Infection with Human Papillomavirus: Update on Epidemiology, Diagnosis, and Treatment

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Human papillomavirus (HPV) infection is the most common sexually transmitted viral disease worldwide. Low-risk types of HPV (eg, HPV-6 and HPV-11) are the causative agents of genital warts, whereas high-risk types (*eg*, HPV-16 and HPV-18) have been associated with anogenital cancer, particularly cervical cancer. Cervical cancer remains the second most common cancer in women worldwide. Recent advances have led to a better understanding of how HPV causes cancer on a molecular level and of the immunologic response to HPV. Methods to detect HPV infection have been improved, and a new treatment method for genital warts has been developed. The production of empty capsids of HPV done using recombinant technology has led to the development of serologic assays for HPV. The empty capsids are now the basis of clinical trials of vaccines to prevent HPV infection and disease.

Introduction

Human papillomavirus (HPV) infection is the most common sexually transmitted viral disease; its prevalence ranges from 10% to 50% in sexually active women. More than 70 types of HPV have been defined and differ by more than 10% in DNA sequence. Approximately 30 types infect the anogenital tract and are subdivided according to their oncogenic potential. Human papillomavirus-6 and HPV-11 are low-risk viruses associated with more than 75% of genital warts and are not considered oncogenic [1]. High-risk HPV types, such as HPV-16 and HPV-18, account for more than 90% of cervical carcinomas and are involved in other types of anogenital cancer [1]. Cervical cancer is thought to progress from a low-grade squamous intraepithelial lesion (SIL) on cervical smear or a cervical intra-epithelial neoplasia (CIN) grade I on cervical biopsy to high-grade SIL or CIN grade II or III; carcinoma in situ; and, finally, invasive cervical cancer. Detection and treatment of the premalignant lesions is the basis for Papanicolaou (Pap) smear screening, which is thought to be partly responsible for the decline in the incidence of cervical cancer in the United States over the past 30 years [2]. HPV is a double-stranded DNA virus that is 8.0 kilobases in length and is divided into six early and two late proteins (Fig. 1). The early proteins are E1 and E2, which are involved in viral replication; E4 and E5, whose function is not yet defined; and E6 and E7, the oncogenic proteins. The late proteins L1 and L2 are involved in capsid formation. In the past year, numerous advances have been made in the epidemiology, pathogenesis, diagnosis, and treatment of HPV infection.

Viral Structure and Replication

Human papillomavirus cannot be routinely propagated in the laboratory, perhaps because of late gene expression that occurs only in terminally differentiated epithelial cells. Recent advances in cell and organotypic culture and the use of nude mouse systems have led to in vitro propagation of some HPV types. HPV-16 xenografts can be propagated in severe combined deficiency mice, and the histologic characteristics of the lesions produced are similar to those of intraepithelial neoplasia [3]. In addition, a collagen raft system that allows differentiation of skin supports the replication of HPV-positive laryngeal cells [4]. In the future, these model systems may be used to test potential therapeutic agents.

Expression of the late proteins of HPV in various systems has led to the production of empty capsids, or virus-like particles [5]. These capsids can disassemble into capsomers and reassemble; reassembly requires disulfide bonds and perhaps divalent cations [6,7]. With this technique, investigators have encapsidated DNA into the capsids [8]. The resulting pseudovirions can be used to investigate the tissue tropism of HPV and to determine requirements for DNA encapsidation.

