

The Biology and Significance of Human Papillomavirus Infections in the Genital Tract

RICHARD REID, M.D.,^a AND MICHAEL J. CAMPION, M.D.^b

^a*Director, Cervical and Vulvar Dysplasia Unit, Sinai Hospital of Detroit, Detroit, Michigan;* ^b*Director, Clinical Research and Education (Gyn), Regional Cancer Center, St. Joseph's Hospital, Atlanta, Georgia*

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A variety of human papillomavirus (HPV) types infect the anogenital mucosa, giving rise to lesions that differ in clinical appearance, histology, and risk of malignant progression. Certain high-risk types (HPVs 16, 18, 31, 33, 35, and 39) have a strong association with high-grade epithelial neoplasia and invasive carcinomas of the anogenital tract. Cancer appears to have a multifactorial etiology, and HPV infection alone is probably insufficient for malignant transformation. The consistent association between HPV infection and anogenital cancers emphasizes, however, that the sexually transmitted papillomaviruses may have a necessary role in carcinogenesis. Hence, there is a prospect that vaccination programs may one day allow public health control of HPV infection, thereby eliminating an important risk factor.

INTRODUCTION

Invasive squamous cell cancer of the uterine cervix is one of the most common cancers of the female genital tract. Worldwide, cervical cancer is the greatest cancer killer of women under the age of 40 years, and the frequency of the disease in younger women appears to be increasing [1,2,3]. When cervical cancer does occur in this age group, the tumor tends to be poorly differentiated and has a proclivity to more rapid systemic spread [3,4]. This process carries an attendant threat of more drastic therapy, including chemotherapy.

Cervical cancer has long been considered a sexually transmitted disease [5,6]. Epidemiological research has shown that sexual intercourse is pivotal in determining risk [7,8]. Specific sexual behavioral characteristics, such as early age of coitarche [9,10] and multiple sexual partners [11], define women at high risk of cervical neoplasia. Rotkin, in 1967, therefore proposed the concept of the male as the vector of some carcinogenic agent transmitted to the female sexual partner at the time of intercourse [12], an agent showing a marked affinity for the unstable squamous metaplastic cells of the immature cervical transformation zone.

Vulvar cancer remains uncommon. Within the last forty years, however, the incidence of vulvar intraepithelial neoplasia grade 3 (VIN 3; severe dysplasia/carcinoma in situ) has increased, while the modal age at diagnosis has fallen by two decades, to 30 years of age [18]. Women with vulvar neoplasia show an increased risk of cervical neoplasia, suggesting a field effect by a common causative agent [19].

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Abbreviations: CIN: cervical intraepithelial neoplasia E region: early region EV: epidermodysplasia verruciformis HPV: human papillomavirus L region: late region ORF: open reading frame SPI: subclinical papillomaviral infection URR: upstream regulatory region VIN 3: vulvar intraepithelial neoplasia grade 3

Address reprint requests to: M.J. Campion, M.D., Director, Clinical Research, 5669 Peachtree-Dunwoody Road, N.E., Atlanta, GA 30342

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Still less is known about the etiology of the less common squamous genital cancers, particularly vulvar and penile carcinoma. Although penile cancer is much less common, the incidence of cervical and penile cancer actually correlates in a number of countries [13]. A man with either penile cancer or carcinoma in situ of the penis places his partner at increased risk of cervical neoplasia [14,15,16]. Conversely, 5–10 percent of male partners of women with cervical neoplasia will have penile intraepithelial neoplasia (severe dysplasia/carcinoma in situ) [17].

The association between cervical, vulvar, and penile neoplasia implies transmission of an infectious oncogenic agent. Herpes simplex virus was previously suspected as this infectious carcinogen. Two decades of research, however, have failed to produce evidence that convincingly relates herpes viruses to cervical cancer. The candidacy of the papillomaviruses was suggested over ten years ago by Zur Hausen [20]. Since papillomaviruses could not be propagated in cell culture, however, virologists of the day had no effective tools with which to study this hypothesis. Advances have only occurred since the advent of molecular biology. This exciting new technology has helped to unravel the plurality of papillomaviruses, their prevalence in various tumors, and aspects of their biology. Such data have strengthened speculation regarding a role of papillomaviruses in the etiology of genital cancer.

The human papillomaviruses (HPVs) are site-specific DNA viruses that produce characteristic proliferations on epidermal and mucosal surfaces. Papillomaviral infection of the genital tract is common among sexually active women [21]. Since clinically detectable condylomata acuminata are seldom seen on the cervix, it was traditionally believed that HPV infections were limited to the production of papillomas affecting the lower vagina, vulva, and anus. In the past, cervical HPV infections were overlooked because the lesions are most frequently macroscopically flat and entirely invisible without the aid of acetic acid [22]. When seen at colposcopy, these changes were either dismissed as physiological variations or misinterpreted as mild dysplasia. In the mid-1970s, however, it was recognized that 1–2 percent of all smears from women with a clinically normal cervix displayed cytologic signs of HPV infection [23,24,25] and that many mild dysplasias showed only viral proliferation, without any real evidence of neoplastic transformation [22,24]. That is to say, it was now apparent that the most common manifestation of sexually transmitted HPV infection was a non-papilliferous hyperplasia of the metaplastic epithelium of the transformation zone [26]. The only major difference between clinically overt and subclinical condylomas is their macroscopic appearance. Both variants show essentially identical histology, both forms are infectious, and both lesions denote a risk of neoplastic transformation.

