The Role of Human Papillomaviruses in the Generation of Genital Cancer

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Human papillomaviruses (HPV) are the causative agents of a variety of conditions ranging from warts to cervical carcinoma. Infection by this virus occurs in 10 to 20 per cent of the population. The induction of premalignant and malignant changes by papillomavirus has led to important insights into the mechanisms of carcinogenesis. This review outlines the biology and clinical aspects of this important human pathogen. The aspects of HPV that will be discussed include:

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I. ROLE OF VIRUSES IN HUMAN MALIGNANCY

The concept of an association between viral agents and cancer has a long and interesting history dating to the early years of this century [1]. The first reports of tumor induction by filtered tumor extracts came in 1908 from Ellerman and Bang working with avian eythroleukaemia [2] and in 1911 from Peyton Rous working with an avian sarcoma [3] These studies were the first steps on a path leading to the identification of the RNA-containing retroviruses and to an understanding of their oncogenic potential. They laid the basis over 60 years later, to the cellular origin of transduced retroviral oncogenes [4] and the identification of cellular oncogenes themselves [5].

The first evidence for the existence of DNA viruses with oncogenic potential also came from animal systems. The key findings were made on tumors which occurred naturally at a relatively high incidence in the wild. In 1933, Shope reported that extracts of cutaneous papillomas of the cotton-tail rabbit induced similar lesions on experimental transmission to cotton-tail of domestic rabbits [6]; moreover these lesions frequently progressed to frank carcinoma [7]. A few years later Lucké published his work showing that the renal carcinomas commonly found in leopard frogs (*Rana pipiens*) were similarly transmissible within the natural host species [8]. The path from these original findings to our present understanding of DNA viral oncogenes was less direct than the identification of retroviral transduced oncogenes. Here the crucial experiments were carried out using oncogenic proteins discovered in a variety of other DNA virus families. These were mouse polyomavirus [9], the simian polyomavirus SV40, [10] and certain human adenoviruses [11], which could be readily propagated in cell culture. Each could induce tumors on experimental inoculation into rodent species and transform cultured embryonic fibroblasts from these species into permanently established cell lines *in vitro* [12]. The studies of polyoma and adenovirus provided the conceptual basis for current work on the role of DNA viruses associated with human tumors. The principal agents now being studied in this latter context are not polyoma or adenoviruses, but members of the same DNA virus families first studied by Shope and by Lucké. The following table identifies those viruses for which there is now strong evidence of an etiological link with particular forms of human cancer.

Viruses and Human Cancer

Virus type	Tumor	% virus positive	Latency period
Human papilloma viruses			
HPV types 16, 18	Genital carcinomas	>75%	5-30 years
HPV types 5, 8	Skin carcinoma of EV patients	>90%	5-30 years
Epstein-Barr virus			
EBV types 1, 2	Immunoblastic B cell lymphoma	100%	0.1-10 years
EBV types 1, 2	Burkitt's lymphoma, endemic	>95%	3-10 years
	sporatic	20%	
EBV types 1, 2	Hodgkin's disease	40%	3-60 years
EBV types 1, 2	Nasopharyngeal carcinoma	100%	30-40 years
Hepatitis B virus (HBV)	Hepatocellular carcinoma	>80%	30-50 years
Human T lymphotropic virus			
HTLV type 1	Adult T cell leukemia	100%	20-50 years

It is worth stressing that, cumulatively, the above virus-associated human tumors constitute a substantial fraction (perhaps 15-20%) of total tumor incidence worldwide [13, 14]. In many of the above cases, therefore, the hope is that tumor incidence can be substantially reduced by some form of immune intervention directed against the associated virus. This may be achievable by conventional vaccination to induce virus-neutralizing antibodies and thereby prevent viral infection, as is happening in the case of hepatitis B virus, or by other strategies, for instance activating cytotoxic T-cell responses capable of recognizing viral antigens which continue to be expressed in the tumor cells.

II. ANALYSIS OF THE GENETIC STRUCTURE OF HPV

Papillomaviruses are widespread in the animal world and there are representatives infecting many different species including fish, birds, and a whole variety of mammals such as dogs, sheep, cattle, monkeys, and man [15]. Virus replication takes place in benign epithelial proliferations which develop after viral infection. To date no *in vitro* system for virus propagation has been developed. Therefore biochemical and serological characterization of the virus particles is only possible in those cases where sufficient quantities can be obtained from the naturally occurring lesions. With the introduction of recombinant DNA technology, the viral genomes were molecularly cloned [16] and thereafter, by reverse genetics, some gene functions could be identified. However, the failure to successfully propagate significant quantities of virus in tissue culture has greatly hindered our understanding of the biology of papillomavirus.

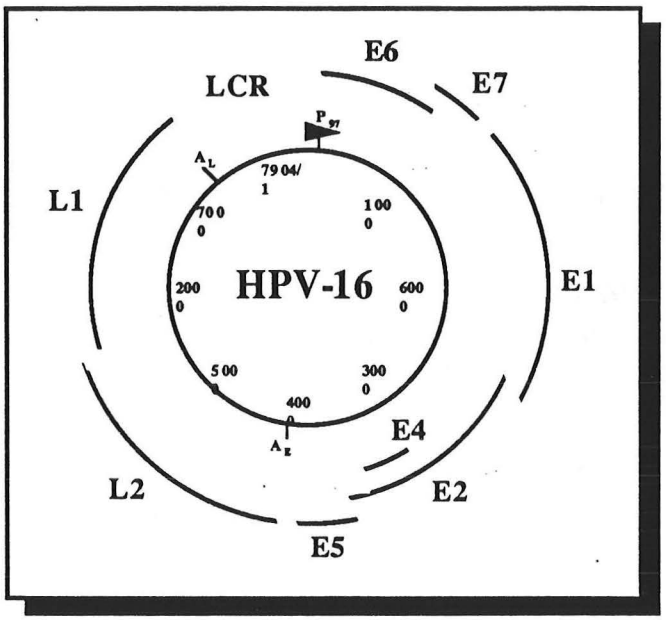
Distribution of HPV Types by the Degree of Genetic Relatedness

Group	Types	Location and characteristics of lesions
A	1	Skin; mainly plantar warts
B	2, 27, 29, 57; 3, 10, 28	Skin; flat and common warts
C	4	Skin; common warts
D	5, 8, 12, 14, 19-23, 25, 36, 46, 47, 49; 9, 15, 17; 37, 38; 24	Skin; most isolates are from EV patients
E	6, 11; 13, 44, 45	Genital tract (6, 11, 44, 45) and oral cavity (13)
F	7, 40	Skin, common warts
G	16, 31	Genital tract
H	18, 32, 42, 45	Genital tract (18, 42, 45) and oral cavity (32)
I	26, 51	Skin
J	30, 53	Larynx
K	33, 52	Genital tract

Though all human and animal papillomaviruses have similar genetic organization, different degrees of sequence homology between individual papillomavirus genomes has revealed that extensive heterogeneity exists within this family of viruses [17]. DNAs of different human papillomaviruses are cross-hybridizing under conditions of reduced stringency [18]. A variety of different HPV types have been identified using this procedure. Depending on the preferential site of infection, human papillomaviruses can be divided broadly into skin and mucosal types. It is not clear which factors (genomic diversity, cellular receptors, intracellular activation, or immunological phenomena) determine viral tropism for target tissues [19-21].

The icosahedrol capsid structure of papillomavirus encloses an approximately 8000 bp circular double stranded DNA molecule. This molecule contains two regions ('early' and 'late') with a number of open reading frames (ORF) as well as an intermediate DNA stretch of about 1000bp between the early and late regions. This latter portion of the part of the genome does not contain any ORF of reasonable size, but it does contain *cis*-acting elements which are required for the control of DNA replication and mRNA transcription.

Genomic Map of HPV-16



This region has been variously designated the non-coding region (NCR), upstream regulatory region (URR), or long control region (LCR). The early region was originally defined as a 69% fragment of the bovine papillomavirus (BPV) genome which is sufficient for transformation of rodent cells *in vitro* [21]. It was through genetic analysis of the BPV that the functions of the individual ORFs were unraveled. *In vitro* transforming activity was allocated to the 5' and 3' ends of the early region containing the E6/E7 and E5 ORFs respectively. There are at least two proteins encoded by the E1 ORF and both proved to be involved in DNA replication and plasmid maintenance. The E2 ORF codes for two proteins which act as activators and repressors, respectively, of viral mRNA synthesis. Although encoded within the early region, the E4 protein is distinct from the early viral proteins in that it is produced in large amounts within the HPV-induced lesions [22] and seems to be involved in virus particle maturation. The E6 and E7 are the transforming proteins of HPVs and are always expressed in HPV-associated invasive cancers and in cell lines derived from these tumors. The ability of the oncogenic viruses HPV-16 and HPV-18 to transform rodent cells, immortalize human keratinocytes, and produce carcinoma *in situ*-like lesions in organ cultures of human epithelium is localized to their E6 and E7 genes. The late region encodes two proteins L1 and L2. These proteins form the viral capsid and probably mediate viral functions such as attachment to susceptible cells, host range, immune response, and neutralization.

Functions Assigned to the Papillomavirus Open-reading Frames

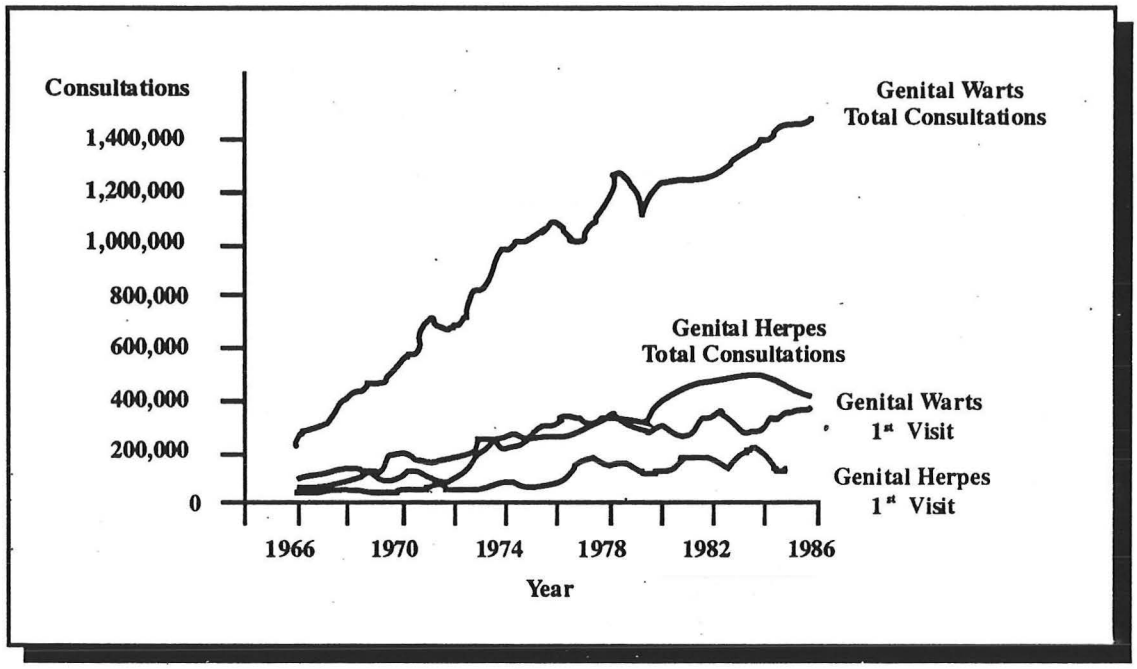
Function	ORF
Plasmid replication	E1
Regulation of transcription	E2
Transformation	E5, E6, E7
Coding for capsid proteins	L1, L2
Coding for late cytoplasmic protein	E4
Not yet known	E3, E8

III. EPIDEMIOLOGY OF HPV INFECTION

Human papillomavirus infections are not reportable to public health departments, and given the difficulty in diagnoses accurate incidence figures are difficult to obtain [23],. The most easily recognized clinical manifestation of HPV infection is overt genital warts or condyloma acuminatum. Surveys of consultations by private physicians for genital warts conducted by the National Disease and Therapeutic Index have shown an increase from an estimated 169,000 consultations in 1966 to 1,150,000 consultations in 1984 [24]. The majority of patients were between the ages of 15 and 30, and more women sought treatment than men. Because these estimates do not include patients who elect not to seek treatment, they represent minimum figures. This same survey also calculated that about 450,000 patients were seen for genital herpes simplex virus (HSV) infections in 1984 [25], suggesting that genital warts may be more common than genital herpes.