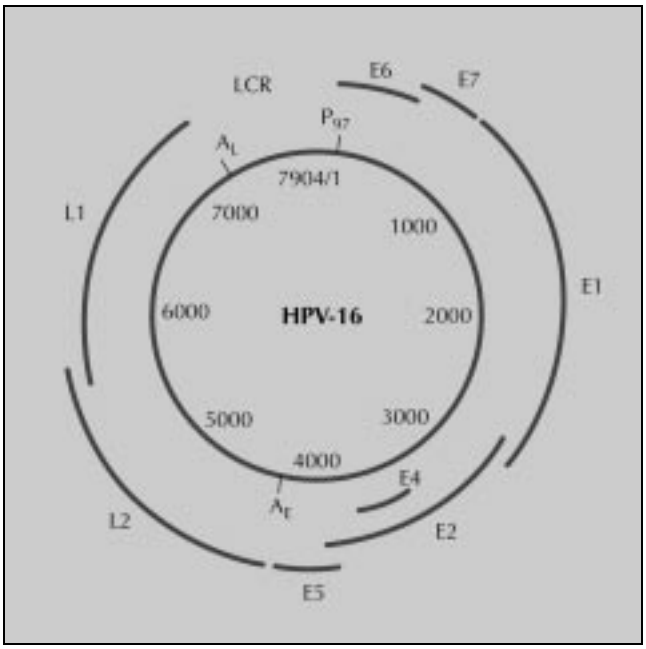

Figure 1. Genomic organization of human papillomaviruses (HPV). HPV-16 is used as an example. Six early genes and two late genes are expressed from overlapping reading frames.

Epidemiology

Numerous studies have further delineated the risk factors for current or previous HPV infection. Results of the National Breast and Cervical Cancer Early Detection Program [2], conducted between 1991 and 1995, showed that of 300,000 Pap smears analyzed, 3.8% (n=1140) were abnormal. They also showed that CIN rates on cervical biopsy were highest in young women, whereas cervical cancer rates were highest in women older than 50 years of age. These findings underscore the importance of Pap smear screening for all women.

Longitudinal studies in college-age women have found a surprisingly high cumulative rate (43% over 2 years) of incident HPV infection (positivity for HPV DNA), with infection persisting for an average of 8 months [9••]. Another investigation [10] showed an infection rate of 35% in college-age women; only 5% of these cases progressed to high-grade SIL. Finally, Watts *et al.* [11] followed 151 pregnant woman–infant pairs for possible vertical transmission of HPV. Vigorous HPV DNA testing done using polymerase chain reaction (PCR) of both mothers and infants showed no significant vertical transmission.

Cross-sectional studies have shown high rates of detection of HPV DNA (especially HPV-16 DNA) in women with abnormal Pap smears or CIN [12]. Cervical intra-epithelial neoplasia III was shown to be increased in smokers, decreased in condom users, and decreased in patients who consume α-tocopherol (vitamin E) (odds ratio, 0.15) or ascorbic acid (vitamin C) (odds ratio, 0.46). HPV DNA was detected in 30% of women who have sex with women [13•], and SIL was detected in 14% of these women. Although lesbian women may generally be at low risk for sexually transmitted diseases, this study emphasizes the need for Pap smear screening in this population.

The serologic assays that detect HPV capsid antibodies have been used as epidemiologic tools. Serum antibodies to HPV-16, HPV-18, and HPV-33 were all associated with SIL, CIN, and the same type of HPV DNA detection [14]. Detection of HPV-16 IgA and IgG antibodies was more common in women who were repeatedly positive for HPV DNA [15]. We recently published a seroepidemiologic study of risk factors for HPV-16 infection in a cohort of more than 2500 pregnant women [16] participating in the Vaginal Infection and Prematurity study of the late 1980s. The overall rate of seropositivity was 28%; bivariate risk factors were predominately markers of sexual activity. Multivariate models determined that age, number of sexual partners, length of sexual activity, and history of gonorrhea were independent risk factors for HPV-16 antibody detection. Overall, seroepidemiologic studies have shown that HPV antibody prevalence is driven by degree of sexual activity (Table 1).

HIV and Human Papillomavirus Infection

Women positive for HIV have increased rates of HPV infection (67%) and HPV-related disease, particularly if they have lower CD4 cell counts [17•,18]. Longitudinal studies have noted an increased incidence of new HPV infections, persistence of HPV DNA (especially from high-risk HPV types), and infection with multiple types of HPV in HIV-positive women [18]. In addition, an increased risk for progression from low-grade SIL to high-grade SIL and a higher relapse rate after treatment for high-grade SIL have been found in HIV-infected women [18,19]. The current recommendation is HIV-positive women have Pap smear screening at 6-month intervals; this recommendation is based partly on an increased rate of false-negative Pap smear results in this population [19]. One small study [20] noted a reduced prevalence of CIN but persistent HPV DNA detection in women receiving highly active antiretroviral therapy. Whether patients with regressing CIN lesions will have relapse due to HPV persistence is not yet known. Men positive for HIV, particularly those with lower CD4 cell counts, have an increased incidence of HPV infection (>90%), have a higher HPV viral load, have more persistent detection of HPV, and are more likely to have multiple types of HPV compared with HIV-negative men [21•,22]. The rate of anal dysplasia in HIV-positive men approaches that of cervical dysplasia before the onset of routine Pap smear anal screening. These studies underscore the need for routine screening of HIV-positive men.