At first, this new insight led to the widespread assumption that the task for the future was to define morphologic criteria by which benign papillomaviral infections could be differentiated from real pre-malignancies. There never was, in fact, any experimental basis for such a belief. Conversely, there has been a rapid accumulation of histologic, serologic, and virologic evidence linking both minor and major grades of cervical neoplasia with papillomaviral infection [27]. As an additional complexity, the common genital varieties of papillomavirus (types 6 and 11) were mainly associated with either venereal warts or minor grades of dysplasia, whereas high-risk HPVs (types 16, 18, 31, 33, 35, and 39) have been found mainly in high-grade pre-malignancies or invasive cancers [28]. While these data suggest that only a fraction of cervical HPV infections are potentially malignant, it must be remembered that there is substantial morphologic overlap between lesions caused by the different viral types. In essence, the

trriage of the abnormal Papanicolaou smear amounts to the differentiation between benign warty proliferation, minor-grade dysplasia, and full-thickness intraepithelial neoplasia [29]. Hence, a clear understanding of the biology, morphologic expression, and natural history of papillomaviral infection is essential to the rational management of patients with lower genital tract neoplasia.

BASIC VIROLOGY OF THE PAPILLOMAVIRUSES

Taxonomy

Papillomaviruses are small, double-stranded DNA viruses. Because of superficial similarities in electron microscopic appearance and biologic properties, papillomaviruses were originally classified within the same family as polyomaviruses, this group being termed *papovaviruses* [30]; however, papillomaviruses (genus A) have a larger chromosome (7,900 versus 5,200 base pairs), a larger virion capsid (55 versus 44 μm), and a completely different genomic organization [31]. Hence, it would be best to regard papillomaviruses as a distinct and unique family.

Polyomaviruses (genus B) are readily passaged in tissue culture, transform cell lines *in vitro* and are oncogenic in animals of other species; genus B viruses do not, however, usually cause widespread disease in their natural hosts. The main scientific importance of the polyomavirus subgroup has been as laboratory models of viral oncogenesis. In contrast, papillomaviruses cannot be propagated in tissue culture and do not generally induce infection in other species. Rather, the papillomaviruses are host- and site-specific viruses that cause benign skin tumors (warts) in their natural hosts, some of which tumors evolve into squamous cancer.

In reality, papillomaviruses represent a divergent group of evolutionarily related viruses which manifest qualitatively similar biological characteristics; however, individual papillomaviruses show enormous differences in species specificity, site of predilection, and degree of oncogenicity. All papillomaviruses exhibit a similar pattern of genetic organization, in that sequencing of the different types has shown preservation of broadly equivalent areas of protein coding potential (known as open reading frames or "ORFs"); however, the actual nucleotide sequences within these ORFs are widely disparate [31]. Hence, papillomaviruses are named according to *species* and subclassified into *types* according to nucleotide sequence. Within the same species, any new isolate which exhibits less than 50 percent homology (by solution hybridization) with existing members is designated as a new type and numbered in order of discovery. Human papillomaviruses (HPV) comprise the largest group, with more than 55 known types. Other members of scientific importance include the cottontail rabbit, bovine, canine, ovine, equine, elk, and mastomys papillomaviridae.

Virion Structure

Papillomavirus particles, whether isolated from infected rabbits, cattle, or humans, are remarkably similar in overall appearance. Virions consist of a central core of viral DNA, enclosed within an outer capsid of viral protein. The viral capsid is composed of 72 subunits (capsomeres), which are arranged in symmetrical, 20-sided (icosahedral) pattern that gives the individual virion an almost spherical shape on electron microscopy (Fig. 1). The virion does not have an outer membrane such as is found in some other viruses (for example, human immunodeficiency virus). This fact may account for the low antigenicity of papillomavirus infections (Figs. 2 and 3).

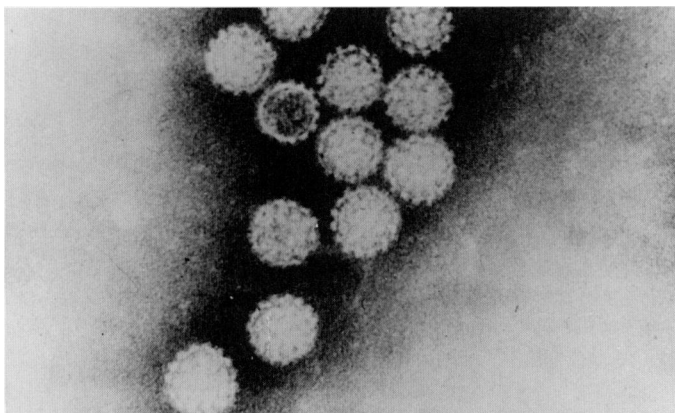


FIG. 1. Electron micrograph of formed virions in a condyloma acuminatum. The icosahedral shape of the viral capsid is apparent.

Biochemical analysis of the viral capsid has revealed two different families of structural protein: a *major* protein (molecular weight, 54,000 daltons) and a *minor* protein (molecular weight, 76,000 daltons) [32]. Denatured major capsid proteins of all animal and human papillomavirus are highly cross-reactive and serve as genus-specific antisera. In contrast, the minor capsid proteins appear to be highly type-specific and might one day provide a suitable target for immunocytochemical typing of biopsies.

DNA Organization

All papillomavirus chromosomes are covalently closed, circular, double-stranded DNA molecules with a molecular length of about 7,900 base pairs and a molecular weight of about 5.2×10^6 daltons (about half a million times smaller than the genome of a human cell). In contrast to mammalian chromosomes, all of the viral genetic information is contained on just one of the paired DNA strands.