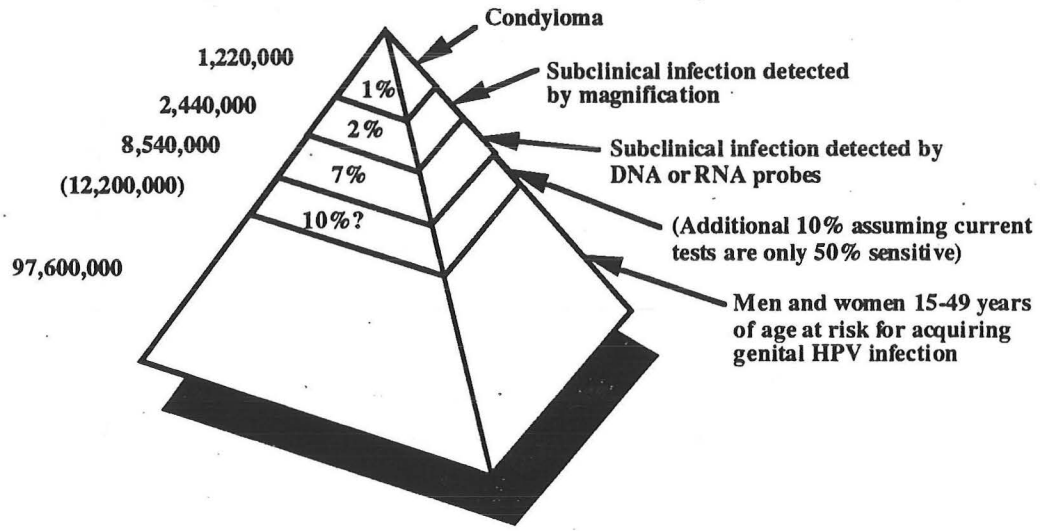
The incidence of genital warts is only an indirect indicator of the true incidence of HPV infection of the genital tract. The spectrum of HPV infection is much wider and also includes minimally symptomatic lesions, subclinical infections, and latent infections. Although the exact proportion of individuals with each type of clinical manifestation is not known, it has been estimated that genital warts may represent only 10% or less of the total spectrum of genital tract HPV infections [26]. Because there is no simple, sensitive, and accurate test for the presence of HPV that can be applied to large populations, direct measurements of the prevalence of HPV are not available. However, studies of selected

Estimates of First Office Visits in the U.S.



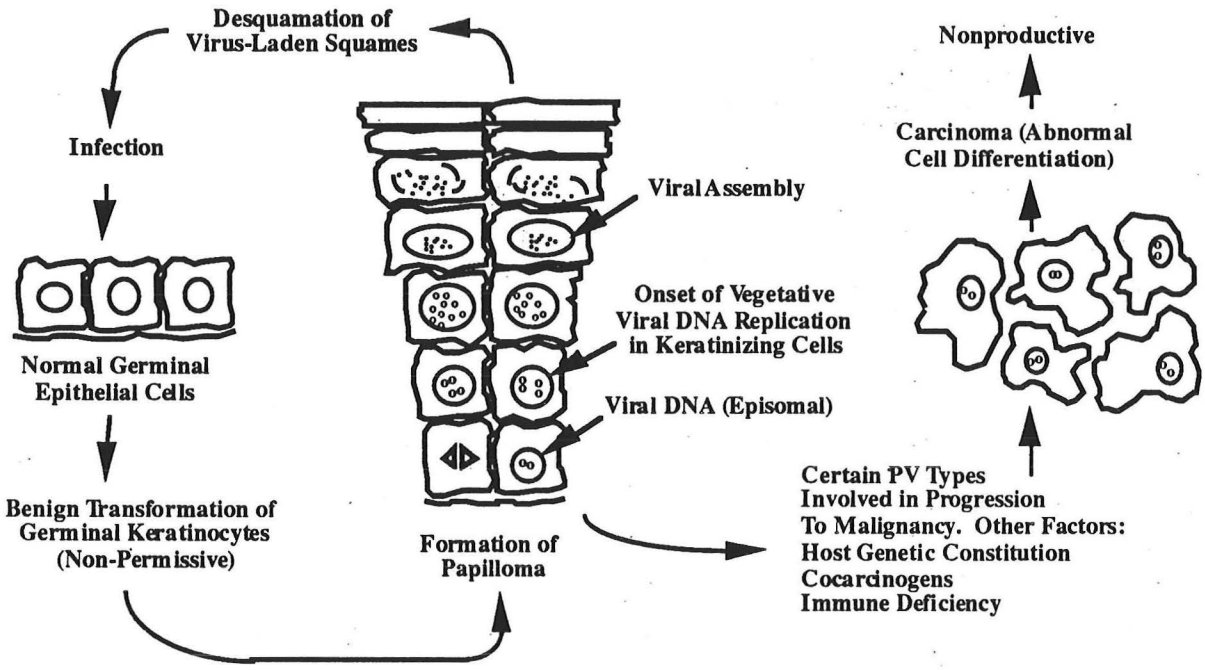
populations have provided some estimates of prevalence that suggest that a significant number of individuals may be infected. Such estimates include 9% of unselected women presenting for cytologic screening [27], 9% and 11% of women attending a university student health clinic and an STD clinic, respectively [28], 23% of women attending a family planning clinic [29], 11% [30] or 28% [31] of unselected pregnant women, and 82% of repeatedly sampled prostitutes [32]. Although each of these studies suffers from limitations due to patient population or HPV-detection method employed, it would not be unreasonable to suggest that a minimum of 10% to 20% of sexually active women may be infected with HPV. Although there are fewer studies in men, those available suggest that nearly two thirds of male sexual partners of HPV-infected women have genital lesions suggestive of HPV infection [33, 34]. Thus, the prevalence of HPV infection in men is likely to be similar to that in women.

Estimated Prevalence of Genital HPV Infection Among Men and Women Between the Ages of 15 and 49 years in the United States in 1987



The natural history of genital HPV infections has been reviewed by Oriel [35]. Although it is clear that genital HPV infection is transmitted sexually, the exact mechanism of infection at the level of the virus-host cell interaction is not known. It is generally assumed that the virus gains entry to the basal cell layer of epithelial surfaces through small or microscopic abrasions, leading to transformation of one or more basal cells. Genital lesions usually appear after an incubation period of approximately 3 months, with a range of 3 weeks to 8 months [35]. Studies aimed at the localization of transcription of HPV genomes in condyloma acuminatum reveal mRNA species from several early-region ORFs in the basal layer of cells, whereas late transcripts, which signify virion production, appear only in the more differentiated layer of epithelium [36]. Only the top layers of epithelium contain infectious viral particles [37, 38]. The infectious viral particle is probably released along with desquamated cells that are shed normally from the skin or mucous membranes.

Life Cycle of HPV in Productive Lesions



The immunobiology of HPV infections has recently been reviewed by Kirchner [39]. The natural history of HPV infection can be extremely variable. Spontaneous regression of papillomatous growths is commonly observed [40, 41]. Trauma or surgical manipulation of one in a group of warts may lead to regression of other warts in the group. Trauma may cause the dissemination of viral antigens, causing a boost in host immunity. Evidence from immunodeficient patients points toward a role for cell mediated immunity (CMI) in controlling HPV infections [42-44]. In 105 immunosuppressed renal transplant recipients, evidence of HPV infection was found in 17.5% and evidence of genital neoplasia was found in 9.5% [45]. The incidence of HPV-related disease in these patients was 17 times greater, and the risk of cervical neoplasia 9 times greater than in a matched immunocompetent population. In another study of 132 female renal transplant recipients, 11 (8.5%) developed cervical condylomata; 6 of these 11 patients developed cervical neoplasia [46]. Patients with Hodgkin's disease, lymphoma, or chronic lymphocytic leukemia have an increased incidence of warts [43]. Patients infected with the human immunodeficiency virus (HIV) have a high incidence of genital HPV infection and epithelial dysplasia [47, 48]. Existing HPV infections often worsen during pregnancy, but it is not clear whether this is due to alterations in the immune system, hormonal changes, or other factors [49, 50]. In contrast to defects in CMI, defects in humoral immunity do not have a significant effect on the natural history of HPV infections [51].

A role for HPV in the etiology of cervical cancer was also proposed a number of years ago [52]. Over the last few years, this agent has become the leading candidate. Although numerous studies have found HPV DNA in the vast majority of cervical carcinoma tissues, there have been few controlled epidemiologic studies of this association similar to those that initially implicated HSV-2. The major factor that has limited such studies has been the technical difficulty of testing for HPV in large population. One case-controlled study of Latin American women with invasive cervical cancer detected HPV DNA in 91% of cancer patients as compared to 63% of controls (P=0.002) [53]. Human papillomavirus type 16 or 18 was found more commonly in cancer patients than in controls (67% versus 43%; P=0.03). Another study attempted to correlate the incidence of cervical HPV infection with the incidence of cervical cancer in Greenland and Denmark [54]. Although the incidence of cervical cancer in Greenland is nearly six times that in Denmark, the incidence of cervical HPV-16 and HPV-18 infection was found to be slightly higher in Denmark (13% versus 8.8%), whereas the incidence of other HPV types was similar.

A large epidemiologic study was conducted by performing cytologic examinations and HPV DNA detection in over 9000 women at three clinics in Germany. Cervical cytology was normal in 94.2%, 2.1% had only cytologic changes suggestive of HPV infection (koilocytosis), and 3.7% had dysplasia or invasive carcinoma. Nine percent of patients with normal cytology were positive for HPV, whereas 35% of those with dysplasia or cancer were positive for HPV. Thus, although the epidemiologic studies conducted to date have not provided a conclusive link between HPV and cervical neoplasia, the consistent identification of HPV DNA in dysplastic and neoplastic lesions and *in vitro* studies showing dysplastic-like changes in cultured epithelia into which HPV DNA has been introduced all provide strong support for the hypothesis that HPV has an etiologic role in cervical carcinogenesis. Associations of HPV with vulvar carcinoma, penile carcinoma, and anal carcinoma have also been demonstrated, but the data are less complete.

IV. CLINICAL MANIFESTATIONS OF HPV INFECTION

The spectrum of clinical disease of the genital tract associated with HPV infection ranges from latent, asymptomatic infection, to overt malignancy of epithelial surfaces. The disease entities associated with HPV infection vary with the anatomic area of the genital tract that is infected. Although condyloma acuminatum has been recognized for many years, it is now clear that individuals with subclinical HPV infections far exceed the number with condyloma acuminatum.

