REVIEW

Cervical Cancer: Etiology, Pathogenesis, Treatment, and Future Vaccines

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Abstract

Cervical cancer is a sexually transmitted disease caused by the human papillomavirus (HPV), especially HPV-16 and -18. Of the half million new cases of cervical cancer reported yearly, 20% occur in India. Mass cancer screening programs to detect and treat cervical cancer and its precursor lesions are not available in India and most other developing countries because of the lack of resources. Curative and palliative treatments are not the same for all patients with cervical cancer because the result depends on the immunological response of the patient. This article describes the natural history of cervical carcinogenesis and the rational behind various modalities of prevention and treatment for the practising gynecological oncologist. Prophylactic vaccines against HPV-16 and -18 and therapeutic vaccines against cervical cancers should be able to overcome the logistical problems that now exist to screen, diagnose and treat cervical cancer and its precursor lesions.

Key Words: Human papillomavirus - pathogenesis - cervical cancer - treatment - prophylactic vaccines - therapeutic vaccines

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Introduction

Cervical cancer is the major cause of cancer deaths in women worldwide (Jenson & Lancaster, 1990; Schlegel, 1990; zur Hausen & DeVillier, 1994; Bosch et al., 1995; Wallboomers et al., 1999; Munoz, 2000; Tyring 2000; Adams et al., 2001; Lehtinen et al., 2001). The global estimate for 2000 was 470,600 new cases of cervical cancer and 233,400 deaths (Ferlay et al., 2001). Of the half million new cases of cervical cancer reported yearly, nearly one-fifth are detected in India alone. Removal of the primary cancer by surgery and the immunological response to the metastatic cancer cells remaining after surgery play a major role in the morbidity and mortality of patients with cervical cancer (Delgado, 1978; Da Silva et al., 2001; Graflund et al., 2002). The 5-year age-standardized (0–74 years) relative survival from cervical cancer varies from 28.0% (Philippines) to 70.1% (U.S. white population).

The standard treatment regimen for cervical cancer is radical hysterectomy with pelvic lymph node dissection (Kamura et al., 1993) for early stage disease with the addition of radiation, chemotherapy (most frequently cisplatinum based [Park & Thigpen, 1993]), or both for advanced stages (Ferenczy & Jenson, 1996). However, some physicians do not use chemotherapy unless absolutely necessary because it adversely suppresses the immune response against cancer cells (Emens et al., 2001; F. Price, personal communication). Metastatic cervical cancers that are confined to the lymphatics and induce a protective immune response in lymph nodes (Ilyin et al., 1979; Kinugasa et al., 1991) appear to have a better prognosis than cancers that are not impeded by the immune response or are found in extranodal sites, stimulating angiogenesis (Dinh et al., 1996; Cooper et al., 1998; Di Leo et al., 1998; Garozzo et al., 2000).

In 1978 Delgado reviewed the world's literature and compared the survival of 1523 patients with stage 1B cervical cancer 5 years after radical hysterectomy and pelvic node dissection with and without metastasis. None of the patients was treated with radiation or chemotherapy. Of the 1523 patients, 179 had positive nodes and 1344 had negative nodes; 5-year survival was 48.1% for patients with positive nodes and 88.6% for patients with negative nodes. Our interpretation of these findings is that the removal of the primary uterine cancer resulted in persistent, metastatic cervical cancer, most likely confined to the lymph nodes, where the formation of new cancer cells was in equilibrium with the destruction of preexisting cancer cells, presumably by the immune system. This phenomenon (sometimes designated tumor dormancy) has been reported in various cancer patients, particularly those with antigenic cancers

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such as melanoma and renal cell carcinoma.

Certain medications such as corticosteroids may upset the delicate balance between renewal and destruction of cancer cells by the immune response, innate or otherwise. A marker to distinguish which cervical cancers are persistent or less progressive would enable physicians to better utilize scarce resources for treatment such as radiation and chemotherapy.