Whether isolated from disrupted virions or extracted from transformed cells, the viral DNA is combined with histones (derived from the cellular pool of the natural host), to form a small chromosome [33]. Thus, viral DNA constitutes only 12 percent of the virion by weight. When the nuclear proteins are stripped away, the viral DNA assumes a super-coiled shape (form I). Super-coiling is visible on electron microscopy

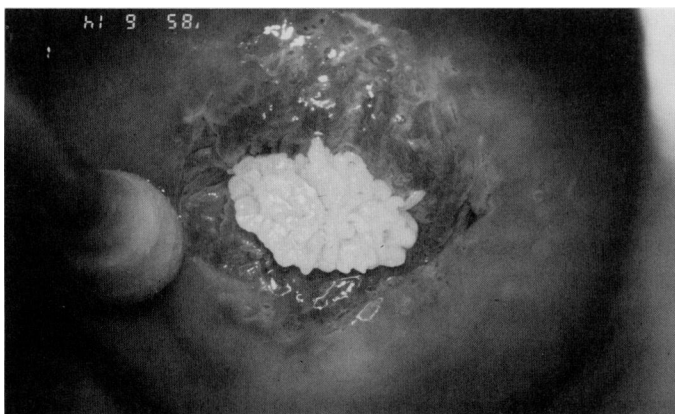


FIG. 2. A clinical condyloma acuminatum on the cervix of an 18-year-old woman. Such a lesion was previously considered very rare.

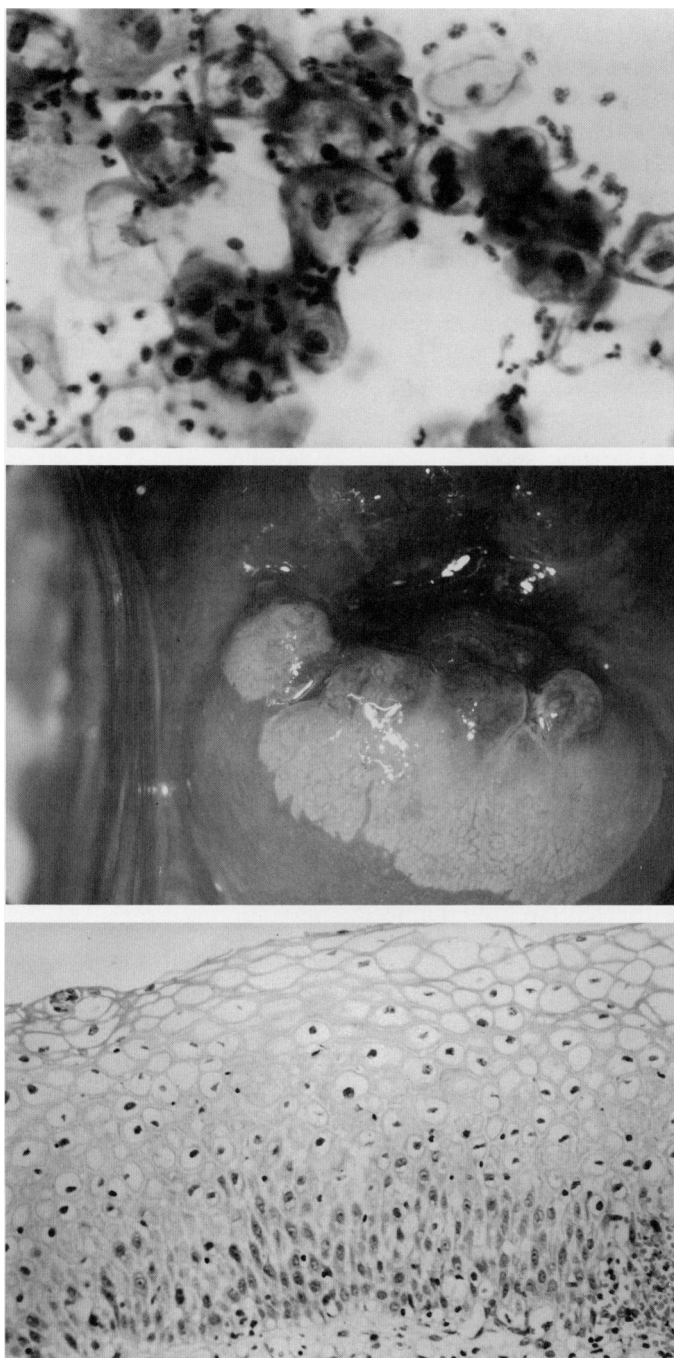


FIG 3. A. A Papanicolaou smear showing cytopathic effects of HPV infection, specifically koilocytotic atypia (K) characterized by nuclear atypia and peripheral condensation of cytoplasm. B. A colposcopic photograph of the cervix of this 24-year-old woman showing a minor-grade lesion with an irregular feathered border and trivial vascular pattern. C. Histology of this lesion reveals basal hyperplasia and koilocytotic atypia amounting to HPV infection without associated CIN; however, HPV 16 DNA was extracted from this minor-grade lesion.

and is also detectable at electrophoresis because of the relatively rapid mobility of these tight circular molecules [31]. Cleavage of only one DNA strand by bacterial enzymes (restriction endonucleases) results in a relaxed circle (form II), while cleavage of both strands at a single site produces a linear molecule which migrates more slowly in electrophoretic gels (form III).

Viral Genetic Function

As previously mentioned, alignment of sequenced papillomaviruses' DNAs revealed a highly conserved pattern of protein coding potential, these areas with protein coding potential being termed *open reading frames* (ORFs). In essence, each of these ORFs represents a viral gene, which will determine such characteristics as host range, tissue tropism, and the clinicopathologic consequences of infection.

Studies of the non-productive interaction of BPVs with mouse fibroblasts has shown that the viral genome can be subdivided into three functional portions. While it has not been experimentally demonstrated that the same relationship holds for HPV infection of epithelial cells, it is still valuable to examine human disease in relationship to this model.

The upstream regulatory region (URR) The upstream regulatory region is a non-coding segment representing about 15 percent of the viral genome. This region contains the origin of DNA replication, several promoters (sequences needed to initiate viral mRNA synthesis), and several enhancers (sequences that increase the rate of RNA transcription) [34,35,36]. From the functional viewpoint, enhancers fall into two groups: those which bind to the viral genome (thereby regulating the production of other virus-encoded proteins) and those which bind to host receptors (thereby inducing a conducive host cellular milieu).