Classes of Papillomaviruses

- **Cutaneous (non-genital) - HPV 1-4**
  Benign - plantar, common, flat warts
- **EV - many HPV types**
  (Benign - HPV 3, 10)
  Malignant potential - HPV 5, 8
- **Mucosal (genital, oral, laryngeal)**
  Mainly Benign - HPV 6, 11
  Malignant potential - HPV 16, 18, 31, 33

A. HPV Infection in Men

Human papillomavirus can infect the penis, urethra, scrotum, perianal, anal, and rectal areas in men. Condyloma acuminatum is usually an easily recognized clinical entity, appearing as soft, sessile tumors with a surface that ranges from smooth to very rough with multiple finger-like projections. Perianal condylomata acuminatum are usually rough and cauliflower-like, whereas penile lesions are often smooth and papular. Penile condylomata acuminatum are usually 3 to 5 mm in diameter and often occur in groups of three of four lesions. Lesions usually cause no symptoms, but some patients complain of pruritus, irritation, or bleeding as a result of trauma. Although classic condyloma acuminatum is easily recognized, a second type of HPV-induced lesion known as a flat keratotic plaque may be more difficult to detect. These lesions project only slightly above normal epithelium and have a rough surface that may be somewhat pigmented. Subclinical HPV infection is common in men, and presents as diffuse foci of epithelial hyperplasia that are invisible on routine examination [55].

Rosemberg et al [56] evaluated 291 men for the presence of HPV infection of the genital area. This group consisted of 92 men with self-recognized disease and 199 who were referred for evaluation after HPV was detected in their female sexual partners. Of the 199 male partners of HPV-infected women, 99 had visible lesions and 47 showed only subclinical HPV infection. Only 53 patients (27% of the referral group) had a negative initial examination, and 8 of these 53 patients developed lesions during a 6-month follow-up period. Thirty-nine penile shaft lesions and 60 cases of scrotal involvement were visible only after the application of dilute acetic acid, a procedure performed in all patients. Biopsy samples from 30 patients (10 condyloma acuminatum and 20 subclinical lesions) were analyzed for the presence of HPV genomes with probes for HPV type 6, 11, 16, 18, and 31. All the condyloma contained HPV genomes (eight with HPV types 6 or 11, and two

with an unidentified HPV type). Penile cancers have also been shown to contain HPV DNA sequences [57, 58].

It is not known whether HPV can be transmitted from men with subclinical infection to sexual partners. Such a transmission is theoretically possible, and men with subclinical HPV infection may represent a major reservoir in the population. Intraurethral condylomata represent another potential reservoir for HPV infection. Treatment failure in women may represent, in some cases, reinfection by a male sexual partner with one of these occult forms of HPV infection. Therefore, it is essential that men with condyloma acuminatum and male sexual partners of women with known HPV infection be evaluated are fully and treated.

B. HPV Infection in Women

As in men, the spectrum of the clinical disease associated with HPV infection is broader than previously recognized. Although the classic exophytic lesions of condyloma acuminatum of the external genitalia are easily recognized, detection of other forms of HPV infection requires careful colposcopic and sigmoidoscopic examination. As in men, malignancies of the genital epithelia in women often contain HPV DNA sequences. There appears to be a stepwise correlation between the degree of pathologic expression (from histologically normal skin to overt neoplastic features) and the detectability of HPV DNA in biopsies of genital lesions in women [59]. The clinical entities caused by HPV have been reviewed by Campion [60]and include vulvar, vaginal, cervical, and rectal infection.

Vulvar condyloma acuminatum appears as soft, whitish sessile tumors, either papular or with fine, finger-like projections. They are most common in moist areas such as the introitus and labia. In nonmucosal areas, lesions appear similar to those usually seen in men on the penile shaft. Large areas of disease form from multiple, coalescing lesions . Subclinical HPV infection of the vulva can be identified by application of dilute acetic acid followed by colposcopic examination. Human papillomavirus types 16 and 18 are detected in vulvar papules that histologically show epithelial dysplasia [61-63]. Vulvar carcinoma is associated with HPV in a high percentage of biopsies. Reid et al [59] found HPV-16 in 80% of vulvar carcinomas, while Sutton et al [64]demonstrated the presence of HPV-6 or HPV-11 in 78% of these cancers.

Condyloma acuminatum of the vagina occurs in approximately one third of women with vulvar condylomata [65]. Multiple lesions are usually present. Vaginal discharge, pruritus, and postcoital bleeding may occur, although most vaginal condylomata are asymptomatic. Human papillomavirus types 6 and 11 are most commonly detected in these lesions. Several patterns of subclinical vaginal HPV infection are identifiable by acetic acid

staining of the vaginal mucosa followed by colposcopic examination [60]. Elongated vaginal papillae are epithelial projections analogous to individual fronds of condylomata. Acetowhite epithelium is a second form of subclinical vaginal HPV infection that appears as sharply defined flattened white patches. Reverse punctation is a third form of subclinical vaginal HPV infection that appears as multiple tiny acetowhite spots on the vaginal walls visualized with the colposcope. Vaginal malignancy has been reported in association with HPV infection [66 67].

Condyloma acuminatum of the cervix occurs in approximately 20% of women with HPV infection in other areas of the genital tract [65]. Condyloma acuminatum of the cervix appears as papillary epithelial proliferations in the cervical transformation zone and in the original squamous epithelium (Fig. 4). Irregular vascular loops are often seen beneath the translucent surface epithelium. Single or multiple lesions may be present on the cervix. Condyloma acuminatum of the cervix is usually caused by HPV-6 or HPV-11, with HPV-16 being detected in less than 10% of patients [57].

Condyloma acuminatum may also be present in the perianal, anal, or rectal area of women. As in men, the anal squamous epithelium is another site at which infection with HPV (most often HPV-6, HPV-16, and HPV-18) is associated with malignant transformation [68]. Without sigmoidoscopy, lesions of the rectum will not be seen. Subclinical HPV infection in the perirectal and rectal areas can be detected by acetic acid staining. Flat white lesions are noted that reveal the typical histologic features of HPV infection on biopsy.

C. Subclinical Cervical Hpv Infection (SPI)

It is now generally accepted that most cervical HPV infection is subclinical, becoming visible only after the application of acetic acid [69]. Whereas cervical condylomata acuminata are uncommon, cervical HPV infection is one of the commonest sexually transmitted diseases. Cytopathic effects of HPV infection, specifically koilocytotic atypia, dyskeratosis, and multinucleation, are detected in 2% to 3% of routine cervical smears [70; 69, 71]. 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.

Colposcopic differentiation 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. The Reid colposcopic index [72] greatly aids in differentiating SP1 and more significant degrees of carcinoma *in situ* (CIN). This method uses four colposcopic signs (lesion margin, color, vascular pattern, and iodine staining) as

a means of directing biopsy to the most severe area of disease. SPI is characterized colposcopically by either indistinct acetowhitening 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 mosiacism and punctation characteristic of CIN 2-3. However the vascular pattern of SP1 is composed of uniform, fine-caliber vessels, loosely and randomly arranged often as a horizontal mesh reminiscent of bizarre spider webs. Nondilated 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 [28, 47, 73, 74].

SPI apparently represents the earliest stage in the CIN continuum and should be seen as the earliest cervical lesion capable of progressing to invasive cancer [28, 47, 73]. Women with a history of koilocytotic atypia on a cervical smear have an increased risk of CIN 3 and invasive cancer [75]. However such retrospective studies do not provide conclusive evidence of the progressive potential of SP1. Approximately 30% of women whose smears show only koilocytotic atypia will have cervical neoplasia confirmed at directed biopsy [76].

Evans and Monaghan demonstrated that 16% of histologically proven cervical SP1 progressed to CIN 2-3, including one microinvasive carcinoma within a 12-month period [77]. This progressive potential of mild cervical atypia was also documented by Campion and colleagues in a recent prospective study [78]. Of women with cytologic and colposcopic evidence of mild cervical atypia, 26% progressed to histologically proven CIN 3 within a 2-year period. In 85% 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 [78]. Moreover, the spontaneous regression rate was very low (11%). 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 [79], who found an increased incidence of CIN 3 and invasive cancer in young women in the United Kingdom over the past decade [80].

The large excess of SPI and CIN over cervical cancer suggests that the minor cervical lesions have a low malignant potential, progression to cancer being unlikely within a woman's lifetime. However, any lesion with proven potential for progression and associated karyotypic anomalies must be accepted as a true precursor of squamous cancer [81]. Certainly, the invasive potential of CIN 3 is established [82] and the progressive potential

of SPI to CIN 3 has been documented [78,77].

Studies relating ploidy to progression within histologically proven biopsies of cervical SPI have been confusing, some demonstrating a positive predictive value [83, 28, 47, 73] and others failing to demonstrate any association [77]. Flow cytometry is a useful technique for analyzing a large number of cells. However, the cell suspension usually contains a mixture of normal and abnormal cells. Combining both techniques increases the sensitivity of ploidy analysis. Using this approach, as many as 68% of biopsies showing histologic evidence of HPV-induced cervical atypia without associated dysplasia, have been reported to contain aneuploid cells [84].

D. Carcinoma *In Situ*

The characteristic morphologic features of mild dysplasia (CIN 1) include a slight degree of proliferation of basal and parabasal cells showing mild nuclear atypia and koilocytosis (koilocytotic atypia) in most instances. The koilocyte is characterized by nuclear enlargement, hyperchromasia, coarsening and margination of the chromatin, an irregular nuclear membrane, and cytoplasmic necrosis producing a perinuclear halo. Because replication of the virus is linked to squamous epithelial cell differentiation, the manifestations of productive infection are most prominent in the superficial layers, as demonstrated by *in situ* hybridization for HPV DNA and immunohistochemical localization of viral structural proteins [85, 86]. Nuclear pleomorphism, including giant nuclei, binucleation, and multinucleation are common [87, 88]. Variations on this theme occur. For example, although the typical koilocyte may have a centrally located, round nucleus surrounded by a clear zone, more often the nucleus is displaced against the cell membrane. In addition superficial cells, containing HPV structural proteins may have round, flattened or raisin-shaped pyknotic nuclei in which cytoplasmic clearing is inconspicuous or absent [89, 90]. Acanthosis results in a thickened squamous epithelium with an undulating or spiked surface that may exhibit hyperkeratosis. Papillae are formed as a result of the penetration of the epithelium by capillaries in the underlying stoma. The hyperkeratosis and vascular proliferation associated with HPV infection lead to the characteristic picture of white epithelium, punctation, and mosiacism observed on colposcopic examination.

Moderate dysplasia (CIN 2), severe dysplasia (CIN 3) and carcinoma *in situ* (CIS) (CIN 3) constitute a morphologic continuum that merges with CIN 1. Despite the subtle changes exhibited by the different grades of lesions along this morphologic spectrum, CIN 2 and 3 lesions display morphologic and molecular features that separate them from CIN 1. Koilocytosis may be present in CIN 2 and 3, but occurs less frequently than in CIN 1. Whereas CIN 1 is characterized by abnormal differentiation apparent in the superficial

layers of the epithelium, it is the nearly complete or total lack of differentiation that characterizes the higher grade CIN lesions. In the latter, abnormal parabasal cells replace the lower half or the full thickness of the epithelium and mitotic activity is increased. There is a rough correlation between the grade of CIN the level at which mitotic figures are observed and the presence of abnormal mitotic figures. Specifically, CIN 1 lesions usually do not display mitoses much above the basal layer, whereas in CIN 3, mitoses are often present in the superficial levels. Abnormal nitoses, characterized by aberrations in spindle number (tripolar, multipolar) or nature (uneven division), are occasionally observed in CIN 1, but are frequently seen in CIN 2 and 3 [91].