Susceptibility of the Cervix to Human Papillomavirus Infection

Virtually all cervical cancers are sexually transmitted diseases (Dillner et al., 2000) caused by carcinogenic human papillomaviruses (HPVs) that are unimpeded by barrier contraceptives and infect unstable (metaplastic) cervical squamous epithelium of the transformation zone (Jenson & Lancaster, 1990). Young women have large areas of immature metaplastic cervical epithelium, which appear to be the most susceptible of all squamous epithelia to infection by carcinogenic HPV. If sexual activity begins at an early age, especially with multiple partners harboring carcinogenic HPV, the women are put at high risk for developing cervical neoplasia (Koutsky et al., 1988; Vittorio et al., 1995; Dillner et al., 2000). HPV-induced carcinoma of the cervix can develop within 2 years after initial infection of unstable squamous epithelia of endocervix; however, most cancers develop from or adjacent to precursor lesions that progress from one stage to another over 10–30 years. Over time, uninfected metaplastic squamous epithelium matures and appears to become more susceptible to no-risk or low-risk viruses such as HPV-6 that have a tropism for mature squamous epithelium of the mucosal surfaces (A.B. Jenson, unpublished observations).

It has been shown that 99.7% of all cervical carcinomas bear high-risk HPV when corrections are made for nonrepresentative tissues and inadequate DNA (Walboomers et al., 1999). HPV infection and its sequela are necessary to cause virtually all invasive cervical cancers. Of these, 75% are caused by HPV-16 and -18 (Bosch et al., 1995; Munoz, 2000). These two subtypes of HPV appear to occupy a niche worldwide in their role as the major causes of cervical cancer. It is clear that vaccination, either with a prophylactic vaccine or by therapeutic intervention, will have to target HPV-16 or -18. Induction of neutralizing antibodies by recombinant prophylactic vaccines will prevent infection and the subsequent development of cancer. Therapeutic vaccines in combination with biological response modifiers (Street et al., 1997; Ghim et al., 2001) will be necessary to stimulate an efficient antitumor immune response to cause regression of the tumor.

Screening Programs for Cervical Cancer

Successful mass cervical cancer screening programs account for the difference between the countries having a high incidence of cervical cancer and those having a low incidence. Screening can be accomplished by either Pap smear cytology or testing for carcinogenic HPV DNA (Castle et al., 2002). The latter test is more sensitive because it detects virus in abnormal cells and in adjacent atypical squamous cells of undetermined significance (ASCUS). Normal-appearing cells circumferential to the lesion also contain latent papillomavirus (PV) DNA (Ferenczy et al., 1985). For these reasons, the cross-sectional sensitivity of HPV DNA test in detecting cervical intraepithelial neoplasia (CIN) 2 and 3 and invasive cancer is significantly better than cervical cytology in most studies (Table 1).

The definitive test for cervical cancer is the colposcopically directed biopsy, revealing the architectural arrangement of abnormal cells that were not scraped. Most women who develop cervical cancer have not been screened for at least 5 years and, most importantly, cannot mount an immune response to clear the virus-infected cells from their cervix. In most instances, two consecutive positive screening tests—abnormal Pap smears or DNA tests positive for carcinogenic HPV, especially HPV-16—are warning signs that the woman is at increased risk for developing a high-grade lesion (M. Schiffman, personal communication).

Testing for carcinogenic HPV DNA is more sensitive than the Pap smear for the detection of high grade CIN lesions and cervical cancers in all countries where the two screening tests have been compared (Table 1). The sensitivity of HPV DNA testing is statistically more significant than that of the Pap smear when positive screening tests (HPV [+] DNA tests, Pap [+] ASCUS, CIN 1 through CIN 3) are referred to colposcopy for a definitive diagnosis. The presence of carcinogenic HPV is more frequently associated with CIN 3 lesions and cervical cancers than abnormal Pap smears.

Social customs in different countries and various taboos associated with examination of the anogenital tract of women may make a difference in the effectiveness of well-intended cervical screening programs. Recruitment of males for their

Table 1. Comparison of Performance of HPV DNA Test for Detection of High-grade Squamous Intraepithelial Lesions

Study	Country	HPV DNA Sensitivity (%)	Cytology Sensitivity (%)
Schiffman et al. 2000	Costa Rica	88	78
Belinson et al. 1999	China	98	94
Womack et al. 2000	Zimbabwe	81	44
Ratnam et al. 2000	Newfoundland	68	27
Blumenthal et al. 2001	Zimbabwe	80	44
Wright et al. 2000	South Africa	84	61
Cuzick et al. 2000	UK	95	79

input into Pap smear screening, particularly of sexual partners, may be necessary to develop an effective screening program (A.B. Jenson, unpublished information). The self-collection method of screening, particularly for detection of virus DNA, circumvents social customs that hinder modern medicine. Reasonably sensitive (Sellors et al., 2000; Wright et al., 2000; A.T. Lorincz, personal communication), it appears to be preferred by most of the women screened. Regardless, successful screening for cervical cancer and its precursor lesions depends on analyzing the scrapings or collections of cervical cells that have exfoliated from the endocervical canal. For the screening to be complete, the endocervical cells still have to be processed and identified as abnormal cells by cytology or for carcinogenic DNA.