The URR is the genetic interval most likely to be divergent among viral types, and some of these differences have been correlated with changes in virulence and oncogenic potential [31]. Moreover, these differences provide much of the basis for the type-specific diagnostic probes [37].

The E (early) region The E region is a long segment, representing about 45 percent of the viral genome. This region contains at least seven ORFs, all of which code for proteins that either maintain cell transformation or control viral DNA replication. Each ORF was named according to relative size. Hence, the number assigned to a particular ORF bears no relationship to its actual location within the E region of the viral genome.

Functions of individual ORFs, are as follows (reviewed in [31]):

E1 ORF controls the episomal replication of viral DNA [38]. This viral gene appears to have two parts: a positive control (the E1-C domain) and a negative control (the E1-M domain). Experiments in cell cultures suggest that, shortly after transfection, viral copy numbers rise to about 150–200 (due to E1-C “up” regulation) and thereafter drop to about 20–50 copies per cell (due to E1-M “down” regulation). Preservation of an intact E1 ORF, appropriately located downstream of the URR, appears to prevent integration of the viral genome into the host cell chromosome (see below).

E7 ORF also plays a role in regulating viral copy number during episomal DNA replication [39]. It is not, however, presently clear whether E7 proteins are involved in maintaining “steady-state” levels of HPV DNA in latently infected epithelial cells or in promoting the high copy numbers seen in vegetative viral infections.

A 20,000-dalton E7 protein has been found in human cervical cancers [40], indicating that this viral gene may also play a role in malignant transformation.

E2 ORF produces a pair of competing proteins that bind to the upstream regulatory region and function either to promote or to inhibit viral transcription. The

E2 ORF may have an important role in producing or maintaining “benign” cellular transformations.

E5 ORF also plays a role in cell transformation, apparently by producing a small (7,000-dalton) protein which binds to cytoplasmic membranes. Deletion of the E5 protein appears to impair episomal DNA replication, perhaps favoring integration of viral DNA into the cellular chromosome [31].

E6 ORF produces an 18,000-dalton protein that is involved in “malignant” cellular transformation and in the alteration of growth properties within cell culture. The E6 region has been conserved and is transcribed in all of the HPV-containing human tumor biopsies and cervical cancer-derived cell lines analyzed to date [31].

E4 ORF produces several proteins that are found in relative abundance as parabasal cells differentiate into mature keratinocytes, and it is thought that messages from this region initiate the onset of koilocytotic changes. Not surprisingly, the E4 ORF is usually deleted from carcinomas.

The L (late) region The L region comprises about 40 percent of the viral genome and contains two ORFs that are essential to vegetative viral replication:

L1 ORF encodes the major capsid protein (molecular weight 54,000 daltons). The L1 gene product is highly conserved among most animal and human papillomaviruses. Accordingly, antisera directed against disrupted, denatured HPV-1 capsid are used as cross-reactive, group-specific immunocytochemical reagents. Only about 50 percent of genital condylomas produce detectable L1 protein, however, and this percentage declines with increasing degrees of neoplasia [41].

L2 ORF encodes the minor capsid protein (molecular weight, 76,000 daltons). Marked variation has been found among the nucleotide sequences for the L2 ORF. Hence, L2 proteins might serve as distinguishing targets for immunocytochemical typing of infected tissues. Within the intact virion capsid, however, type-specific epitopes of the L2 protein are shielded and probably could not form the basis for a vaccine [32].

DNA Replication

After transport of viral DNA to the cell nucleus, it appears that the E1-C (positive regulatory) protein binds to the origin of replication in the URR and initiates “runaway” replication [31]. Initial copy number may rise as high as 200 per cell but is thereafter stabilized at about 20–50 copies (as a result of down regulation by E1-M and E7 modulator functions). To this point, it does not appear that there are any steps in viral DNA replication which could be targeted for selective blockage. Hence, any drug which would attack viral DNA replication would probably also disturb host cell function. The best prospect for a drug to block viral replication would appear to be a chemical active against the E7, E1-M, or E1-C viral proteins.

RNA Transcription

RNA transcription mapping is still at a preliminary stage. Current data suggest that papillomaviruses use complex splicing techniques as a means of specifying messages from different ORFs. One important difference in the high-risk viruses HPV 16 and 18 (relative to the low-risk viruses HPV 6 and 11) is the presence of additional splice sites within transcripts spanning the E6 ORF. These additional splice sites lead to a

truncated form of the E6 protein, termed E6* [40]. This E6* protein may have properties important to transformation and carcinogenesis. In addition, a different species of RNA has been identified in tumor cells, wherein part of the viral message has been deleted and replaced by a segment of host cell RNA. This arrangement might account for the increased half-life of viral transcripts in malignant cell lines.

The production of subgenomic RNA probes, small enough to detect messages encoded by the individual ORFs, has now made it possible to study the tissue distribution and relative abundance of the signals from the different viral genes [37]. These studies show continued early gene expression from parabasal to superficial layers. In contrast, messages from the L1 and L2 ORFs are mainly confined to the superficial strata.

CLINICAL GROUPS OF HUMAN PAPILLOMAVIRUSES

From the viewpoint of biologic properties, human papillomaviruses comprise three clinicopathologic groups: cutaneotropic viruses found in immunologically normal individuals, cutaneotropic viruses causing epidermodysplasia verruciformis in immunosuppressed patients, and mucosotropic viruses infecting the genital, oral, and respiratory mucosae.

Cutaneous HPVs in the Immunocompetent Population

Viruses in this group always produce vegetative infections which, by definition, can never be carcinogenic. Viral type correlates with lesion location, clinical features, and histologic appearance.

HPV 1 and 4 are usually found in plantar warts, HPV 1 being preferentially associated with deep, painful lesions and HPV 4 with more superficial varieties.

HPV 2 is found in common warts (*verrucae vulgaris*) which are usually acquired in childhood, causing very keratotic lesions that primarily affect the dorsal skin of the upper or lower limbs.