A number of benign lesions may be confused with CIN. Among these, immature metaplasia and atrophy are most commonly mistaken for high-grade CIN. In immature metaplasia the full thickness of the epithelium is composed of immature parabasal cells. The cells are uniform, usually vertically arranged, and the nuclei are somewhat hyperchromatic. The most important feature distinguishing CIN from immature metaplasia in the absence of nuclear atypia. In addition, immature metaplasia is often covered by a residual layer of nucinous epithelium, a feature generally not present in CIN. Immature metaplasia may be active and display mitotic activity, but abnormal mitotic figures are not present. Atrophic epithelium is thin and composed of basal and parabasal cells showing no differentiation and a high nuclear cytoplasmic ratio. However unlike CIN atrophic epithelium lacks nuclear pleomorphism and mitotic figures. In older women in whom it is difficult to distinguish atrophy from high-grade CIN, a repeat biopsy after a 3- to 6 week course of topical estrogen cream often resolves the problem by inducing maturation in atrophic epithelium.

The prevalence of HPV DNA by Southern blot hybridization in intraepithelial and invasive cancer is 80% to 90% and approximately 15% in histologically normal cervices [92]. Several morphologic and molecular virologic studies have shown a differential distribution of HPVs in genital tract lesions. Thus HPV 6 and 11 account for 70% to 90% of the HPVs associated with exophytic lesions and condylomas [59, 93, 94] and type 16 is found in nearly half of invasive carcinomas [95-97]. However, whereas exophytic condylomas and invasive cancer show a relatively homogeneous distribution of HPV types, there is a heterogeneous distribution of types in CIN that includes those found in exophytic condylomas and cervical carcinomas [93, 95, 97, 98]. It has been shown that approximately 20 HPV types can infect the lower female genital tract and these have all been found in CIN 1 [99]. With increasing grades of severity of the intraepithelial lesions the proportion of HPV 16 increases from 20% in CIN 1 to 40% in CIN 2 and 66% in CIN 3 [91]. The frequency of HPV 16 in CIN 2 and CIN 3 corresponds to that of HPV 16 found in invasive squamous carcinoma [95, 100].

In contrast, HPVs 6 and 11 are found in approximately 15% of CIN 1, less commonly in CIN 2 and very rarely in CIN 3 or invasive carcinoma [91]. HPV 18 is found in approximately 20% of invasive squamous carcinomas, but in only 2% to 3% of all squamous intraepithelial lesions [101]. Other HPV types each account for approximately 15% of all CIN lesions [102, 103]. HPV 31 is found in 5% of squamous carcinomas, whereas the other HPV types other than 16 and 18 are found in approximately 20%. Thus the virologic data show a dichotomous rather than a continuous distribution in CIN. In CIN 1 there is a wide variety of HPV types present, whereas in CIN 2 and 3 there is a homogeneous distribution consisting largely of HPV 16 with approximately 20% of CIN 2 and 3 containing other types. Based on the virologic data, preinvasive lesions therefore segregate into two groups, low-grade (CIN 1) and high-grade (CIN 2 and 3) lesions.

E. Cervical Carcinoma

The most common symptom in patients with invasive cancer of the cervix is vaginal bleeding. This may occur as postcoital bleeding or as irregular bleeding which may be mistaken by younger women as an abnormality of the menstrual cycle. Many patients with this disease, however, have postmenopausal bleeding as the primary symptom. Patients with advanced disease (large-volume stage IIB or stage III) often have a malodorous or bloody vaginal discharge. Additionally, patients with advanced disease often are nutritionally depleted and have a low performance status.

Clinically, cervical cancers may appear as exophytic, endophytic, ulcerative, or polypoid lesions. The exophytic configuration occurs slightly more often in squamous cell carcinomas than the ulcerative lesion (38% and 33% respectively). About 24% of lesions are endophytic in type while 5% are polypoid. Adenocarcinomas of the cervix are not markedly different in their clinical presentation (exophytic 40%, endophytic 32%, ulcerative 19%, polypoid 9%). It is interesting and perhaps surprising that the majority of adenocarcinomas are exophytic and not endophytic in type. This is probably due to the eversion of the histological endocervix in women in the reproductive ages.

Occult carcinomas are small non-palpable endophytic lesions which have not expanded the endocervix. Unlike the microinvasive group of lesions in which nodal metastases are rare (3% or less), occult lesions behave like larger, clinically apparent lesions. Boronow (1977) reported a high rate (20.7%) of pelvic nodal metastases in patients with IB occult tumors. Occult lesions were commonly associated with bilaterally involved pelvic nodes (15%), and involved aortic nodes (8%). Presumably this is related to increased access to lymphatic vessels when the tumor invades the cervical stroma high in the endocervical canal.

Staging Classification for Carcinoma of the Cervix

Pre-invasive carcinoma	
Stage 0	Carcinoma in situ, intraepithelial carcinoma Cases of stage 0 should not be included in any therapeutic statistics for invasive carcinoma
Invasive carcinoma	
Stage I	Carcinoma strictly confined to the cervix (extension to the corpus should be disregarded)
Stage Ia	Micro-invasive carcinoma (early stromal invasion)
Stage Ib	All other cases of stage I. Occult cancer should be marked 'occ'
Stage II	The carcinoma extends beyond the cervix, but has not extended on to the pelvic wall The carcinoma involves the vagina, but not the lower third
Stage IIa	No obvious parametrial involvement
Stage IIb	Obvious parametrial involvement
Stage III	The carcinoma has extended on to the pelvic wall. On rectal examination there is no cancer-free space between the tumor and the pelvic wall. The tumor involves the lower third of the vagina. All cases with a hydronephrosis or non-functioning kidney should be included, unless they are known to be due to another cause
Stage IIIa	No extension on to the pelvic wall
Stage IIIb	Extension on to the pelvic wall and/or hydronephrosis or non-functioning kidney
Stage IV	The carcinoma has extended beyond the true pelvis or has clinically involved the mucosa of the bladder or rectum. A bullous edema as such does not permit a case to be alloted to stage IV
Stage IVa	Spread of the growth to adjacent organs
Stage IVb	Spread to distant organs

Endometrial extension of cervical cancer has been reported by a number of authors to result in a poor prognosis. Perez et al (1977) reported that 9% of patients have extension of their cervical tumors to the lower uterine segment. Baltzer et al (1981) reported that 3 of 4 patients who were found to have ovarian metastases at the time of radical hysterectomy had extension into the endometrium as well. It is difficult to determine the frequency of endometrial extension, since a large number of patients with cancer of the cervix do not undergo hysterectomy. Perhaps because of uncertainty in regard to the significance of this finding, the current FIGO staging system does not alter the stage for its presence.

Several factors are important in the selection of treatment: preservation of sexual function, age of the patient, tumor volume, depth of invasion of the tumor, presence of vascular invasion in the biopsy or conization specimen, palpable extension into the parametrial tissues, and demonstrable extrapelvic disease. The treatment of the majority of patients with cancer of the cervix must be with radiation and efforts should be made by post-therapy vaginal dilation, local estrogens, and local antibacterial agents to minimize the damage to the vagina in order that the patient can resume normal sexual activity, including vaginal intercourse. Hanks et al (1983), summarizing a pattern of care study for radiation-treated cancer of the cervix, concluded that young patients have more complications than older patients, particularly those who have had previous pelvic disease, those who had all the radiation given by external therapy (not intracavitary) and those who are extremely thin.

Surgery, either hysterectomy or more radical surgery, is also frequently used to treat cervical carcinoma. Some surgeons have stated that radical hysterectomy can be performed on women of almost any age. O'Leary & Symmonds (1966) considered age not to be a deterrent to radical hysterectomy and felt that the presence of cardiovascular or renal impairment was the most important consideration in selecting therapy.

Cancer of the Cervix: Percentage in Various Stages

Author	Year	No. of Pts.	I	II	III	IV
Kjorstad	1977	2002	47	37	11	5
Jiminez et al	1979	1227	31	55	12	2
Volteranni et al	1980	417	45	24	30	1
Zander et al	1981	980	77.7	22.5	0.3	—
Shingleton & Orr	1985	1742	54.7	29.1	11.5	4.7

Whether young patients have a lower survival rate than older patients with the same stage cancer of the cervix is still controversial. Kjorstad (1977), studying a group of over 2000 patients, reported that young patients had a more favorable stage distribution and a better prognosis than older ones. Adcock et al (1982) also reported better survival in young women (under age 35) with either squamous cell carcinoma or adenocarcinoma, but a decreased survival rage for those with adenosquamous tumors. Baltzer et al (1982) noted no difference in survival above and below the age of 35 in women with either squamous tumors or pure adenocarcinomas; he also reported a decrease in survival for those with adenosquamous tumors. Gynning et al (1983), however, expressed concern that younger patients have more aggressive tumors and lower 5-year survivals. In most of these studies, there were no matched controls, no adjustment for volume of tumor within stage, and usually small numbers in the younger treatment group. Our young patients with stage IB squamous cell cancer of the cervix have no significant difference in survival from the older group using an age cut-off of either 40 years of 30 years. It is difficult to resolve this question in view of the fact that the current FIGO staging system allows great variation in tumor volume within a given stage, and accurate assessment of tumor volume and extent is impossible except in the surgically treated or surgically staged patients. Thus, the treatment for cervical carcinoma includes both radiation and surgery. Optimal treatment is dependent on the stage of the disease, the age of the patient, and concurrent medical problems.

F. Epidermodysplasia Verruciformis (EV)

An association between papillomaviruses and human skin cancer is found in epidermodysplasia verruciformis (EV) patients. EV patients suffer an obvious, though not yet clearly defined, genetic susceptibility to papillomaviruses and in particular become infected with a subgroup of HPVs which induce characteristic, macular lesions disseminated all over the body. Many EV patients develop squamous cell skin carcinomas, which are often of the *in situ* type, but also grow invasively with heavy destruction of the surrounding tissue. HPV DNA regularly persists in these cancers and EV has been consequently extensively studied as a model for viral cutaneous oncogenesis. In contrast to EV, it is only very rarely possible to detect viral genomes in skin carcinomas of the general population. However there is no reason to disregard HPV as a possible etiologic agent of skin carcinomas, because viruses may play a role at some stage in tumor development, yet be lost from the cells before the fully malignant phenotype is achieved.

Association of HPV Types to Morphology and Location of Skin Warts

Clinical type	Location and characteristics	Associated HPV(s)*
Deep plantar wart	Bottom surface of feet; generally single	HPV-1
Common wart	Mostly on hands; generally multiple	HPV-2, -4
Mosaic wart (superficial spreading wart)	Feet and hands; resistant to treatment	HPV-2
Flat wart	Arms, face, around knees; multiple	HPV-3, -10, -28, -41
Reddish-brown (macular) plaques of EV	Potential for malignancy in light-exposed areas	HPV-5, -8, -9, -12, -14, -15, -17, -19, -20, -21, -22, -23, -24, -25, -36, -47, -50
Butcher's warts	Common warts on hands of butchers and meat handlers	HPV-7

*HPV-26, -27, -29, -27, -39, -46, -49, and -49 are also recovered from skin warts.

During childhood EV patients develop persisting flat warts and also pathognomonic, red, brown, or achromatic plaques with a very characteristic histology showing large, clear, dysplastic cells with vacuolized nuclei. The HPV etiology was proved by the studies of Jablonska and collaborators [77, 104]. HPV 3 and 10 induce the flat

warts in EV patients just as they do in the general population, but they were able to identify at least 18 different HPV DNAs from the disease-specific, macular lesions. Furthermore individual EV patients may be infected with several HPV types at a time.