The screening programs based on cytology face various logistical problems in different countries. Of these, recruitment of qualified screeners who can properly set up laboratories and institute quality assurance programs may represent one of the biggest challenges. Ingenuity almost always provides the answers: highly successfully programs in redeveloping countries have focused on recruiting disabled individuals and training them as highly competent Pap smear screeners. These individuals are mentally competent but are otherwise shunned or barely tolerated by society in general because of physical deformities. They have been among the most efficient and proficient screeners of Pap smears that one of us has evaluated (A.B. Jenson, unpublished observations). However, in most low- or middle-income countries, cytology-based screening programs cannot be implemented because of constraints of funds, lack of trained pathologists, the absence of facilities for further evaluation of the cytology-positive cases, and poor compliance among women at risk—sexually active women (Sankaranarayanan et al., 2001).

Prophylactic and Therapeutic Vaccines

Inexpensive prophylactic and therapeutic vaccines (Frazer, 1996; Schiller & Lowy, 1996; Breitburd & Coursaget, 1999) hold the greatest promise for a successful campaign against cervical cancer in many redeveloping countries where resources can not be extended to screen most sexually active women and to treat dysplasias by the various methods of cervical ablation or excision. Even if a successful prophylactic vaccination program was implemented today, it would be years before there would be a noticeable positive effect. Uninfected girls and young women may be the primary recipients of a recombinant (HPV-16 and -18) vaccine, but all women should be vaccinated if it is financially and logistically feasible. Although women may already be productively infected with HPV-16, only 50% may have circulating neutralizing antibodies against the virus (Cater et al., 2000). If a woman is vaccinated while infected, the vaginal secretions that accompany sexual activity may contain enough neutralizing antibody to partially or completely prevent infection of her sexual partner (A.B. Jenson, unpublished observation). Vaccination under these circumstances may also prevent self-inoculation during intercourse, thereby preventing the spread of infectious virus to the rest of the anogenital tract.

In contrast to prophylactic vaccines, successful therapeutic vaccines could possibly be effective within weeks or months. However, a therapeutic vaccine will be much more difficult to develop because of the steps that the virus—not the cancer cell—has taken to avoid immunosurveillance (Tindle, 2002; Riethmuller and Scilles, 2000; Ghim et al., 2001) and to successfully set up a subviral infection, expressing E6 and E7 oncoproteins at levels necessary and sufficient to maintain the malignant state (Shah & Howley, 1990; zur Hausen & DeVillier, 1994; zur Hausen, 1999; Ghim et al., 2001).

Therapeutic Cervical Cancer Vaccines

In women with cervical cancer, the immune response that has evolved to protect the cervix from infection and development of cancer fails to clear virus from the newly infected keratinocytes (Bal et al., 1990; De Bueger et al., 1993; Nakagawa et al., 1997; Neckoloff et al., 1997; Lechler et al., 2001). Development of tolerance to the virus-specific antigens (VSAs) derived from the virus oncoproteins E6 and, especially, E7 in newly infected keratinocytes continues as tolerance to the very same antigens; these antigens are designated tumor-specific antigens (TSAs) in dysplastic cells and cervical cancers. Infected keratinocytes, cancer cells, and their precursors are not eliminated. The two virus oncoproteins bind to and alter the function of the cellular suppressor genes P53 and RB, respectively, resulting in the accumulation of nonlethal cellular mutations responsible for the pathological findings designated as dysplasias (zur Hausen, 1999). The widely accepted role of therapeutic cancer vaccines is to break tolerance induced by the TSA of the cancer cell. Because VSAs and TSAs are one and the same, therapeutic vaccines should also be capable of preventing the development of precursor lesions of cervical cancer by the induction of VSA/TSA-specific cell-mediated immunity that clears the virus by killing infected keratinocytes, condylomata, and dysplasias.