Pathogenesis: Oncoprotein Function

The E6 and E7 proteins of high-risk HPVs are essential for initiation and maintenance of the transformed state. The

Table 1. Comparison of sexual activity and seroprevalence of human papillomavirus–16

Study population	Lifetime sexual partners, *n*	Human papillomavirus–16 seropositivity rate, %
College-age women	2	9.5
	4	24
Pregnant women	3.3	28.6
Patients at a sexually transmitted disease clinic	10	49
Homosexual men	>50	44

E6 protein binds and degrades the tumor suppressor gene *p53*, whereas the E7 protein inhibits the retinoblastoma protein. These proteins lead to immortalization of the cell and, eventually, to tumorigenesis. One study [23] showed that mutant *p53* with an arginine at position 72 is degraded more readily, leading to a sevenfold increase in risk for cervical cancer. The E7 protein has been noted to sensitize human keratinocytes to apoptosis, in part because of the inflammatory cytokine interleukin-1α [24]; accordingly, measurement of interleukin-1α may be a marker for progression to high-grade disease. The structure of the interaction between E7 and the retinoblastoma protein has been crystallized [25], and it is now possible to design drugs that can interfere with this interaction. Finally, the risk for premalignant lesions is higher in women who have HPV-16 E6 protein detectable on cervical biopsy or E6/E7 mRNA detectable in cervical scrapes [26,27]. These tests may be clinically useful in identifying women who will probably progress to have abnormal Pap smear results.

Diagnosis

Because HPV cannot be routinely propagated in the laboratory, HPV infection must be diagnosed clinically, pathologically (by Pap smear), or by detection of its DNA or an antibody response against it. Thus, most HPV infections may be subclinical (Fig. 2). Examination of the cells collected on a Pap smear can screen for premalignant lesions. Recently developed liquid-based, thin-layer, cervical cytologic techniques (thin prep) have been shown to detect SIL more often than the traditional Pap smear [28]. In addition, these techniques make it possible to detect HPV DNA in the same sample. The clinical course of the additional SIL lesions detected by thin prep is not yet known, but thin prep should help decrease the false-negative rate in Pap smear screening.

Liquid hybridization, hybrid capture (HC, Digene, Silver Spring, MD), or PCR methods can be used to detect HPV DNA in cervical samples and tissues. Hybrid capture detects 13 high-risk HPV types and five low-risk HPV types and compares favorably with PCR, although PCR is more sensitive [29]. The optimal method for detecting HPV in archived, fixed tissues is still unknown, but recent studies showed similar results with in situ hybridization and PCR [30]. In addition, a nested PCR approach has shown increased sensitivity in fixed tissue [31].

Analysis of PCR products for specific types of HPV has traditionally been done with dot-blot hybridization or the restriction endonuclease digestion method. The newly developed reverse-line blot detection technique (Roche Molecular Systems, Alameda, CA) immobilizes 27 HPV probes, a positive control, and β-globin probes on a small nylon strip and then exposes this strip to biotinylated PCR amplicon. This technique is as sensitive and as specific as dot-blot hybridization [32••]. Use of these nylon strips may help reduce interlaboratory variation in the detection of HPV DNA. Variants of a specific type of HPV infection (variants were defined as differing by <10% in DNA sequence) were previously shown to be associated with progression in women [33]; a similar association between men and the risk for anal carcinoma has also surfaced. Future efforts to more fully characterize HPV variant types may be helpful in assessing risk for anogenital cancer.

Immune Response Against Human Papillomavirus

Humoral immunity

The human antibody response to HPV capsid antigens is type specific, reacts mainly against conformational epitopes [34], and develops more than 8 months after productive infection occurs. Indirect evidence suggests that HPV L1 antibody levels may wane over time [34], but no changes in HPV-16 antibody levels were seen in women followed for 4 years [35]. Systemic HPV IgG levels correlate with persistent HPV infection, and one recent study [36] showed that systemic IgA against HPV-16 levels may be protective against HPV infection.