About one-third of the patients develop multifocal skin cancer on average 25 years after the onset of verrucosis, such tumors mainly arising at sun-exposed sites [105]. This suggests a co-carcinogenic effect of UV-light, and indeed carcinomas are rare in black EV patients presumably due to protection by skin pigmentation. In certain regions like the south of Japan the rate of malignant conversion seems to be particularly high (60%) [24]. Crucially the DNA of HPV types 5 or 8 is found in more than 90% of the EV-associated skin cancers studied to date in Europe and the United States. Clinically apparent infections with HPV 5, HPV 8, and related viruses are largely restricted to EV patients. No EV-specific macular lesions were described so far in the general, immunocompetent population. This striking contrast to the number of HPV types detectable in the benign lesions has been interpreted as indicating a higher oncogenic potential of these particular viruses in comparison to the other related types [105]. Skin cancers of EV patients contain within the limits of detectability by Southern blot hybridization only episomal viral genomes [105]. This is in contrast to the frequently observed integration of HPV DNA in cervical cancers, and excludes carcinogenic mechanisms such as promoter or enhancer insertion for the activation of cellular oncogenes and insertional mutagenesis for the inactivation of tumor suppressor genes.

Cell-mediated immunity appears depressed in most EV patients, and this is assumed to facilitate infection by the EV-specific HPV 5 and related types [105]. It was therefore reasonable to look for these viruses particularly in patients who were immunosuppressed for other reasons. There is a high prevalence of warts in organ transplant recipients, with up to 90% of patients affected after prolonged immunosuppression [33, 106]. Furthermore squamous cell skin carcinomas are about 30-40 times as frequent as in the normal population, in contrast to basal cell carcinomas which are only 3-4 times as frequent [107]. Interestingly HPV 5, HPV 8, and other EV-specific HPVs were indeed detected in skin lesions of renal allograft recipients [33, 106, 108, 109]patients with Hodgkin's disease [110], and HIV-infected individuals [78, 111]. The positive lesions represented red plaques, macular lesions, verrucous or actinic keratoses, or warts. Consistent with this finding is an increased prevalence of anti-HPV antibodies in immunosupressed patients [112]; G. Steger, H. Pfister, unpublished). Most importantly HPV 5 or HPV 8-specific DNA could be demonstrated in at least some of the skin cancers to which these patients are prone [113, 114].

However, HPV DNA is usually not detectable in the majority of skin cancers apart from the EV-associated subset and severely immunocompromised patients. This may be

for any of three reasons: (1) the etiology of these cancers is frequently unrelated to HPV; (2) the relevant HPV types are not yet characterized and persisting viral DNA is not detected due to its lack of cross-hybridization with presently available probes. This possibility is supported by the detection of apparently genus-specific antigens of papillomaviruses in 28 out of 144 cases of Bowen's disease, in contrast to the low detection rate of viral DNA in these lesions [115]; (3) cancer development is triggered by HPV infection but viral functions are not necessary for maintenance and the DNA is lost from malignant cells. Such a 'hit and run' mechanism is certainly very difficult to prove.

G. Laryngeal Papillomas

Clinical symptoms usually start in infancy as hoarseness or abnormal cry, with subsequent respiratory distress, stridor, and aphonia. If not treated, these lesions can be extensive enough to cause death. The serious nature of laryngeal papillomas results from massive amounts of tissue in the airway, which has a restricted size and can tolerate very little obstruction. Large papillomas can significantly block the airway if they are located on the vocal cords. The most serious form of laryngeal papillomatosis involves extension into the trachea, bronchial tubes, and lungs. Weiss and Kashima [116] have reported that in their studies, 26% of the patients had tracheal involvement, 5% had bronchial lesions, and 8% (3 of 39 patients) had pulmonary involvement. Pulmonary disease, while rare, is extremely difficult to manage. It is frequently fatal, even in patients receiving medical treatment.

The most notable characteristic of laryngeal papillomas is their tendency to recur following surgical removal. It has been frequently stated that recurrent disease is restricted to children, and it is often referred to as juvenile laryngeal papillomatosis. However, this concept is not accurate. Holinger et al. [117] reported that among their patients were nearly equal numbers of juvenile and adult-onset disease. In a study of 45 patients with laryngeal papillomavirus, equal numbers of children and adults presented. It is unusual for papillomas to present in the adolescent years and there were none in this series. Sex distribution in juvenile-onset patients is equal, while adult-onset is more common in males. The period of active disease ranged from 1 year to 22 years and two of the patients with juvenile-onset disease have been symptomatic for 24 years with no signs of remission. Remission can be temporary, or can last for the lifetime of the patient. The average age of juvenile-onset patients who did enter remission was 10.2 years but only about 50% of these patients went into remission.

The epidemiology of laryngeal papillomas is only partially resolved. Cook et al. [118], and Quick et al. [119] have data which suggested that there is a link between genital

condyloma and laryngeal papillomas. Two-thirds of the patients in the study by Quick et al. [119] had a positive maternal history of condyloma, and those with a definite negative history were primarily adult-onset patients. The source of infection in the adult-onset patients is not known. It is postulated that infants born to women with condyloma are infected during birth. However there are instances where newborns who were delivered by Cesarean section, developed laryngeal papillomas during the first weeks of life. Thus, it is possible that the fetus can be infected in utero.

HPV Genomic Sequences in Respiratory Cancers

Tumor (reference)	Number tested	Number HPV-positive	HPV type
Larynx, squamous cell carcinoma (97)	42	1	30
Larynx, squamous cell carcinoma (19)	60	3	11 and 16 related
Larynx, control (19)	53	2	11 and 16 related
Larynx, squamous cell carcinoma (183)	36	1	16
Larynx, squamous cell carcinoma (218)	116	15	6, 11, 16
Lung, squamous cell carcinoma (155)	34	1	16
Other head and neck (97)	40	0	

Laryngeal papillomatosis are usually benign. However it is possible for them to become malignant. There are reports in the literature documenting malignant transformation either spontaneously or after X-irradiation [120-125]. The incidence of spontaneous transformation is very low. Irradiation with X-rays markedly increases the risk of malignant conversion. In a large study of patients at the Mayo Clinic from 1914 to 1960, 14% of the 43 treated with radiation therapy developed squamous cell carcinoma, while a control group of 58 papilloma patients treated with surgery alone showed no malignant conversion [126]. Thus laryngeal papillomatosis is a serious disease with a high degree of morbidity and a great deal of unpredictability.

V. DIAGNOSIS OF HPV INFECTION

A. Clinical and Histologic Disease

As noted previously, condyloma are readily recognized. In addition to gross observation, however, examination under magnification after the application of acetic acid may identify areas of subclinical HPV infection. This procedure involves soaking the skin of the genital area with 3% acetic acid for 5 minutes and then examining the skin either directly or under magnification. A shiny white appearance of the skin occurs as the result of the acetic acid soaking, called acetowhitening. Acetowhitening represents foci of epithelial hyperplasia. When intraepithelial neoplasia is present, lesions that are made visible by the application of acetic acid may have a dull gray or dull white color.

Beyond clinical observation, cytology and histology can be informative is diagnosing HPV infection. A large cell with a hyperchromatic nucleus and perinuclear clear ring in the cytoplasm, the koilocyte [35], is highly characteristic of an HPV-induced lesion and is virtually diagnostic. It is likely that koilocytosis is the cytopathic effect that is induced by HPV infection of an epithelium. However, there are many HPV-infected tissues that do not exhibit koilocytosis, so a cytologic or histologic specimen that does not exhibit this change cannot be assumed to be free of papillomavirus. An extension of this approach is the use of electron microscopy, which has been used to identify HPV-sized particles in some tissue specimens [97, 127]. This method appears to identify viral particles in only a minority of specimens in which HPV can be detected by other methods [128].

A somewhat more useful method is immunohistochemistry using an antibody to a shared papillomavirus structural protein. Antiserum to HPV proteins can be coupled with horseradish peroxidase or biotin and used to stain tissue sections. This method is more sensitive than routine histologic staining, but it still fails to identify many infected tissues, especially when dysplasia is present [129]. The currently available papillomavirus antisera do not distinguish among different HPV types. However, there have been some type-specific HPV antisera reported [130], and more of these may be developed in the future and used in diagnostic testing.

Frequency of Detection of Genital-tract HPV DNA in Exfoliated Cervical Cells: a Summary of Some Recent Studies of Normal Populations

Population (reference)	Number of Samples	Percents Cytologically Positive	Percent DNA Positive		
			All Samples	Cytologically Negative	Cytologically Positive
France, mean age 46 (165)	381	14	6	1	33
Germany, all ages (48)	9,295	6	10	9	34
U.S., inner city (129)	204	6	16	9	92
U.S., inner-city teenagers (134)	89	24	13	3	48
U.S., STD clinic (109)	454	12	11	5	35
U.S., college women (109)	545	8	9	6	29
Trinidad, mean age 37 (126)	313	26	7	6	11
Denmark, 20-39 years (111)	661	4	15	-	-
Greenland, 20-39 years (111)	586	4	10	-	-

[a] Cytologically positive category includes koilocytosis, CIN-1, and higher-grade lesions.
[b] Cytologically negative category includes nonkoilocytic and inflammatory atypias.

B. DNA Hybridization Methods

The most commonly used test for detecting and typing HPV, is the Southern blot [19]. In this assay, DNA is extracted from the specimen (such as a biopsy) and cut into specific fragments that are then separated according to size; the separated fragments are transferred to a solid membrane support. The membrane is then exposed to labeled, cloned HPV DNA of a known type, and, if there is HPV DNA in the test specimen, the labeled probe binds to the membrane and can be detected. This method is usually quite specific, and it can detect as little as one HPV DNA molecule per 10 cells or less. The major limitation of the procedure is that it is rather slow and labor-intensive; thus, it is difficult to apply to large numbers of specimens. A variant of this method, the dot blot, applies the extracted DNA to a single spot on the membrane and thus is somewhat less cumbersome, but it is also somewhat less sensitive and less specific.

Another commonly used method is the tissue *in situ* hybridization assay [131]. In this procedure, a tissue section is mounted on a microscope slide and is specially treated and exposed to a labeled HPV DNA probe. After treatment to detect the binding of the labeled DNA, the slide is examined microscopically for evidence of HPV DNA. This method is moderately labor-intensive but can be done using procedures similar to those routinely used in histopathology laboratories. It has the advantage of allowing correlation of the presence

of HPV DNA with the histology of the lesion. The sensitivity of the method is difficult to compare with that of the Southern blot because, in theory, one infected cell in a tissue section could be detected; however, that one infected cell would need to have at least 10 to 50 copies of HPV DNA to bee seen over the background.

C. Polymerase chain reaction

The polymerase chain reaction (PCR) technique has recently been used successfully to diagnose HPV infection [132, 133]. PCR is a procedure in which bacterial enzymes are used to amplify a small segment of HPV DNA, if there is HPV in the specimen. The amplification process makes this method exquisitely sensitive. PCR has been reported to detect as little as one viral genome in 10^5 cells [134]. Because parts of the procedure can be automated, the method can be applied to large numbers of specimens. Shibata et al. [135] developed a PCR assay for the detection of HPV in paraffin embedded tissue. Using simple deparaffinization, the tissue was directly rendered suitable for the PCR. In all biopsies positive for HPV by the DNA *in situ* hybridization technique, the same HPV type could be detected by the PCR. The sensitivity of this PCR assay was about one HPV genome per cell. Using the PCR on paraffin embedded tissue, Cornelissen et al. [136] found HPV positivity only in those sections derived from severe cervical dysplasia which actually contained premalignant of koilocytotic cells. Compared to the DNA *in situ* hybridization technique [130, 137] and the Southern blot technique [136], the PCR on paraffin embedded tissues had an increased sensitivity.