Circumvention of Immunosurveillance

Evidence suggests that tissue-infiltrating lymphocytes that recognize either VSAs or TSAs are actually rendered unresponsive (tolerized) by infected keratinocytes. Unlike antigen-presenting cells, infected keratinocytes do not have receptors for VSAs and TSAs and co-stimulatory molecules to activate and expand appropriate lymphocytes into antiviral and antitumor lymphocytes. Because of this, potential antiviral and antitumor lymphocytes become anergic (unresponsive) by a phenomenon known as peripheral tolerance (Bal et al., 1990; DeBuegei et al., 1993; Doan et al., 1999; Nakagawa et al., 1997; Neckhoff et al., 1997; Lechler et al., 2001). This circumvention of immunosurveillance is probably adopted by HPV-induced warts and oral papillomas, enabling them to persist for long periods before undergoing regression, most likely when tolerance is broken by trauma and the resulting inflammation

in the vicinity of the wart or papilloma (Ghim et al., 2001). The major difference is that the cutaneous lesions invariably remain benign, causing no more than unsightly cosmetic problems.

In the cervix, tolerized lymphocytes cannot clear infected cervical cells, and a lesion will persist unless some type of trauma intervenes to cause a proinflammatory environment, such as ablation of the cervix by cauterization and caustic chemicals. Tolerance is probably broken when virus proteins and other chemoattractants from dead or dying epithelial cells attract phagocytic antigen-presenting cells into the area and, because of the presence of co-stimulatory molecules, prime and expand the antivirus and antitumor lymphocytes that clear the virus and cause regression of cervical cancer precursor lesions. If the lesion is not detected clinically and develops into a high-grade dysplasia, the major histocompatibility complex (MHC)-1 of the cellular immune system is either specifically or nonspecifically downregulated and the E6 and E7 (TSA) antigens are no longer presented to the immune response in the context of the MHC-1 molecules (Conner and Stern, 1990; Cromme et al., 1994a and 1994b, Honma et al., 1994; Keating et al., 1995). At this time, it no longer matters whether peripheral tolerance persists or is broken because antitumor lymphocytes have no target to attack on the neoplastic cell and the cell-mediated response is downregulated. In many of these patients, virus-type-specific E6 and E7 antibodies can now be detected in the systemic circulation (Ghim et al., 2001; Ghim et al. unpublished observations).

Detection of circulating antibodies against the virus oncoproteins may be an important prognostic factor in cervical cancer (Gaarenstroom et al., 1994; Baay et al., 1995; DeGruijl et al., 1996; Scott et al., 1999: Zumbach et al., 2000; Hapfl et al., 2000). The immunoglobulin (Ig) G isotype reactivity with the E6 or E7 oncoprotein appears to reflect the prognosis. Reactivity of recombinant E6 and E7 with IgG1 is a component of the Th-2 response, or antibody-mediated response, which in this setting is inefficient for altering the course of the tumor but may exist to eliminate E6- and E7-IgG1 immune complexes from the circulation. On the other hand, a predominant IgG2 response is part of the Th-1 response, or cell-mediated immune response (Romagnani, 2000), and is likely to be an indicator of antitumor immunity, particularly when the cancer is confined to the lymph nodes. A predominant Th-1 response to virus oncoproteins during intraepithelial neoplasia is usually associated with the clearing of virus and the intraepithelial lesion from the cervix. Both IgG1 and IgG2 reactivities with virus oncoproteins are virus type specific, helping type the virus and potentially providing a marker for effectiveness of traditional treatment or vaccination. In the few selected patients we followed with these serological tests, a predominant IgG2 response against E7 was associated with periods of tumor stability, especially when the cancer was confined to the lymph nodes. An IgG1 response was usually associated with various rates of progression of tumors, particularly in extranodal sites (A.B. Jenson & S. Ghim, unpublished observations).

Therapeutic intervention of cervical neoplasias has three known hurdles to be overcome, all related to the virus infection within the neoplastic cells (Ghim et al., 2001). An effective therapeutic vaccine given at a site distant from the cervix can break peripheral tolerance, activating and expanding antitumor lymphocytes. Biological response mediators or their equivalent will be used to upregulate the target of cell-mediated immunity—the presentation of virus antigens in the context of upregulated MHC-1 molecules—and the existing Th-2 response to virus proteins will have to be switched to a predominant Th-1 response to target the TSA presented in the context of upregulated MHC-1.