HPV 3 occurs in flat warts (seen as minimally elevated red patches on the hands and face).

HPV 7 produces common warts in meat and animal handlers.

Such site specificity probably reflects the fact that susceptibility to viral infection depends upon the types of keratin produced during terminal differentiation. Hence, the relationship between viral type and lesion morphology is quite constant. Nonetheless, occasional exceptions occur, such as reports of HPV 1 or 4 in common warts, or HPV 2 in plantar or genital warts.

HPVs Affecting the Anogenital and Aerodigestive Mucosae

About a dozen of the known HPV types are mucosotropic, infecting either anogenital skin or mucous membranes. These viruses are also associated with specific disease patterns:

HPV 6 and 11 are primarily responsible for two types of disease: papillomas of the upper airways and benign exophytic condylomas affecting the external genitalia, lower third of vagina, and anal canal. Detection of HPV 6 or 11 in minor lesions of the transformation zone has fostered a mistaken belief that these types account for the majority of minor cervical atypia. In fact, HPV 6 or 11 probably causes only between 15 and 50 percent of "flat condylomas" or mild dysplasias [42].

HPV 16 is the viral type detected universally with greatest frequency in high-grade intraepithelial neoplasia and invasive cancers. Nonetheless, HPV 16 is also found in 15–40 percent of minor-grade cervical lesions [21,43]. Moreover, in a high-risk British population, HPV 16 was also found in 40 percent of subclinical lesions on the vulva and penis and in up to 10 percent of condylomata acuminata, particularly recalcitrant lesions.

HPV 18 shows a bimodal distribution pattern, being present in about 5 percent of invasive cervical cancers (especially aggressive adenocarcinomas of young women) and about 5 percent of minor-grade genital lesions. HPV 18 is, however, significantly underrepresented in patients with only cervical intraepithelial neoplasia, suggesting that this virus may produce lesions that progress too rapidly for reliable detection by mass screening programs.

HPV 31, 33, 35 and 39 exhibit intermediate oncogenicity, with a predilection for the squamous cells of the cervix or vagina. The “thirties” viruses are a little overrepresented in cervical intraepithelial neoplasia (CIN) (about 30 percent) and underrepresented in cervical cancer (about 10 percent).

Cutaneous HPVs in Immunosuppressed Individuals

Almost half of the presently known HPV types have been isolated from the skin of immunosuppressed individuals. The main source of these novel cutaneotropic viruses has been patients suffering from a rare disease termed epidermodysplasia verruciformis (EV). This disease is characterized by multiple flat warts of non-genital skin, generally affecting patients with congenital impairment of their cellular immunity. Malignant conversion of these flat warts occurs in association with infection by specific “high-risk” HPV types, as discussed below. Similar HPV types have also been isolated from warts in patients taking immunosuppressive drugs and in skin cancers occurring in renal allograft recipients. Since it is highly unlikely that these HPV types could persist in nature if they infected only EV patients and other immunocompromised individuals, it is reasonable to speculate that latent infection of normal individuals may serve as the reservoir for these rare viruses.

MALIGNANT CONVERSION OF PAPILLOMAVIRUS INFECTIONS

Some papillomaviruses induce tumors which may progress to carcinoma. Skin warts of cottontail rabbits were first shown to convert into carcinomas in 25 percent of infected animals, long-persisting papillomas becoming malignant after about one year [42]. Chemical carcinogens (such as tar) greatly accelerated the conversion rate [43]. This rabbit system reveals many basic characteristics of papillomaviral oncogenesis. In most experimental models, these viruses were weakly oncogenic alone; effective carcinogenesis usually depended upon exposure to other physical or chemical carcinogens (such as ultraviolet light, X-rays, or special dietary components).

The rare hereditary skin disorder, epidermodysplasia verruciformis, provides an excellent human model for study of malignant warts [46,47,8]. Unlike other warts, these flat lesions do not regress. Rather, lesions persist for life, spreading over the entire body. Experience has shown that 25–33 percent of affected individuals will develop skin cancer, usually within sunlight-exposed areas. Lag time between the appearance of flat warts and progression to squamous carcinoma averages about 25 years.

Although more than 15 different HPV types have been isolated from skin lesions in

the patients, HPVs 5 and 8 are detected in 90 percent of malignancies [47,48]. The preferential location of skin cancers within sunlight-exposed areas is another example of synergism between papillomavirus infection and extrinsic factors.

Infants born to women with HPV 6- or 11-induced genital condylomata acuminata are at a small but definite risk of developing laryngeal papillomatosis [49,50]. Because of the often incurable nature of laryngeal papillomatosis, this disease was once treated by radiation therapy. Malignant conversion of the benign tumors induced by "low-risk" genital HPV types has been reported in such patients [49]. This process is a further example of synergism between an HPV infection and a cofactor in human carcinogenesis.

HPV AND GENITAL NEOPLASIA

HPV Types in Genital Tumors

The site-specific preference of the individual HPVs sometimes reflects a degree of similarity in viral genomic organization. For example, HPVs 6 and 11 have identical tropism and pathogenic properties. Although these viruses are classified as different types (because they show only 25 percent cross-reactivity when analyzed by reassociation kinetics in liquid phase), actual sequence analysis has demonstrated almost 90 percent homology of nucleotide base pairs [50,51]. Likewise, HPVs 16 and 31 are also closely related at the nucleotide level [52]. HPV 33 is distantly related to HPV 16, but this fact poses no problem for differentiation. HPV 18 and HPV 35 have no close relatives among other known HPVs. Nonetheless, viruses originally isolated from skin warts are occasionally found in genital tumors. HPVs 1 and 2 have been detected in condylomata acuminata [53]. HPV 10 has been reported in both condylomata acuminata and cervical cancer tissue [54]. HPV 34 was originally cloned from Bowen's disease of the skin but has also been reported in an isolated case of vulvar intraepithelial neoplasia grade 3 [55].