The value of the application of the PCR in the detection of HPV in archival clinical material is substantial. It seems that the method of fixation does not affect the specificity of the PCR [130]. Using this PCR assay, a direct correlation between the presence of HPV in the primary cervical tumor and metastases was found [130]. Recently, Haase et al. [138] described a combination between the *in situ* hybridization technique and the PCR. Using multiple primer sets to generate DNA fragments with overlapping cohesive termini, they were able to amplify specific viral sequences *in situ*. This *in situ* amplification may have interesting future applications in research on the pathogenesis and expression of HPV infections. At present, the most widely used application is the detection of HPV in cervical scrapes. Comparison of PCR with Southern blot analysis revealed that the results for all the HPV samples positive by Southern blot analysis under high stringency conditions were verified by the PCR. However an additional 27% of HPV positive samples was found by the PCR [139]. Automated PCR will likely be suitable for large screening programs.

VI. TREATMENT OF HPV INFECTION

Whereas a variety of treatment modalities are available for HPV infection of the genital tract, none has been shown to be completely safe and effective. Even so, because HPV may be associated with genital neoplasia, treatment should be offered to all infected patients. Careful follow-up of all patients treated for HPV is necessary because it has not been proved that treatment eliminates the risk of malignancy. The overall goals of treatment include the eradication of condyloma or dysplastic tissues in any location, the reduction of symptoms, and an attempt to prevent transmission of HPV to uninfected individuals.

Conventional therapies for condyloma acuminatum involve local destruction of exophytic growths and usually succeed in eliminating visible sessions. Frequent recurrences, probably due to latent HPV infection, demonstrate the need for therapy that eliminates HPV from normal-appearing tissue as well as from obvious genital lesions. Evaluation of therapies for HPV infection is hampered by a lack of complete natural history data. Certain features of infection, such as virus type, location of lesions, duration of disease, and the immune status of the infected patient, may be important in the natural history of this virus.

The currently available therapies for condyloma acuminatum are described in the following section. It should be kept in mind that each modality is associated with certain adverse effects and that none has been shown to eradicate HPV from underlying and adjacent tissue. It is therefore unclear at the present time which therapy is optimal for an individual patient.

A. Podophyllin

The resin from the North American *Podophyllin peltatum* and Indian *Podophyllin emodi* plants contains several compounds that arrest mitosis in metaphase and cause subsequent epithelial cell death. Podophyllin has been used to treat anogenital warts for many years. Most practitioners use podophyllin in tincture of benzoin to apply directly to anogenital warts. Application of podophyllin to condylomata causes an acute inflammatory reaction to occur, leading, over the next 24 hours, to intracellular and intercellular edema and abnormal mitotic [140]. This process continues over the next several days until lesions resolve. Some lesions may not respond to podophyllin and usually undergo no morphologic changes following treatment. Most patients require several applications to eradicate all condylomata acuminatum.

Few studies have been done comparing podophyllin with other forms of therapy for condyloma acuminatum. Jensen [131] compared surgical excision with application of podophyllin in a randomized, prospective study of patients with first episode perianal condyloma acuminatum. Treatment was repeated weekly, for a total of 6 weeks.

Podophyllin cleared genital warts in 77% of patients; surgical excision cleared 93% of patients. Significantly more patients experienced recurrence of lesions with podophyllin than with surgical excision (65% versus 29% at 12 months). Both regimens were well tolerated in this study.

The application of podophyllin is not an entirely safe treatment for condyloma acuminatum. Local reactions include severe necrosis and scarring in the anogenital area, fistula in ano, dermatitis, and hyperplastic infiltrative reactions [137]. Balanitis and phimosis have occurred in men treated with podophyllin [71]. Systemic reactions to podophyllin also occur, usually when large amounts are applied to extensive areas of skin or when podophyllin is left on the skin for long periods of time [16]. Various modifications in topical podophyllin treatment have been used to minimize local and systemic reactions, including washing treated areas at varying times after application, and the use of ointments to protect surrounding skin from contact with podophyllin.

B. Cryotherapy

Treatment of HPV infection of the genital tract with cryotherapy has been studied in a number of settings. The usual cryogen is liquid nitrogen, generally applied directly to a condylomatous lesion. When applied with a cryosurgical device (directed 5-to-10-second bursts of liquid nitrogen are applied) lesions are frozen, resulting in local cellular death. After two or three weekly sessions, warts usually disappear. Local anesthesia may be used but is not often necessary. Cryotherapy is nontoxic, and other than local pain, is not associated with significant adverse reactions.

Cryotherapy has been compared with podophyllin for the treatment of condyloma acuminatum in 572 men and women [141]. Cryotherapy eliminated warts in 79% of patients, compared with 51% of podophyllin-treated patients. Fewer treatments were required to eradicate warts in the cryotherapy group. No patient in either treatment group suffered from serious adverse effects.

In pregnant women, cryotherapy has been used to treat condyloma acuminatum, including lesions of the cervix, with good results. Bergman et al [142] treated 34 women in the second trimester (4 cases) and third trimester (30 cases) for condyloma acuminatum with cryotherapy. The entire lesion, including the base 1 to 2 mm of surrounding tissue, was frozen in several 30- to 60-second applications. Treatment was repeated every 2 weeks until lesions disappeared. No significant bleeding, infection, or scarring occurred, nor did premature rupture of membranes occur in any patient. At 6 weeks postpartum, no patient had recurrence of condyloma acuminatum. Bergman et al [143] treated a second group of 28 pregnant women with cervical condyloma acuminatum, with equally good results.

Patients with cervical intraepithelial neoplasia (CIN) have been treated with cryotherapy. In a study of 1675 women treated over an 11-year period, 94% of patients had normal cytologic and colposcopic findings 1 year after completion of therapy [71]. Of the patients who were available for reassessment, 89% and 85% did not have recurrence of dysplasia at 5 and 10 years, respectively.

C. Laser Therapy

The precision of laser treatment, when performed by an experienced operator, affords destruction of condylomata as well as preservation of normal tissue [144]. Patients usually tolerate the procedure with local anesthetics, but general anesthesia is required when extensive lesions are present. Most series have reported to the efficacy of laser therapy for external genital lesions to be in the 60% to 90% range [80, 145], with recurrence rates of 5% to 10%. However, one series compared laser therapy and conventional surgical excision [146]and found no significant difference in eradication of lesions (43% with laser versus 36% with surgery). Condyloma recurred in the area originally treated in all patients except one. Complications of laser therapy may include local pain, vaginal discharge, periurethral swelling, and vulvar itching and swelling.

Laser therapy has also been used to treat cervical dysplasia. It has been successful when used either to ablate dysplastic lesions [70] or to perform cervical conization [147]. As with patients treated with any modality for cervical dysplasia, frequent, long-term cytologic follow-up of patients treated for CIN with laser therapy is mandatory [23]. However, there is evidence that laser therapy does not eliminate HPV from infected tissue. Riva et al [148]evaluated 25 women who had undergone CO2-laser therapy for condyloma acuminatum. Despite concurrent treatment of their male sexual partners, biopsies done 3 months after completion of therapy showed histologic persistence of subclinical HPV infection in 88% of patients. In another study, biopsies of normal skin 5 and 10 mm from the laser excision site were taken following laser therapy for condyloma acuminatum in 20 patients [13]. Nine patients were found to have persistence of HPV DNA in adjacent normal-appearing skin, and six of these patients suffered from recurrence within 6 months. this study demonstrates that latent HPV can exist beyond the usual laser treatment area and suggests that its presence influences subsequent recurrences.

D. 5-Fluorouracil

The antimetabolite 5-fluorouracil (5-FU) inhibits DNA and RNA synthesis and thus inhibits cellular proliferation [149]. When applied topically, 5-FU inhibits the growth of a variety of cutaneous neoplasms [74]. Although patients with penile or perianal warts were

not able to tolerate therapy because of skin irritation, 13 of 14 men with urethral meatus warts showed complete regression after an average of 3 weeks of treatment.

In a study of patients with vaginal condyloma acuminatum, 90% of 58 women treated with 5% 5-FU cream applied into the vagina had resolution of "papillary" lesions, but only 50% of 12 women with "flat" condylomata of the vagina had resolution [5]. Adverse effects of 5-FU cream included erosive vulvitis in 12.5%, which was believed to be secondary to leakage of 5-FU from the vagina during the night. Twenty-fife percent of women experienced recurrence of vaginal condyloma acuminatum. Krebs treated 20 women with vaginal condyloma acuminatum with 5% 5-FU cream given weekly for 10 consecutive weeks [150]. Three months after treatment, 17 patients (85%) showed no evidence of disease by colposcopic or cytologic examination. Vulvar irritation and vaginal discharge were frequent adverse effects.

Topical 5-FU has also been evaluated as adjuvnat therapy in the treatment of genital HPV infection with somewhat mixed results. Reported recurrence rates in patients treated with 5-FU following another form of therapy have varied from 71% [104] to 13% [79]. Because 5-FU is teratogenic in animals, topical 5-FU should not be used in pregnant women. Although no teratogenic effects have been reported in humans, the potential for such effects exists because 5-FU is absorbed through the skin and mucosal surfaces [149, 151].

E. Interferon

It is clear that in patients who have been treated for condyloma acuminatum, genital epithelium near treated areas with normal colposcopic, cytologic, and histologic appearance can contain HPV [13]. Latent HPV infection is associated with recurrent lesions in patients treated with laser therapy. Therefore, forms of therapy for HPV such as interferon (INF) have been sought that may treat all infected epithelium, regardless of clinical appearance.

Interferons are a group of biologically active glycoproteins with anti-viral, antiproliferative, and immunomodulatory properties [83] which are classified into three types: alpha (produced by leukocytes of lymphoblasts infected with virus), beta (produced by fibroblasts exposed to double-stranded RNA), and gamma (produced by lymphocytes stimulated by mitogens). Recombinant alpha, beta, and gamma IFNs have also been developed. Clinical experience has shown that IFN has activity against human infection with HPV [69, 108]. However, it is not clear if such activity represents antiviral, antiproliferative, or an immunoregulatory effect. Interferons have been studied for the treatment of HPV infection of the genital tract for approximately 13 years. Topical, intralesional, and parenteral forms of each type of IFN have been used. It is difficult to compare the results of these studies because of variations in patient selection, route,

frequency, and duration of treatment, and the type and dosage of IFN used. However, several placebo-controlled, prospective, randomized studies have shown that there may be a role for IFN in the therapy of HPV infection of the genital tract.

Most investigators have studied IFN-alpha in the treatment of condyloma acuminatum. Intralesional recombinant IFN-alpha cleared the treated condylomata in 53% of 30 patients treated with 1.0×10^6 IU three times a week for 3 weeks, compared with 19% of 32 patients receiving 1.0×10^5 IU and 14% of 29 patients receiving placebo [152]. The activity of intralesional natural IFN-alpha in the treatment of condyloma acuminatum has been evaluated. Intralesional natural IFN-alpha at 2.0 to 5.0×10^5 IU/25 mm of wart area or placebo was given twice weekly to 158 patients for 8 weeks or until warts resolved. Natural IFN-alpha eliminated warts in 62% of 86 patients compared with 21% of 72 placebo-treated patients. Eron et al conducted a trial of intralesional IFN-alpha for condyloma acuminatum in 296 patients. Injections of IFN-alpha (1.0×10^6 IU) were administered three times weekly for 3 weeks. Patients treated with IFN-alpha experienced a 62.4% reduction in wart area compared with a 1.2% reduction in the placebo group. At the conclusion of the study, the mean wart area was decreased 39.9% below the initial size in the IFN-alpha group, whereas it had increased by 46% in the placebo group.