In addition to the three hurdles that should be overcome by therapeutic vaccines, other factors may hinder therapeutic successes. Published reports suggest that the earlier a potentially efficacious therapeutic vaccine is implemented the more likely it is to be successful. It is well-documented that the immunological response necessary for successful therapeutic vaccination becomes suppressed to various degrees after surgery, radiation, and chemotherapy (Decker et al., 1996; Hensler et al., 1997; Brune et al., 1999; Emens et al., 2001). The presence of extranodal cancer may be a major factor in the response of the cancer to immunotherapy and biological response modifiers. The persistent subviral infection, which makes cervical cancer cells so highly antigenic and sensitive to the immune response, generates a high rate of mutational events because of continual overexpression of E6 and E7 oncoproteins and eventually induces cancer cells that are refractory to chemotherapy and the existing immune response (Ghim et al., 2001).

Future Therapeutic Vaccines

Different combinations of therapeutic modalities may be used to break tolerance and switch classes from a Th-2 to a Th-1 response. The immune system may have to be stimulated by vectors continually expressing the E6 and E7 proteins. DNA vectors containing HPV E6 and E7 genes have been shot by gene guns into somatic cells that subsequently express E6 and E7. The biggest hurdle facing many of these techniques is the presence of circulating E6 and E7 antibodies that form an insoluble immune complex with E6 and E7 in antibody excess, rendering the antigen incapable of being taken up and processed by antigen-presenting cells. This problem also may exist with chimeric virus-like particles (VLPs) with E7 fused to a structural virus protein internal to the pseudocapsid. Unlike authentic virions, the pseudovirion is porous to antibodies (Ghim et al., 1996), and the anti-E6 and -E7 antibodies are most likely capable of forming antigen-antibody complexes with internalized oncoproteins carried by chimeric VLPs, leaving the antigenic determinants bound as immune complexes within the VLP. Regardless, the therapeutic vaccine will be composed of different combinations of therapeutic agents (i.e., E6 and E7 delivered by live vectors in peptides [Velders et al., 1998] or proteins either directly or indirectly in the form of specific

nucleic acids, chimeric VLPs [Nieland et al., 1999; Rudolph et al., 2000], or cell-based vaccines [Ling et al., 2000] with biological response modifiers [Street et al., 1997; Ghim et al., 2001; Tindle, 2002]) to address the various pathways that the virus has taken to overcome immunosurveillance.

Prophylactic Vaccines

Until recently, the major impediment to developing a prophylactic vaccine was the inability to mimic the neutralizing conformational epitopes that determine the serotype of authentic virus particles. Development was previously hampered by the inability to replicate and manipulate HPV in cell culture to produce virus mutants (such as Sabin did) or formalized purified virus (such as Salk did) or to transmit HPV to animal models (such as Jenner did). Recently, prophylactic vaccines have been produced by recombinant techniques that instruct structural virus proteins to be expressed by one or more engineered structural virus genes in cultured cells. Under these conditions, the expressed major capsid (L1) protein folds into a native conformation in the intracellular milieu, self-assembling into a VLP.

