy in a process known as adoptive cellular transfer (Hines et al., 1998). This approach (Boursnell et al., 1996; Krul et al., 1996) provided some protection against HPV 16 and 18 E6 and E7-positive tumors in mouse models.

**VACCINE STRATEGIES**

**Prophylactic vaccines** Protective anti-HPV antibodies to prevent infection are induced by HPV subunit vaccines. Antigenic targets in most prophylactic vaccine studies consist of L1 and L2. Fusion proteins, vaccinia virus recombinants, plasmids, and VLP are used in preparation of these vaccines. Molecular techniques (Rose et al., 1993) are used to produce VLP that are spherical 50 nm structures resembling hollow viral capsids. These VLP lack oncogenic DNA, but possess structurally intact viral capsid proteins and may be used in enzyme-linked immunosorbent and hemagglutination assays to detect humoral responses to HPV (Sherman et al., 1998).

**Therapeutic vaccines** Therapeutic vaccination may be used to eliminate residual cancer, cause regression of existing CIN or warts, or prevent progression of infection or low-grade disease to higher-risk lesions. Potential targets in the development of therapeutic vaccines include HPV E6 and E7 epitope peptides selectively maintained and expressed during malignant progression. Human leukocyte antigen (HLA)-specific, human cytotoxic T lymphocytes (CLT) induced against E6 and E7 peptides have been shown to cause lysis of HLA-specific HPV-positive cervical carcinoma cell lines (Feltkamp et al., 1993; Alexander et al., 1996) in in vitro studies.

**ANIMAL MODELS**

Because HPV is species-specific, it does not cause disease in animals, so extrapolating data to humans must be done with caution. Encouraging results have been obtained, however, using species-specific PV. Experimental infection in vivo can be prevented with immunization with proteins produced in bacteria or immunization with vaccinia vectors that express L1 and/or L2, although low levels of neutralizing antibodies are induced (Pilacinski et al., 1986; Jarrett et al., 1991; Lin et al., 1992). On the other hand, VLP vaccines are strongly immunogenic. Experimental
infection with native virus specific to beagle dogs (COPV) (Bell et al, 1994; Lin et al, 1994; Suzich et al, 1995), cottontail rabbits (CRPV) (Donnelly et al, 1991; Lin et al, 1993; Breitburd et al, 1995; Christensen et al, 1996), and calves (BPV) (Kirnbauer et al, 1991; 1994; 1995), in beagle dogs (COPV) (Bell et al, 1994; Lin et al, 1994; Suzich et al, 1995), cottontail rabbits (CRPV) (Donnelly et al, 1991; Lin et al, 1993; Breitburd et al, 1995; Christensen et al, 1996), and calves (BPV) (Kirnbauer et al, 1991; 1994; 1995). These observations are as follows: 1 more persistent and more frequent clinical expression of HPV infection (which is usually more difficult to treat) results from immunosuppression; 2 vaccine studies with animal papillomavirus systems have produced favorable results; 3 many infected humans never express their clinical lesions; 4 external genital warts, nongenital warts, and HPV-related cervical lesions often regress spontaneously; 5 infiltrates of regressing warts often contain CD4 lymphocytes and macrophages; 6 autologous vaccines have been reported to be successful in humans.

The possibility that a genital HPV vaccine might not be effective, on the other hand, is suggested by the following factors that make HPV vaccines particularly challenging:

- **There is no clear definition of protective immunity, nor is the necessary response to particular antigens well understood.** Antibodies appear to protect against infection in animal systems, and cell-mediated immune responses are required for wart regression or protection against the development of warts.
- **With HPV the route and frequency with which the immunized subject is exposed naturally may be more chronic and repetitive than with other viral infections for which we have effective vaccines.** The level of immunity required to protect against frequent genital exposure is unknown. Viral infections for which we currently have vaccines are not characterized by frequent (potentially daily) exposure for years.
Although both prophylactic and therapeutic vaccines are feasible (Table II), prophylactic vaccines are the most appealing, based on experience with animal PV and in humans, with a number of viral vaccines in clinical trials. A vaccine to control genital HPV probably would need to involve immunization of both men and women. Our knowledge of detection of high-risk HPV in women, the attack rate of HPV in women, and the natural history of HPV in women, however, is much greater than our information about infectivity, attack rate, and natural history of HPV in men.

Development of vaccination strategy and rational design of clinical trials is difficult without this information. The lack of a readily available standardized and validated serologic test to determine who is "immune" and who is susceptible is another obstacle to HPV vaccine development and implementation. VLP ELISA and pseudovirus infectivity assays serve as surrogate markers. In addition, the detection of viral DNA and cervical lesions will characterize patients at entry and serve as markers to determine vaccine efficacy.

There are major psychosocial challenges in addition to the scientific challenges. We do not know if the public is ready to vaccinate their children for sexually transmitted diseases. In addition, small but vocal groups question the value of current childhood vaccinations. There is very little awareness of HPV among the general public, and the risk of acquiring HPV and the reality that cervical cancer is a sexually transmitted disease are unfamiliar concepts outside the medical community.

POTENTIAL VACCINES

Peptide vaccines Peptide vaccines have obvious therapeutic appeal, particularly if capable of binding to HLA and generating cytotoxic T lymphocytes. Peptide vaccines are in early trials as candidate therapeutic vaccines for cervical cancer.

Proteins Production of fusion proteins of all HPV gene products is now possible, but which of these or which combinations should be studied in clinical trials is not known. An HPV6 L2-E7 fusion protein has been shown to be immunogenic, and that until very recently there have been no studies prospectively powered and designed to establish the frequency of or time to recurrence following treatment of genital warts with any modality, although published recurrence rates with most modalities appear to be high. In order to design vaccine trials adequately, this information will be required.

High-risk HPV types Prevention of cervical cancer is the obvious aim of vaccination against high-risk HPV types, but it is not a practical end-point for human vaccine trials. Because the biopsy may alter the natural history, histologic end-points are not feasible. Several potential surrogate end-points are of potential use: the acquisition of infection, the virologic persistence, and a decrease in the proportion of subjects who experience cytologic abnormalities.

In theory the best way to prevent cervical cancer is to prevent the acquisition of high-risk HPV infection, but this may also be the greatest challenge. The lack of a serologic test to accurately identify those who are either susceptible or immune is one of the many problems with this end-point. While virgins would be susceptible, limiting enrolment to virgins has some logistic limitations.

The persistence of HPV, not just the acquisition of a short-lived infection, is a critical step in the pathway to cancer, which may help explain why only a minority of those infected develop cervical cancer. Cervical cancer may thereby potentially be prevented using a vaccine to decrease the proportion of the population with persistent HPV. Inherent limitations in terms of interpretation and reproducibility exists with the use of a screening cytologic test as a clinical end-point.

CONCLUSION

We have come a long way on the path to a vaccine considering that HPV cannot be grown in vitro and that until very recently there have been no serologic tests for HPV. Although large-scale clinical trials are now underway, it is difficult to predict how soon we will have effective prophylactic and/or therapeutic HPV vaccines.

REFERENCES


Bourneu M, Rutherford E, Hickling J, Rollinson E, Munro A, Rolley N:
Tindall RW, Croft S, Herd K, Malcolm K, Ge czy AF, Stewart T, Fernando G: A vaccine conjugate of ISCAR immunocarrier and peptide epitopes of the E7