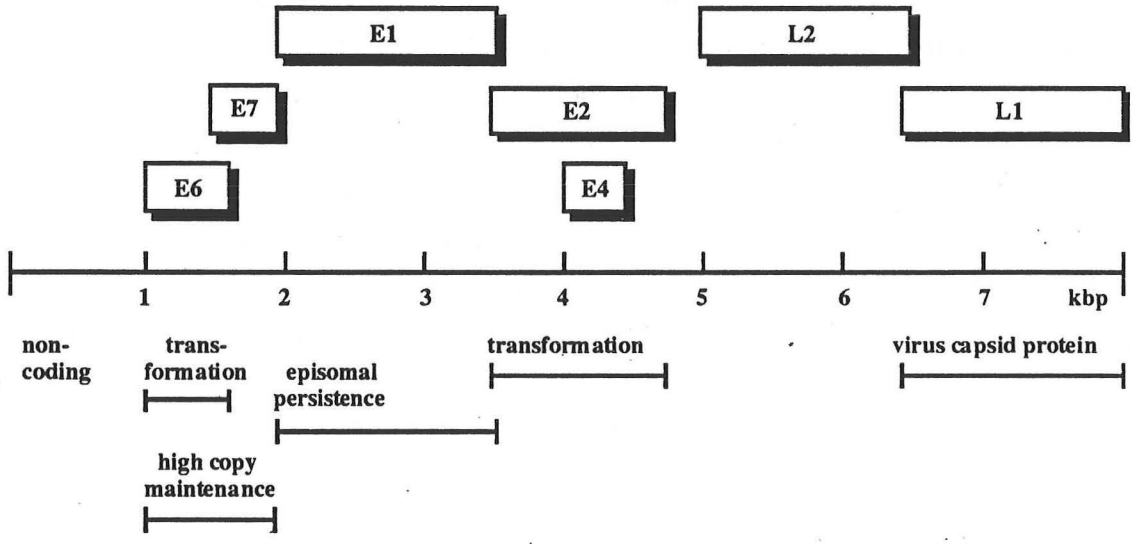
Parenteral IFN has been studied for condyloma acuminatum. Because intralesional IFN produces detectable levels of IFN in serum, there may be activity of IFN against HPV in noninjected lesions [153]. Alternately, IFN could act by an immunologically mediated mechanism when given intralesionally. Intramuscular IFN-beta has been evaluated in a study of 22 patients with condyloma acuminatum. An intramuscular dose of 2.0×10^6 IU of IFN-beta or placebo was given for 10 consecutive days. Condylomata resolved in 9 of 11 patients given intramuscular IFN-beta and in 2 of 11 patients given placebo. None had recurrence of warts after 12 months. Mild fever, myalgia, and headaches occurred in about 50% of those receiving IFN-beta. Kirby et al [88]treated 28 patients with refractory condyloma acuminatum with intramuscular recombinant IFN-gamma. Response to therapy was noted in 14 patients (53%). Flu-like symptoms were common, but were well tolerated.

Gross et al [82]evaluated subcutaneous recombinant IFN-alpha in 14 patients with condyloma acuminatum in a crossover study. Patients received daily subcutaneous injections of either 1.5×10^6 or 18.0×10^6 IU of IFN-alpha for 1 week. After a 4-week observation period, retreatment was given to those who had remaining lesions. Those in the low-dose group received 18.0×10^6 IU and those in the high-dose group received 3.0×10^6 IU of IFN-alpha daily for another 7 days. Eight of 14 patients had a complete remission after an interval of 4 weeks: five patients of the low-dose group and three

patients of the high-dose group. However, Southern blot analysis indicated the persistence of HPV genomes during therapy with natural IFN-alpha suggesting that IFN may not eliminate latent HPV at the doses used in these studies. Recombinant IFN-alpha is approved by the FDA for intralesional treatment of condyloma acuminatum involving external surfaces of the genital and perianal areas. The recommended dosage from the manufacturer (Schering) is 1.0 x 10^6 IU of IFN-alpha into each lesion three times a week on alternate days, for 3 weeks.

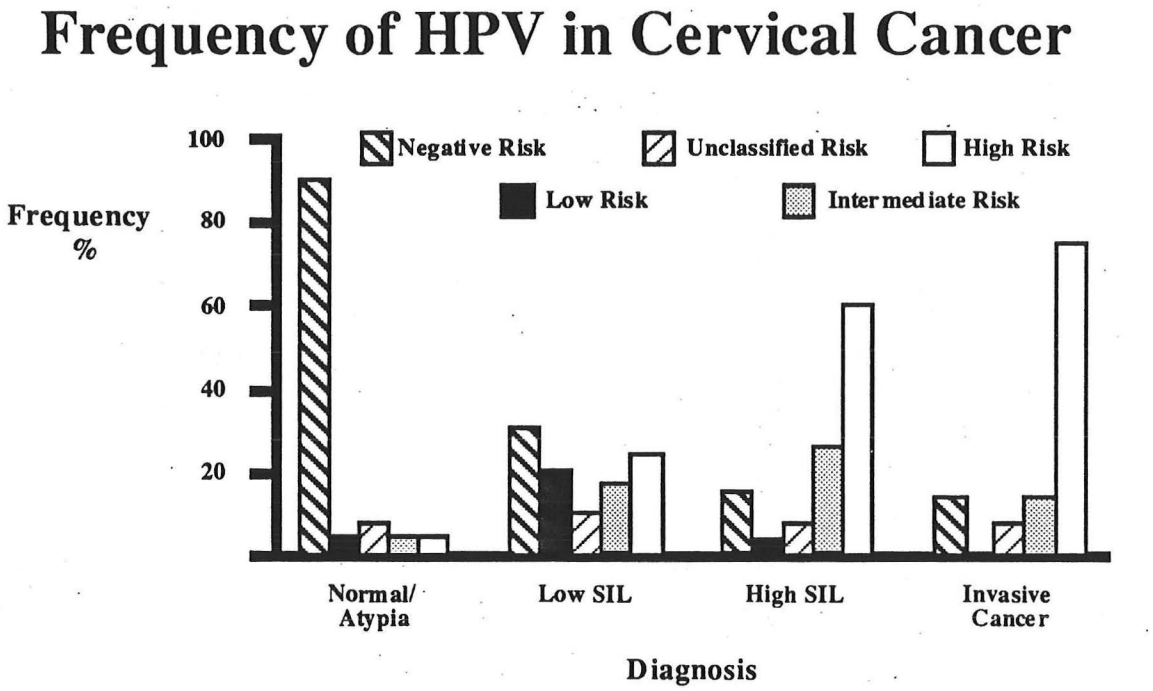
VII. MECHANISMS OF HPV-INDUCED MALIGNANCY

Consensus Genome Organization of Papillomaviruses



The initial evidence to link papillomavirus with cervical cancer was provided by the frequent detection of HPV DNA within the tumor cells. In fact, most of the high-risk papillomaviruses were first identified when DNA extracted from cancer biopsies was analyzed by hybridization using other HPV types as probes [154]. In turn these newly identified papillomavirus genomes were molecularly cloned and subsequently used as probes to screen additional samples. Samples from various parts of the world were analyzed in different studies and in each case HPV DNA was found in the majority of cervical cancer biopsies[155]. The most prevalent virus types proved to be HPV 16 (found in about 50-70% of biopsies) and HPV 18 (found in 4-20% of the samples). Interestingly, there is a certain geographic dependence of the HPV types found in cervical cancer. HPV 18, for instance, is more frequently detected in tumor biopsies obtained from African or South American women as compared to European samples [72, 155, 156]. This difference possibly reflects an uneven

distribution of HPV 18 throughout the world, since this virus type was also detected at higher prevalence in cervical swabs obtained from asymptomatic African women [156]. Underlying these geographic differences in the relative prevalence of HPV types may be variations in susceptibility to HPV infections within the individual ethnic groups because of their different range of HLA polymorphisms. In fact, the prevalence of HPV 18 in cervical cancers of black American women is similar to the figures reported from Africa [97].



Other characteristic features of HPV 18 distinguishing it from HPV 16 have also been reported. HPV 18 was detected in more aggressively growing cervical cancers [152, 157]. The molecular basis of this property may be an elevated level of HPV 18 E5/E7 gene expression *in vivo* since the transcriptional activity of the HPV 18 URR was shown to be stronger when compared to the HPV 16 URR. [158] In this context, it is interesting to note that an equal number of established cervical carcinoma cell lines contain HPV 18 DNA as contain HPV 16 DNA despite the more frequent occurrence of HPV 16 in tumor biopsies. In addition, there is a preponderance of HPV 18 in adenocarcinomas of the uterine cervix. It remains to be seen whether the individual papillomavirus types are infecting different cervical target cells at slightly different stages of differentiation, or whether the viruses themselves are able to influence the subsequent direction of differentiation of a common target. It is clear that the normal pattern of squamous cell differentiation is disturbed in HPV-associated cervical lesions[159] and furthermore this effect can be reproduced in an *in vitro* system for keratinocyte differentiation when HPV DNA is introduced into the cells

[160]. Productive HPV infection in the superficial layers produces a morphologically characteristic cytopathic effect, which has been termed koilocytotic atypia and consists of cytoplasmic vacuolation, which causes perinuclear halos, nuclear wrinkling, irregular chromatin clumping, and hyperchromasia [88]. Multinucleation and nuclear enlargement are also characteristically seen in superficial HPV-infected cells in clinical samples.

HPV genomes are not just persisting as silent passengers within malignant cells but are transcribed into poly-adenylated cytoplasmic mRNAs with the capacity to encode the E6 and E7 proteins [161]. There is expression of the HPV RNA and the E6 and E7 proteins products in HPV positive cervical cancers and [29, 162-164]. Antibodies to the HPV 16 and HPV 18 E6 and E7 proteins are clearly found in patients with cervical cancer [165-168].

HPV 16 and 18 are frequently associated with cervical carcinomas. HPV 6 is only rarely found in these cancers.

HPV 16 and 18 transform established cells and immortalize human keratinocytes. HPV 6 has low transforming activity and does not immortalize keratinocytes.

HPV 16 and 18 containing cervical carcinoma-derived cell lines express the E6 and E7 genes. Both genes are required to immortalize human keratinocytes. The E7 gene encodes the ability to transform established cells.

Integration of HPV genomes into the host chromosome has been observed in the majority of tumor biopsies analyzed to date and in all established cervical cancer cell lines [161]. Episomal molecules have also been detected in cervical cancer biopsies [169], but it cannot be excluded that in these cases precancerous lesions adjacent to the malignant tumor were also collected. In the majority of cancer biopsies integration occurs within the 3' part of early region 2. It has been speculated that the interruption of the E2 open reading frame, and thus the effective removal of E2 as a transcriptional repressor, facilitates the constitutive expression of the viral oncoproteins E6 and E7. *In situ* hybridization has demonstrated that an increased level of E6/E7 transcripts was observed in high grade cervical lesions and cervical cancer as compared to the undifferentiated keratinocytes of low grade cervical lesions [170, 171]. These data indicate that unregulated expression of HPV early proteins is indeed necessary for tumor progression. Since integration of the viral DNA in precancerous cervical lesions, even of high grade, is only rarely detected this implies that, depending on the state of progression towards neoplasia, that different

mechanisms are responsible for deregulation of HPV expression [17, 172].