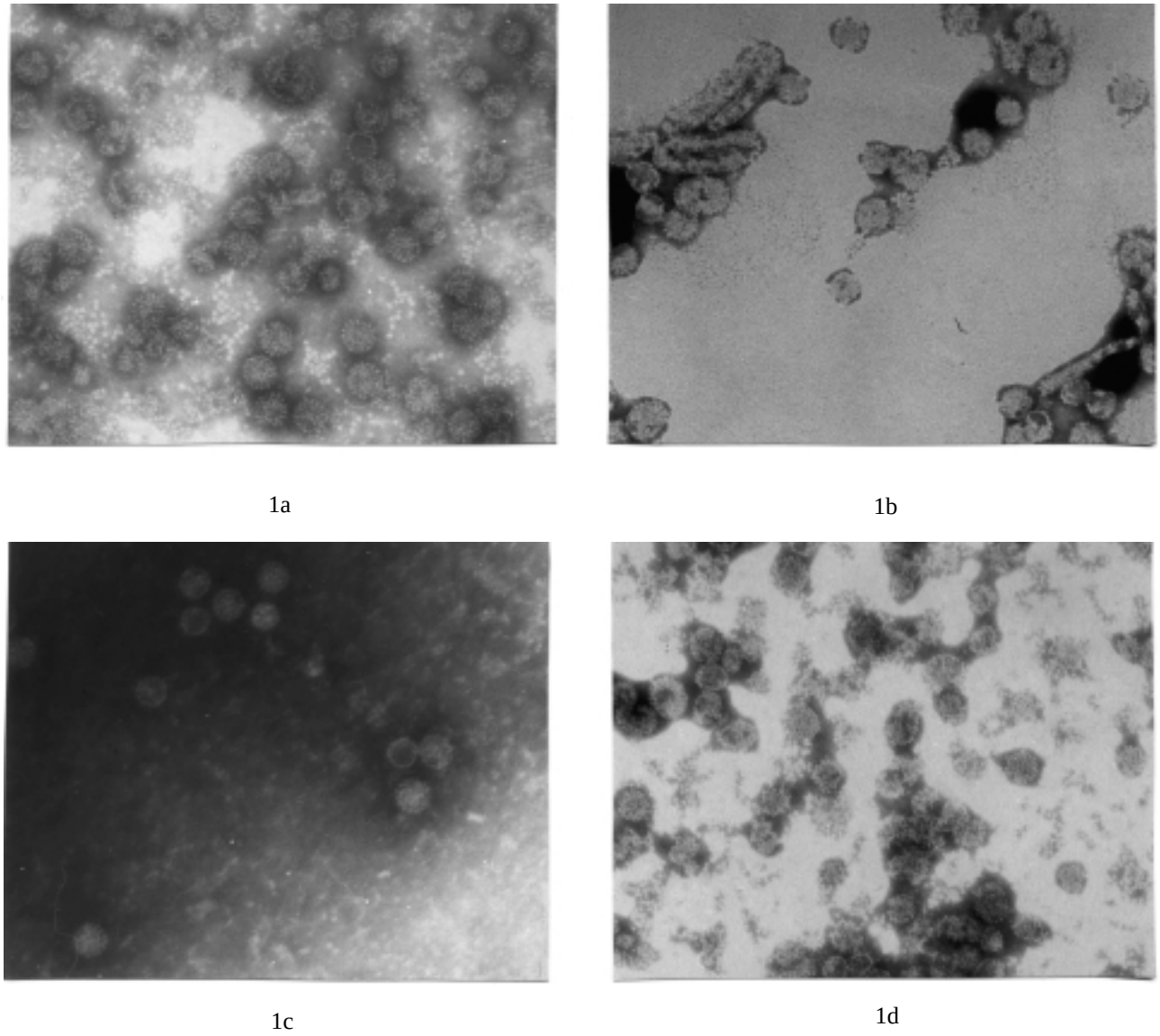
The expression of L1 by genetically engineered constructs that are transfected or infected into cultured cells results in the translocation of recombinant L1 protein into the nuclei. In monkey cell lines (designated Cos), these conformational proteins are diffusely distributed throughout the nucleus, presumably as single proteins intermixed with capsomeres and capsids (Ghim et al., 1992). However, insect cells infected by recombinant baculoviruses contain the L1 gene downstream of the polyhedron promoter, the strongest promoter of the baculovirus; the L1 protein is localized in the nucleus by the forces that aggregate baculovirus proteins into intranuclear factories (Schiller & Lowy, 1996). The proximity of the L1 proteins facilitates self-assembly into VLPs (Figure 1). In both VLPs and native virions, L1 proteins fold into conformaπtionally dependent epitopes capable of inducing neutralizing antibodies. The VLPs can also be used as a substrate for serological screening to detect neutralizing antibodies in the serum of currently infected or previously immunized individuals. The HPV-16 and -18 VLPs produced by recombinant baculoviruses in insect cells express the immunodominant immunogens used as the basis of the ongoing clinical phase vaccine trials. These trials have been highly successful but much of the information remains confidential.