Morphology of Genital HPV-Induced Disease

Condylomata Acuminata Exophytic condylomas usually present as soft, pink or whitish, vascular, sessile tumors with multiple, fine, finger-like projections. They occur primarily in moist areas, especially those exposed to coital friction. Thus, the most common sites are the posterior part of the introitus, the adjacent labia minora, throughout the vestibule, and on the perianal or anal skin. Less commonly, condylomas occur in the clitoral region (even under the clitoral hood) and may extend on to the mons pubis. In the non-mucosal areas, the condylomas may be more keratotic and less papilliferous, similar in appearance to the typical hand warts (*verruca vulgaris*).

Condylomata acuminata now represents one of the most common sexually transmitted diseases. The viral etiology has been clearly proven [56]. Over 60 percent of sexual partners of infected individuals develop lesions after an incubation period of four to six weeks. The vast majority of exophytic condylomata acuminata are induced by HPV 6 (65 percent) or HPV 11 (20 percent). Mixed infections involving more than one genital HPV type are not uncommon. Importantly, HPV 16 is detected in up to 10 percent of these common, benign genital proliferations [57].

The reported incidence of vulvar condylomata acuminata has greatly increased throughout the Occidental world over the past 20 years. In the United Kingdom, the reported incidence has risen 10 percent per year, and there has been a 460 percent



FIG. 4. A colposcopic photograph of extensive cervical HPV infection in a 23-year-old woman who was currently 28 weeks pregnant. HPV II DNA was extracted from a cervical smear from the lesion. This finding has implications for delivery to minimize the risk of neonatal transfer and laryngeal papillomatosis in the newborn.

increase in the United States over the last 15 years [57]. Age incidence peaks between 16 to 25 years, as does that of other sexually transmitted diseases.

Exophytic condyloma acuminatum of the cervix was once considered a very rare tumor; however, careful examination has revealed exophytic papillomas in up to 15 percent of women with vulvar lesions [58]. Women with vulvar condylomata acuminata are at a greatly increased risk of having associated cervical intraepithelial neoplasia (CIN) [59]. Cervical cytology and colposcopy should be part of the work-up of women presenting with condylomata acuminata. Similarly, women who are the sexual contacts of men with penile condylomata acuminata are at greatly increased risk of cervical neoplasia and should also be offered cervical cytology and colposcopy [60].

Progression of condylomata acuminata to cancer is well documented in both sexes; however, the relative risk is very low [20]. Rarely, large "condylomas" may show excessive growth with associated local invasion. Such slowly spreading, genital masses are termed giant condylomas of Buschke-Lowenstein or verrucous carcinomas. These lesions, which are usually induced by either HPV 6 or 11, represent indolent malignancies that rarely metastasize [58].

HPV-Induced Papular Lesions HPV infection of the vulvar skin can also produce small (3 to 7 mm), smooth, flat papules. Lesions may be pigmented or non-pigmented, or, in dark-skinned women, associated with depigmentation. Papules frequently occur in association with condylomata acuminata but may occur in the absence of other HPV-induced clinically apparent disease. Lesions frequently are multiple, sometimes coalescing to produce a larger area of disease (Fig. 4).

The histology of these papular lesions is quite variable, ranging from the features of benign HPV infection to significant epithelial dysplasia. Up to 90 percent of vulvar papules contain HPV 16 DNA. Hence, these inconspicuous lesions may represent an important male and female reservoir for oncogenic HPV types [57].

Subclinical Papillomavirus Infections It is now generally accepted that most genital HPV infection is subclinical, becoming visible only after the application of acetic acid. Cytologic and histologic features of overt and subclinical infections are essentially the same, koilocytotic atypia and dyskeratosis being prominent microscopic features of both forms of sexually transmitted HPV infection.



FIG. 5. A colposcopic photograph of a major-grade lesion with a well-defined regular border and widely spaced punctation and mosaicism. Histology revealed CIN III, and HPV 16 DNA was extracted.

Colposcopic differentiation between subclinical papillomaviral infection (SPI) and high-grade CIN is relatively difficult. Although this diagnosis will ultimately be made by histology, a colposcopic impression is important in order to direct biopsy to areas of most significant disease. On the cervix, subclinical HPV infections are characterized colposcopically by either indistinct aceto-whitening or a shiny, snow-white lesion, by an irregular outline with jagged, angular, or feathered margins, and by the presence of satellite lesions extending beyond the transformation zone. Capillary patterns may be pronounced and are often confused with the mosaicism and punctation characteristic of CIN 2–3; however, the vascular pattern of SPI is composed of uniform, fine-caliber vessels, loosely and randomly arranged, often as a horizontal mesh reminiscent of bizarre spider webs. Non-dilated capillary loops may also run vertically toward the surface, maintaining a uniform vessel caliber throughout their course. Staining with quarter-strength Lugol's iodine is a further aid to colposcopic diagnosis. Positive or partial staining denotes glycogenation, in contrast to the negative staining shown by areas of significantly transformed CIN.

Cervical SPI represents a heterogeneous group of lesions. The majority are due to infection by low-risk or unspecific HPV types, for example, HPV types 6 or 11. Current evidence suggests that, despite their role in causing abnormal Papanicolaou smears, such lesions are not common antecedents of cervical cancer.

In contrast, the 15–50 percent of subclinical cervical lesions induced by HPV 16, 18, 31, 33, 35, or 39 do indeed represent the earliest point in the pre-cancerous spectrum [43,57]. While some workers have suggested that minor-grade lesions containing high-risk HPVs are distinguishable from “non-precursors” by the presence of atypical mitotic figures [61], this finding has not been our experience [42,57,62,63].

Women with a history of koilocytotic atypia on a cervical smear have an increased risk of CIN 3 and invasive cancer [64]. Such retrospective studies do not, however, provide conclusive evidence of the progressive potential of SPI. If colposcopy is scheduled, over 30 percent of women whose smear showed only koilocytotic atypia will have cervical neoplasia confirmed at direct biopsy.

