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Genital human papillomavirus infections and cancer: Memorandum from a WHO Meeting*

There is increasing evidence from molecular biology and clinical investigations that infection with specific types of human papillomavirus (HPV) is associated with anogenital cancers, especially cancer of the cervix uteri. Human papillomavirus types 16 and 18 have been found in the majority of cervical cancers investigated; specific viral mechanisms appear to be involved in the malignant conversion of these tumours. However, evidence from epidemiological investigations is still inconclusive. Concerted efforts to study the etiology and natural history of lower genital tract neoplasia and its association with HPV, together with the development of vaccines against specific viral types, are needed in order to make it possible in the future to prevent cervical and other anogenital cancers.

Cervical cancer is the second most frequent cancer in women and ranks fifth among all cancers in humans. It contributes to approximately 10% of the worldwide tumour burden, and up to 25% in the developing countries. Annually, about 460 000 women develop this disease (*1*). Although declining in incidence and mortality as a result of early-detection programmes in many developed countries, it remains the most important cancer in many parts of the developing world. The occurrence of precursor lesions (dysplasia or cervical intraepithelial neoplasia (CIN)) exceeds the rate of invasive cancers.

Epidemiological studies have suggested a relationship between cervical cancer and cancers of other anogenital sites (e.g., vulvar, vaginal, penile and perianal), although the latter occur with much lower frequency and in higher age groups. For more than a century there has been the suspicion that sexually-transmitted agents may contribute to the etiology of cervical cancer. During the past 20 years much attention has been focused on the role of viruses in the causation of human anogenital cancer, initially on human (alpha) herpesviruses (herpes simplex viruses) but, since 1974, increasingly on human papillomavirus (HPV). The demonstration of the heterogeneity of the HPV group and the isolation of specific types regularly found in cervical, vulvar, penile and perianal cancer biopsies markedly stimulated interest in their possible role in cancer induction. Today a number of experimental studies appear to provide substantial evidence for the role of HPV in cervical cancer.

As observed in other malignant tumours in animals and man linked to virus infections, the infection *per se* by these rather ubiquitous viruses may not be sufficent for tumour induction. Additional changes, possibly involving cellular genes, may be required to promote the outgrowth of a malignant cell clone. A number of mutagenic and perhaps other factors may contribute to this development. If a virus infection, however, represents a necessary, although not sufficient, factor in the induction of anogenital malignancies, this may pave the way for new strategies in the control and therapy of cervical cancer and those neoplasms linked to the same infections.

CLINICAL MANIFESTATIONS

Lesions caused by HPV may be of a clinical nature and easily visible, or they may be subclinical and not recognized by the usual clinical diagnostic methods, requiring the use of exfoliative cytology and colposcopy with or without associated biopsy. The most common clinical lesions in both sexes is the condyloma acuminatum, while the detection of subclinical lesions will depend on the genital organ studied and on the methods of examination.

The diseases resulting from the infection of anogenital epithelia by HPV are to some extent determined by the viral type involved. Thus, infection by HPV-6 or HPV-11 is commonly associated with "genital warts". These are essentially benign condylomas or small sessile lesions often appearing on multiple sites. The same viral

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types may also be associated with more subtle, clinically inapparent or subclinical disease which requires special methods such as colposcopy for diagnosis.

Less is known about the earliest stages of infection of genital epithelia by HPV-16 or HPV-18. These viral types are present in some pigmented or non-pigmented lesions which histologically are carcinoma *in situ*. They are also often present in other premalignant and malignant genital lesions. Whether these are the only clinical manifestation of infections by these viral types is not known.

Infection by HPV-6/11 and HPV-16/18, with their associated lesions, may occur simultaneously. It is also known that the total area of infected epithelium probably greatly exceeds the area displaying lesions.

In women

Infection by most sexually-transmitted HPV types occurs throughout the lower female genital tract but type-specific differences in site, nature and severity of disease at each particular site are discernible. Often multiple sites are involved.

On the cervix, clinically apparent condylomata acuminata, the classic lesions associated with HPV-6/11 infection, are uncommon and when present, they are often small and best assessed using the colposcope, after application of 5% acetic acid; many cervical HPV infections are subclinical and detected only by colposcopy especially after application of acetic acid (*2*). Condylomata may be single or multiple, scattered or confluent. Exuberant growth may produce thick, fleshy folds, particularly in pregnancy. Lesions are frequently detected within the squamous metaplastic epithelium of the cervical transformation zone but may be detected on the original squamous epithelium of the ectocervix. They may occasionally appear to extend into the endocervical canal.

In the vagina, condylomata acuminata occur and are detected by careful examination in as many as one third of women presenting with vulvar lesions. Most vaginal intraepithelial neoplasias (VAIN) are either associated with clinically visible HPV lesions or contain histological evidence of associated HPV infection.

In the vulvar region, condylomata acuminata range from small, benign, readily treated lesions to an extensive, superficial growth covering large areas of skin with multiple local recurrences and almost relentless progression (*3*). Colposcopic examination of the vulva, after application of acetic acid, permits identification of two distinct types of subclinical HPV-associated lesions: single or fused papillary-like lesions and acetowhite epithelium. The single papillae and fused papillae are often evident by close examination of the vulva without the colposcope, but are best assessed using magnified illumination after application of acetic acid. They can be symptomatic, causing intense pruritus.