New capture enzyme-linked immunoassays have been developed for the HPV-16 and HPV-18 E6 and E7 proteins; more than 50% of women with cervical cancer have antibodies to one of these four proteins [37]. HPV-16 E7 antibody levels decline after surgical intervention, and this enzyme-linked immunoassay may aid in predicting tumor burden [38]. A similar report showed that both L1/L2 HPV-16 capsid and HPV-16 E6 antibodies were found in equal numbers in patients with cervical cancer (51% to 56%), while fewer patients (33%) had HPV-16 E7 antibodies

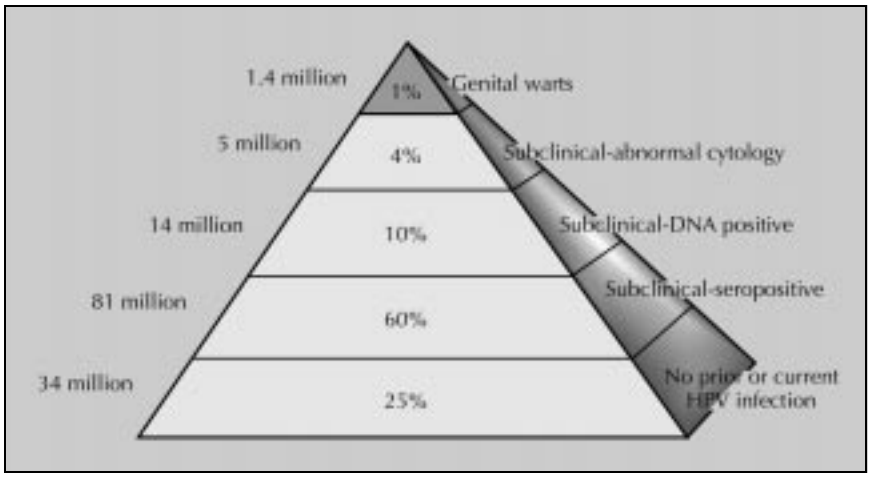

Figure 2. The "iceberg" of human papillomavirus (HPV)–related disease. Most HPV infections are not clinically detectable.

detected [39]. These assays may serve as tumor markers for cervical cancer.

Cellular immunity

The existence of a genetic predisposition to cervical cancer is supported by the finding that HLA-DRB1*1501 occurs in higher proportions in both older (33%) and younger (28%) women with cervical cancer than in historical controls (19.8%) [40]. Colony-stimulating factor (CSF)-1 is a glycosylated protein growth factor produced by many cell types, including fibroblasts, that can stimulate macrophage formation and function. Serum levels of CSF-1 are lower in normal women than in women with CIN or women in whom HPV DNA has been detected in the cervix [41]. The chemokine monocyte chemoattractant protein (MCP)-1 is important in the recruitment of monocytes in tumor tissue; a negative correlation of MCP-1 expression in cervical tissue and expression of HPV-16 E6 and E7 genes has been found [42]. Lack of MCP-1 may play an important role in failure to control HPV infection effectively. In vitro production of interleukin-2 by CD4+ helper T cells has been shown to be associated with viral persistence and disease progression [43]; the highest levels are seen in women with CIN III (93%). These methods are all being used in attempts to better distinguish between HPV-infected woman who will probably progress to an abnormal Pap smear result and HPV-infected women who will "handle" their viral infection.

Treatment

The development of new treatment regimens for HPV infection is hampered by lack of an animal model. Recently, however, a dermal skin-graft model of cottontail rabbit papillomavirus onto nude mice has become a relatively easy way to test therapeutic agents against genital wart–like growths [44•]. This system has identified ribavirin and vinorelabine tartrate (an antitumor agent) as potentially effective agents. Unfortunately, the compounds that were potentially efficacious in this model were also the most toxic.

Production of HPV virions requires epithelial cell differentiation that facilitates late HPV gene expression; lack of differentiation may increase the potential for oncogenic transformation. Thus, agents that induce differentiation may prevent cervical cancer. Dietary supplements that induce differentiation may also help prevent cancer [45], but folic acid and β-carotene have failed to show any consistent differences in CIN rates. These studies on dietary supplements suffer from small sample sizes and brief follow-up periods. In contrast, retinoic acid increased CIN regression rates and reduced HPV expression in cultured cells [46]. Finally, a recent trial showed that 5-fluorouracil gel applied to the cervix in HIV-infected women with CIN II significantly reduced progressive cervical disease (Robinson W, Personal communication). These compounds deserve further study.