Evans and Monaghan demonstrated that 16 percent of histologically proved cervical SPI progressed to CIN 2–3, including one microinvasive carcinoma within a 12-month period [65]. This progressive potential of mild cervical atypia was also documented by Campion and colleagues in a recent prospective study [43]. Of women with cytologic

and colposcopic evidence of mild cervical atypia, 26 percent progressed to histologically proved CIN 3 within a two-year period. In 85 percent of the women whose disease progressed rapidly, a cervical smear was positive for HPV 16 on filter hybridization at the outset of prospective follow-up. Moreover, the spontaneous regression rate was very low (11 percent). Those women who did regress remained at high risk of future recurrence of their cervical disease. These results were very similar to the earlier prospective study of Richart and Barron [66], but the transit time to CIN 3 for this modern group of women was shorter. The progressive potential of CIN 3 is established [67]. This fact may reflect the increased incidence of CIN 3 and invasive cancer in young women in the United Kingdom and elsewhere in the Western world over the past decade.

Unless HPV typing becomes a clinical routine, it is best to regard cervical SPI and CIN 1 as biological equivalents; that is to say, the earliest stages in the pre-malignant spectrum representing a heterogenous group of lesions with differing natural history. Prospective follow-up, in summary, suggests about one-third of minor-grade lesions will progress to CIN 3, about one-third will regress, and about one-third will persist unchanged for years. Hence, a policy of empiric transformation zone ablation still represents the safest and most cost-effective management for such minor-grade atypia. Further research is required to determine whether such minor-grade lesions can be safely triaged for treatment or follow-up according to HPV type.

Subclinical HPV infection is also common in the vagina and vulva. Vaginal lesions present in one of these forms: (1) elongated vaginal papillae, (2) flat aceto-white epithelium, and (3) reverse punctuation.

Vulvar SPI can be either micropapillary or completely flat and therefore invisible without the aid of acetic acid soaking. The subclinical vulvar lesions are sometimes symptomatic, producing chronic pruritus, burning, or post-coital pain.

Penile Skin and Anal Region The concept of cervical neoplasia as a sexually transmitted disease has resulted in contact tracing of male partners [68,69]. Such studies have shown that 10–15 percent of the current male consorts of women with CIN will have overt penile condylomas; another 50 percent will have subclinical penile lesions which can only be seen after the male genitalia have first been soaked with white vinegar. It is especially noteworthy that 5–10 percent of male partners of women with CIN 3 will have severe dysplasia or carcinoma in situ of the penis [69].

In that penile cancer remains a rare disease in Western society, it can be assumed that the progressive potential of these penile lesions must be low. When penile squamous cancer does occur, however, over 50 percent of tumor biopsies contain HPV 16 or 18 [70].

Latent Genital HPV Infection Genital HPV infection is the most common viral sexually transmitted disease. It is clear that assessments of prevalence represent a gross underestimate of exposed individuals. Filter hybridization of cervical smears has detected latent HPV 16 infection in 17 percent of a random normal population [72] and 29 percent of antenatal patients [73]. Forty-eight percent of women attending a London sexually transmitted disease clinic were positive for HPV DNA despite having negative cytology and colposcopy; 25 percent were positive for HPV 16 DNA [Campion MJ: unpublished data]. A similarly large pool of latent HPV infection exists in the vaginal, vulvar, and penile epithelia.

The high prevalence of HPV types, including “high-risk” types, in clinically normal women and men may argue against the virus being causally related to genital

neoplasia. Alternately, when considered in view of the evidence implicating specific HPVs in genital carcinogenesis, the high prevalence of latent infection may define a very large at-risk group. It is clear that exposure to HPV alone may not be sufficient to produce neoplastic transformation and malignancy. Observations in animal and human models suggest a synergism between specific HPV infection and chemical or physical carcinogens. Thus, a search for cofactors is critical to understanding the significance of latent and subclinical HPV infection in relation to genital carcinogenesis.

HPV AND GENITAL CANCER: CASUAL OR CAUSAL ASSOCIATION

Specific HPV types are incriminated as the etiologic agent for a group of epithelial proliferations which may progress to anogenital malignancy. The detection of HPV DNA within genital tumor tissues must be distinguished from various statistical associations between other sexually transmitted disease and cervical, vulvar, and penile cancers. The relationship between HPV infection and malignancy is quite strict [58]. More than 90 percent of cancers contain oncogenic HPVs, and the same HPVs are detected in malignancies throughout the world, despite the heterogeneity of the genus. Archival cervical cancer specimens from the 1930s can also be shown to contain the same HPV types [Collins J, McCance DJ: personal communication]. The cloning of new papillomavirus types has increased the HPV DNA detection rate to 90 percent; still fewer cancers will test negative as more probes become available.

The persistence of viral DNA in malignant cells has also been shown by the detection of HPV DNA in cervical cancer-derived human cell lines. Seven of nine such cell lines contain HPV DNA, including HeLa cells (derived from an HPV 18-containing adenocarcinoma) and Ca-Ski cells (an HPV 16-containing squamous carcinoma) [74].

The precursor lesions of anogenital malignancies frequently harbor the same specific HPV types as those detected in the malignancy. In HPV 16- and HPV 18-associated neoplasia, there are alterations in physical state of the viral DNA within the host cell between pre-malignant and malignant disease. Within pre-malignant tissue, the viral DNA exists as a free episome (i.e., a self-replicating, extrachromosomal, nuclear plasmid). In contrast, most of the viral DNA within invasive cancers is integrated into the host chromosomes (i.e., spliced into the host genome by covalent bonds, often in tandem arrays) [74]. This condition occurs in particular with HPV 16- and 18-containing tumors; however, some evidence suggests that HPV 10, HPV 11, and HPV 33 may persist as an episome within cervical cancers, suggesting integration may not always be a prerequisite for malignant transformation.