Recombinant VLPs mimic the authentic virus particles both antigenically and structurally. Electron microscopy has shown the particles to be approximately 50–55 nm in diameter with icosahedral symmetry. When the L1s that form the VLPs are significantly shortened at the carboxyl terminus (greater than 75 base pairs) by enzymatic scissors, the secondary structure of these L1 proteins still displays icosahedral symmetry but the neutralizing activity of the VLP is lost (Chen et al., 1998). L1 proteins with mutations in essential structural amino acids that are highly conserved and necessary for folding into an icosahedron are not neutralizing because conformationally dependent epitopes are not formed when they attempt to self-assemble into VLPs. Whether a single L1 protein folded in a native conformation can also induce neutralizing antibodies is unknown, but induction of neutralizing antibodies probably requires the L1 protein to be in at least a capsomeric form. VLPs and virus-like capsomeres induce a neutralizing antibody response that is immunodominant in the natural host. HPV L1 VLPs (1a-c) were expressed and self-assembled during infection of insect cells by appropriate recombinant baculovirus vectors. VLPs average 55 nm in diameter with some thinner rod-shaped forms in HPV 16 (1a) preparation. COPV virions (1d) were isolated and purified from productively infected canine oral papillomatosis lesions. All PV VLPs and authentic virions appear structurally similar.

Use of Animal Models

Because the genetic organization of all PVs is similar and the gene products have similar functions, the virus-host interactions in PV animal models can be used to test various formulations of prophylactic vaccines (Suzich et al., 1995). Challenging immunized animals with infectious virus is a standard way to measure vaccine efficacy. However, it is not feasible to carry out similar challenges in humans because of the carcinogenic nature of viruses like HPV-16 and -18. In animal models, spontaneous regression is PV type specific. Both cell-mediated regression and the simultaneous production of neutralizing antibodies against the PV type provide protective immunity against a recurrence of the lesion. Efforts to develop a prophylactic vaccine have focused on the role of the major (L1) and minor (L2) structural virus proteins in the induction of neutralizing antibodies. Although intact virus induces neutralizing antibodies, antigenic determinants on denatured or disrupted recombinant L1s induce only high-titered nonneutralizing antibodies. In animal models, recombinant L2 protein appears to be neutralizing, but the hyperimmune serum in animal models does not consistently induce protective antibodies and when it does, the titers are low (A.B. Jenson, unpublished observations)..

Animal models have been used to test the ability of VLPs to protect the native host against PV infection. Although the Shope PV and bovine PV are two common animal models for proof of practice of the neutralizing capacity of VLPs, canine oral papillomavirus (COPV) is the animal model used to simulate human mucosotropic HPV infections (Suzich et al., 1995). After two subcutaneous inoculations of VLPs, the systemic immune system produces neutralizing IgG antibodies that protect naïve weanling beagles from massive oral challenges with COPV. The induction of the hyperimmune neutralizing serum is dose dependent, with very small dosages being potent enough to protect vaccinated animals for longer than 3 years. Passive transfer of fractionated neutralizing IgG antibodies induced by the COPV vaccine or by regressing oral papillomas protect naïve beagles from challenge, thereby confirming that protection



1a 1b

1c 1d

Figure 1. HPV L1 VLPS (1a- HPV 16; 1b- HPV 18; 1c- HPV 6) (1d- COPV virions)

from PV infection depends on humoral and not cell-mediated immunity. Surprisingly, naïve beagles inoculated by intranasal spray developed IgA-specific antibodies that were not protective against COPV challenge, suggesting that vaccination of the mucosal immune system is not necessary to protect dogs against COPV infection (R. Schlegel & A.B. Jenson, unpublished observations).

Human Prophylactic Vaccine Trials

The results obtained during phase I and II vaccine trials using HPV-16 L1 VLPs suggest that the VLPs are highly protective against HPV-16 incident infections in high-risk populations. A controversy over awarding a license for VLP vaccines focuses partly on whether HPV-16 VLP vaccines should be designated as vaccines that protect against sexually transmitted disease infections or precancerous lesion such as CIN 2. It is unclear why an HPV vaccine trial should have an endpoint of moderate dysplasia, given the intra- and interobservational difficulties of the pathological diagnosis of moderate dysplasia/CIN 2 and the cost and amount of time spent on following up patients until they develop CIN 2 lesions not caused by HPV-16. Nevertheless, the early results from the human phase trials are very encouraging.

Systemic vaccination appears to be sufficient to successfully protect individuals from HPV-16 and other carcinogenic HPV infections. This will be a much more efficient way of preventing the development of cervical cancer than screening for and treating cancer and its precursor lesions. The most troublesome issue appears to be the projected cost of a recombinant vaccine for the developing countries where cervical cancer is most prevalent. It awaits the ingenuity of the international community to determine how to make vaccination affordable for those who need it the most.

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