The most exciting new treatment for genital warts is imiquimod (Aldara, 3M Pharmaceuticals, St. Paul, MN) that can be applied by the patient. This agent is thought to function by inducing local interferon production. A recent multicenter, randomized, double-blind study [47••] showed that a 5% solution of imiquimod was more effective than control (clearance rates, 52% compared with 4%). Further studies showed that imiquimod-induced wart regression was associated with local interferon and tumor necrosis factor–α production as well as lower HPV DNA and gene expression [48]. Treatment options for cervical cancer are limited and consist mainly of ablative or surgical approaches. A recent trial comparing cryotherapy, laser vaporization, and loop excision found all three approaches to be equally effective [49]. Three recent studies [50•–52•] reported decreases in the 5-year mortality rate of patients with invasive cervical cancer who received both chemotherapy and x-ray therapy; this may quickly become the standard of care.

Table 2. Human papillomavirus vaccines in clinical development

Company	Components	Type	Development stage
Apollon, Malvern, PA	Plasmid DNA types unknown	Unknown	Preclinical
Cantab Pharmaceuticals, Cambridge, UK	Liver recombinant vaccinia virus expressing E6 and E7 genes of HPV-16 and HPV-18	Therapeutic	Phase I/II
Cantab	Recombinant protein of L2 and E7 from HPV-6	Therapeutic	Phase IIa
MedImmune, Gaithersburg, MD	VLP (L1) of HPV-11	Prophylactic	Phase I
Merck Research Laboratories, West Point, PA	VLP (L1) of HPV-11 and HPV-16	Prophylactic	Phase I/II
Merck Research Laboratories	Naked plasmid DNA, HPV types unknown	Unknown	Preclinical

HPV—human papillomavirus; VLP—viruslike particle.

Vaccine Development

In vitro production of HPV empty capsids has led to the development of vaccines against various types of HPV (Table 2). Recent reviews have summarized vaccination development strategies [53]. Prophylactic vaccines against HPV will probably target the empty capsid as antigen but will need to be multivalent to be effective. To this end, Medimmune, Inc. (Gaithersburg, MD) is currently conducting clinical trials with a HPV-11 and combined HPV-16, -18 vaccine, and Merck Research Laboratories (West Point, PA) currently has an HPV-11 and an HPV-16 vaccine in phase I/II trials. The target for therapeutic vaccines is less well defined; L1, L2, E1, E2 , E6 and E7 are all feasible. Further development of a therapeutic vaccine that uses vaccinia virus–expressed E6 and E7 proteins is underway.

Modified empty capsids may also be vaccine candidates. Epitope mapping done using monoclonal antibodies has shown that HPV-11 capsomers retain neutralizing epitopes and that these subunits could serve as a vaccine [54]. HPV-16 E7 conjugated to the L2 protein and assembled with L1 into empty capsids protects mice from tumor challenge with cells that express HPV-16 E7 [55]. This approach has the potential advantage of combining a prophylactic and a therapeutic vaccine. Empty capsids have been introduced to various mucosal surfaces of mice with and without cholera toxin, and development of a mucosal immune response has then been studied [56]. To date, nasal immunization had produced the greatest vaginal humoral response, but whether this will be protective against HPV mucosal challenge remains to be determined. Human papillomavirus vaccine development is progressing quickly, and many more human trials are planned for the near future.

Conclusions

Research into the epidemiology, pathogenicity, diagnosis, and treatment of HPV infection is increasing tremendously, and we now possess many tools with which to investigate this difficult pathogen. HPV infection is a widespread health care issue, and HPV serologic examination and DNA detection can better define the epidemiologic risk factors for HPV infection and analyze trends over time. An improved understanding of how HPV causes cancer will, ideally, lead to improved diagnostics and better therapeutic agents with which to prevent cancer. The clinical usefulness of HPV DNA testing is still being determined, and this testing may ultimately be used to better identify those at higher risk for progression to cervical cancer. The treatment of genital warts has improved dramatically with the introduction of imiquimod, and similar agents to control premalignant cervical lesions may be developed in the future. Advances in the treatment of invasive cervical cancer give hope to patients with advanced disease. Finally, vaccine development has undergone tremendous growth, and this, together with current research efforts, should ultimately lead to control of HPV-related disease and anogenital cancer.

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