When viral integration occurs, in the several cases examined, integration occurred near a cellular proto-oncogene (C-src, C-raf, C-myc) [75]. Furthermore, C-myc and/or C-ha-ras oncogenes are amplified in a significant percentage of cervical cancers, and C-myc mRNA levels are also elevated [75]. The integration exhibits an interesting specificity with respect to the viral genetic regions. It is almost always at the downstream (3') end of the E1 open reading frame (the main signal responsible for maintaining viral DNA in an episomal state). Hence spatial disruption of this protein coding area may favor viral integration and subsequent carcinogenesis [31].

All cervical carcinoma cell lines analyzed to date, together with the majority of HPV DNA-positive tumor biopsies tested, contain virus-specific transcripts for open reading frames in the early region of the viral genome [40]. RNA mapping has revealed

transcription of E6 or E6* (a message shortened by interval splicing). In addition, an E7 protein has been identified in several cell lines. Preliminary experiments indicate that continued expression of E6/E7 proteins may be required for maintenance of the transformed state. The fact that transcription takes place in the genomically integrated virus in squamous cell cancers and that a putative transforming segment, the E6, E7 open reading frame, is consistently transcribed argues strongly that the virus is playing a role in the pathogenesis of human genital cancer and is consistent with the action of other oncogenic viruses in other animal systems.

It appears highly probable that specific HPVs play an essential or pivotal role in the etiology of anogenital cancer. It is not possible to decide whether these viruses are necessary for tumor induction, maintenance, or both. As has been discussed, the continued persistence of viral DNA and the fact that the viral DNA is actively transcribed in pre-malignant and malignant cells are highly suggestive of a role in maintenance of the malignant state. It is not possible, however, to exclude the possibility that HPV may just be extremely efficient at persisting after initiation of tumor progression. What is certain is that it is necessary to dispense with a unifactorial theory for genital oncogenesis and consider the possible role of co-carcinogens in the etiology of these cancers.

THE POSSIBLE OPERATION OF OTHER CO-CARCINOGENS

Given the probable role of papillomaviruses, it does seem unlikely that HPV infection alone is sufficient to induce a carcinoma in an immunocompetent host. This conclusion is suggested by the long lag time between initial infection and eventual malignant conversion and by the spontaneous regression of many primary lesions. The risk of a given viral type producing a carcinoma can be roughly estimated from the ratio of the prevalence of that type in the normal population versus the prevalence of HPV-positive cancers. Such a preliminary calculation indicates that only about one in 100 HPV 16-infected women will develop cervical cancer. The risk of malignant progression of HPV 6, HPV 11, or HPV 31 infections appears to be five- to tenfold lower than for HPV 16. Deriving more reliable estimates of these risks will depend upon prospective studies in HPV-infected women, matched for other risk factors.

Studies of the Shope papillomavirus system in rabbits have shown that carcinogenesis is influenced by the operation of additional etiologic agents [44,45]. Malignant progression was facilitated by exposure to physical (ultraviolet light or X-rays) and chemical carcinogens (dietary components, hydrocarbons). In addition, chronic irritation or inflammation exerted a weaker, less specific effect.

Plausible cofactors within the lower genital tract include tobacco products, infection by other microbial agents, and immunosuppression. Heavy smoking has been implicated as a risk factor, and tobacco metabolites have been detected in cervical mucus [76], which permits a possible direct effect on the target cells within the transformation zone. Whether concomitant herpes virus infection could also act as a co-carcinogen is an open question [77]. Nonetheless, it is conceivable that concomitant herpetic infection might affect papillomavirus genes or cellular control genes, either of which could change viral expression. An amplification of the C-myc or C-ras cellular oncogenes or both has also been observed in the late stage of cervical carcinomas. Such an effect could also contribute to tumor progression.

Both the prevalence of genital condylomas and the risk of developing carcinoma *in situ* are increased in immunosuppressed patients. The increased and accelerated

progression of HPV-induced cervical atypia associated with impaired host immunity contrasts with findings in the Shope papillomavirus, where corticosteroid administration did not influence malignant conversion of papillomas.

PROSPECTS FOR VACCINATION

If HPV infection is indeed necessary for the induction of anogenital cancer, the prospect of controlling the disease by vaccination opens an exciting possibility. In human medicine, healthy women cannot be deliberately infected with an oncogenic papillomavirus. Hence, proof along the lines of experiments conducted in the Shope papillomaviral system will never be possible. Rather, the best evidence of causality in humans would be the demonstration that vaccination of non-exposed individuals reduced the subsequent occurrence of anogenital malignancy. Aside from the question of cancer prophylaxis, the morbidity resulting from condylomas and dysplasia, which are clearly HPV-induced, may be reason enough for a vaccination program.

Observations in spontaneously regressing skin warts are most noteworthy. Histologic examination reveals strong mononuclear cell infiltrates, and electron microscopy has shown activated Langerhans cells and attacks by macrophages on HPV-infected epidermal cells. These data indicate that wart cell-specific surface antigens are being detected by the host's cell-mediated immune system. Once started, regression of multiple warts is usually a systemic phenomenon, and the host thereafter remains immune to reinfection by that HPV type.

Notwithstanding the host's potential to fight HPV infections, the immune response is often weak and slow. This failure could reflect scant induction of HPV surface antigens, perhaps in levels that are too low for immunologic detection. Hence, vaccination might help to teach the immune system how to detect HPV-infected cells. To this end, a vaccine would need to be based on specific virus-coded or virus-induced membrane-bound proteins. Future research must therefore focus on the identification and characterization of such antigens. The Shope papillomavirus-rabbit model is then probably the best system for evaluating the efficacy of any vaccine designed to prevent benign or malignant tumors, or both. In addition, vaccination might also offer the prospect of immunotherapy for established disease.

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