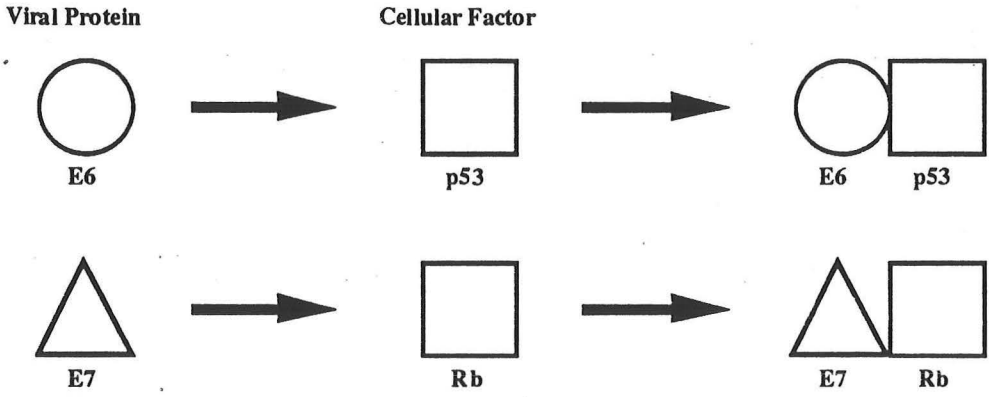
Following the identification of certain genital HPV types as potential human tumor viruses, oncogenic activities encoded by these viruses were identified using rodent cell systems. After transfection of viral sequences into tissue culture cells, both transforming activities (as measured by the ability to induce anchorage independent growth in established rodent cells) and immortalizing activities in primary rat cells (assayed by the establishment of continuous cell lines with or without a cooperating activated ras oncogene) were described. The principal activity in all these assays was localized to the E7 ORF, although weak transforming or immortalizing activities could also be measured following expression of the E6 sequences [78, 110, 173, 174]. The E6 and E7 proteins were therefore identified as the major transforming proteins encoded by the oncogenic genital HPV types.

The studies in rodent cells were important in identifying the transforming potential of HPV encoded proteins, but all such assays suffer from the same criticism; the cell type used is clearly not very relevant to the epithelial cell which the virus normally infects and which gives rise to the cancers. Of greater relevance to the activity of genital HPVs *in vivo* are more recent experiments which have used primary human epithelial cells, either foreskin keratinocytes or cervical cells, as recipients for transfection experiments to examine activities of HPV genes. As with primary rodent cells, HPV DNAs efficiently immortalize primary human cells [83]. Closer analyses again demonstrated that expression of only E6 and E7 sequences is required for this activity, but in these cells cooperation between E6 and E7 is essential for full immortalization [111, 112]. Cells expressing only E7 show a slight extension of their proliferative capacity, but still senesce a few passages after the control cells cease to divide. Cells transfected with E6 sequences alone show no detectable difference in phenotype from the control cells. However, when both proteins are present the cells acquire an unlimited lifespan in culture. Following this immortalization it is also possible to select cells resistant to conditions which induce terminal differentiation in normal keratinocytes. The potential significance of this effect of HPV sequences is most dramatically illustrated when the cells are examined in organotypic systems which allow the development of stratified cell cultures closely resembling the pattern of differentiation seen in epithelium *in vivo*. Primary human keratinocytes in these systems closely resemble normal epithelium, whereas HVP immortalized cells are virtually indistinguishable from CIN, the pre-malignant cervical lesions [175]. The observation that E6 and E7 expression can induce these changes in primary human epithelial cells strongly supports the notion that the HPVs are involved in the development of these lesion *in vivo*.

Transformation studies with HPVs have also demonstrated a correlation between *in vitro* transforming potential of the different viral types and apparent oncogenicity *in vivo*.

In the transformation assays described, E6 and E7 derived from HPV 16 or 18 efficiently transform or immortalize cells in culture whereas HPV 6 or 11 sequences fail to function or show only weak activity [80, 105]. Recent studies in human keratinocytes have shown that both the E6 and E7 proteins that function to immortalize these cells must be derived from an oncogenic HPV type. Substituting either of these proteins for E6 and E7 proteins derived from the benign HPV types results in a loss of immortalizing activity. The use of heterologous promoters to ensure comparable levels of E6 and E7 protein expression between oncogenic and nononcogenic papillomavirus has demonstrated that there is clearly a difference in the function of the proteins between low risk and high risk papillomavirus [80]. Comparison of the sequences of E6 and E7 proteins from oncogenic and non-oncogenic genital HPV types has revealed that changes in specific amino acids in these proteins are critical in determining their oncogenic potential.

Association of Papillomavirus E6 and E7 with Tumor Suppressor Gene Products



The identification of E6 and E7 as transforming proteins in experimental systems supports the observation made earlier that these two ORFs are the only HPV sequences consistently conserved and expressed in cervical tumors. Some evidence has now accumulated to suggest that the expression of E6 and E7 is not only necessary for the establishment of the malignant phenotype but is also required for its maintenance. The down-regulation of E6 and E7 expression partially reverts the transformed phenotype of an established HPV expressing cervical tumor cell line [25]. The role of E6 and E7 in the normal life cycle of the papillomaviruses is poorly understood because of the lack of culture systems for these viruses. However, in addition to extending the proliferative capacity of

infected epithelial cells and interfering with normal patterns of differentiation, there is some evidence from BPV 1 and HPV 8 that E6 and E7 are involved in control of viral DNA synthesis [101, 176].

A. Structure of E6 and E7

E6 and E7 proteins from all papillomavirus types show the same basic primary structural organization, although on average they show only about 50% identity at the amino acid level. Both proteins are very small, around 150 amino acids for E6 and only 100 amino acids for E7, and they show a certain degree of similarity to each other. The most striking feature shared by the two proteins is a repeated cysteine motif which occurs twice in E7 and four times in E6. This similarity has led to the suggestion that E6 and E7 represent duplication and divergence of the same 33 amino acid repeat [102]. Since both E6 and E7 can bind zinc [103] it is possible that the cysteine motifs are involved in zinc binding although the spacing between the cysteines is much larger than is usually found in zinc finger motifs, for example, in transcription factor SP1.

A group of 17 amino acids in the amino-terminal half of E7 shows strong homology with conserved region 2 (CR2) of adenovirus E1A and a region of SV40 large T antigen [173]. Part of the region of homology between E7, E1A, and T antigen is critical for the interaction of these oncoproteins with the cellular retinoblastoma gene product, RB [177-179]. RB displays the properties of a tumor suppressor or anti-oncogene product. Similar point mutations affecting amino acids common to E7, E1A and T antisense destroy both the ability of the proteins to complex with RB and their transforming activities [179]. This indicates that RB binding is critical for the biological activities of E7, although other mutants which continue to bind RB, but are still transformation defective, indicate that RB binding by itself is not sufficient for the transforming activity of E7 [180]. Another activity shared by E7, E1A and LT proteins is their ability to induce cellular DNA synthesis in serum starved cells [27]. One of the proposed roles of RB is in controlling the G1 to S transition, and one function of E7 expression might be to release the cells from this RB dependent block. Other studies have indicated that expression of E7 is sufficient to overcome cell cycle blocks at G2 as well as G1 [174] and complex interactions involving a number of E7 activities are certainly required for full cellular transformation. Finally E7 is capable of transactivating both heterologous viral and cellular promoters in transfection experiments, suggesting that this function may also be important in cellular transformation.

B. Functions of the E6 protein

The activity of the genital HPV E6 proteins is most clearly seen in the cooperation with E7 to immortalize primary human epithelial cells. E6 protein shows some similarity to E7 within the cysteine motifs, but the region of E7 E1A LT antigen homology is not shared. Although there is no strong sequence similarity with transforming proteins encoded by other DNA tumor viruses, some of the activities of the genital HPV E6 proteins suggest that there may be important functional similarities.

E6 proteins encoded by genital HPVs have recently been shown to bind to the cellular tumor suppressor gene product, p53 [181]. As with E7-RB binding, the biological relevance of this association is strongly emphasized by the observation that E6 proteins from the benign HPV types 6 and 11 show no evidence of binding p53. Mutational analyses has been reported to confirm the importance of p53 binding to E6 activity, suggesting that interference of both RB and p53 function is necessary in order to immortalize human cells. There is, therefore, a strong functional similarity between the HPVs and the transforming proteins from adenovirus (where E1A binds RB and E1B binds p53) and SV40 (in which the T antigen can bind to both RB and P53). It seems likely that all three viruses function, at least in part, by interfering with the same cellular pathways.

E6 proteins from several papillomaviruses are able to trans-activate the homologous HPV promoter within the LCR. E6 responsive elements have been mapped in the LCR [13]and trans-activation by E6 probably results in increased expression of both E6 and E7 proteins. However, increasing the expression of E7 is clearly not the only function of E6 in immortalization of human cells. When E6 and E7 proteins are expressed independently from strong heterologous promoters, cooperation between the two remains essential for immortalization [112]. Recent studies have shown that the cysteine residues in the potential zinc fingers are required for the transcriptional activity of E6 [4]. Since the transforming activity of this protein is also destroyed by the same mutations, it seems likely that the transcriptional activity is necessary for transformation. It is possible that the transforming activity of E6 is more closely linked to the activation of cellular gene expression than to the activation of HPV transcription.

VIII. FUTURE PROSPECTS

Clearly, the oncogenic HPVs represent some of the best candidates for human DNA tumor viruses. Two viral proteins, E6 and E7, play an important role in the development of these cancers and the concept that their continued expression is necessary for the maintenance of the malignant phenotype has raised the possibility that the development of

drugs which interfere with E6 or E7 function may be valuable for treatment, as well as prevention, of HPV associated carcinomas. Obviously, the recent advances in identifying which biochemical functions of E6 and E7 are important for transforming activity will be enormously important for this approach to succeed.

An understanding of the action of the HPV transforming proteins may shed light on general mechanisms of oncogenesis in tumors not apparently associated with viral infection. Recent convergence of the fields of DNA tumor viruses and the study of cellular anti-oncogene products such a RB and p53 has demonstrated how similar mechanisms of malignant development can be. Clearly, charges contributing to the progression of HPV associated lesions to full invasive carcinoma will include mutations or alterations in expression of cellular proteins. These may represent previously identified oncogenes such as myc, fos or ras which are known to play a role in many different types of cancers and can all contribute to HPV induce transformation. Alternatively, identification of other cellular genes which can cooperate with the HPV oncoproteins may provide a means of identifying previously unknown oncogenes, which also play a role in a far broader spectrum of malignancies.

Human papillomavirus infection is a major health problem worldwide. The prevalence of this virus and its diverse manifestations make it the subject of intense study. The closer follow-up of patients infected with this virus will make its epidemiology and its relationship to cancer better understood. In addition, the use of polymerase chain reaction will allow a better understanding of the serotypes of papillomavirus responsible for genital malignancy. Now that the viral proteins that are responsible for cervical malignancy have been identified, novel strategies for preventing interactions between viral and cellular proteins may be found. Given the long time interval between infection and the development of cervical carcinoma, papillomavirus infection provides an excellent opportunity to understanding the viral and cellular specific factor responsible for the generation of human genital malignancy.

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