Vulvar intraepithelial neoplasias (VIN) may be clinically obvious because of their leukoplakic character, but many will be diagnosed only after biopsy of seemingly benign condylomatous lesions. Diagnosis (sometimes with the help of colposcopy) must always depend on biopsy evidence of suspicious lesions, such as: leukoplakic areas, condylomatous lesions in a leukoplakic area, condylomatous lesions not responding to medical treatment or those that recur in the same site, or ulcerative or locally destructive lesions.

In men

Condylomata acuminata can easily be identified on the glans, terminal urethra, shaft of the penis, and scrotum. Small sessile warts are more difficult to identify. Examination with a hand lens after the application of acetic acid is a useful diagnostic aid and is essential for the identification of areas of acetowhite epithelium. Anal condylomas occur alone or associated with penile warts; in 50% of cases there are condylomas within the anal canal, so proctoscopy is mandatory.

Subclinical penile lesions, which may be associated with intraepithelial neoplasia, are becoming increasingly recognized and are an enigmatic condition associated with a variety of condylomatous lesions. The malignant potential of the intraepithelial subclinical lesion is unknown; it may be diagnosed by biopsy of certain areas which can be recognized by illuminated magnification after the application of acetic acid. The appearance of these areas is: raised, keratinized and pigmented warty lesions, usually resistant to treatment or recurring after treatment (clinically such areas may be referred to as Bowenoid papulosis); or reddish raised and sometimes ulcerated areas (referred to as erythroplasia of Queyrat), sometimes with the appearance of localized erosive balanitis; or areas of flat white epithelium.

LABORATORY DIAGNOSIS

Because of the absence of suitable *in vitro* methods for HPV propagation, diagnostic procedures for the detection of HPV infections are at present mainly biochemical. Current standard techniques are based on molecular hybridization and on serological procedures.

Molecular hybridization

The presence of the virus, or more specifically of the viral DNA, can be established by hybridizing total cellular DNA (e.g., from biopsies or tissue scrapings) with specific HPV–DNA molecular probes (*4, 5*). In this technique, the complementary strands of DNA obtained from the lesions are separated (denatured) and mixed with denatured radiolabelled viral DNA. If the cellular DNA contains viral nucleotide sequences, they will form base-pair interactions (hybridization) with the complementary strands of the viral DNA probe; the detection of radioactivity associated with the cellular DNA will then be an indication of the presence of viral DNA sequences.

Different approaches have been used to detect the presence of viral DNA sequences. One approach is to hybridize radiolabelled cloned HPV–DNA to cellular DNA which has been fractionated by gel electrophoresis and then transferred to nitrocellulose filters; this technique, known as "Southern blot", permits the identification of specific DNA fragments characteristic of different HPVs. Another technique which has been used is hybridizing radiolabelled total cellular DNA to cloned DNA of different HPV types; in this technique ("reverse hybridization") the presence of radioactivity associated with a particular type of HPV–DNA will identify the virus present in the lesion. In the "dot (slot)-blot" analysis, cellular DNA is concentrated on to nitrocellulose filters in either a "dot" or a "slot" pattern before probing with radiolabelled HPV–DNAs. All three methods, particularly the first one, are highly sensitive. Hybridization conditions can be varied (low stringency) so that hybridization could occur not only with identical HPV–DNA sequences but also with related sequences; in this way unknown types of HPV can be identified and characterized.

The screening of large numbers of samples can be done by "filter *in situ*" hydridization, a technique which does not require prior DNA extraction. In this procedure cells are filtered on to nitrocellulose or nylon filters and, following the denaturation steps, hybridization with radiolabelled HPV–DNA is carried out; again, the detection of radioactivity in the filters is an indication of the presence of viral DNA sequences in the total cellular DNA. Another technique, which allows the detection of HPV–DNA or RNA in specific cells of a lesion, is by "*in situ*" hybridization using tissue sections; HPV–DNA can even be detected in formalin-fixed preparations.

Most studies using the methods above have been done with ^{32}P-radiolabelled probes. However, ^{35}S-radiolabelled probes can be used in the "filter *in situ*" hybridization and biotin-labelled (non-radioactive) probes in the "dot-blot" analysis, although the sensitivity of these probes is significantly lower than ^{32}P-labelled probes. In the "*in situ*" hybridization of tissue sections, ^{35}S, ^{3}H or biotin-labelled probes are being used, again with low sensitivity. The use of single-stranded (strand-specific) probes substantially increases the sensitivity of all the above-mentioned procedures.

Disadvantages of these hybridization methods are the laborious handling procedures and the cost (e.g., "Southern blot") and/or relative insensitivity of the assay system used in detecting low copy numbers of viral DNA (e.g., "filter *in situ*" hybridization). At present, highly trained laboratory personnel are required to do these tests, especially in preparing the probes and interpreting the results. In addition, the sensitivity (in the epidemiological sense, i.e., the proportion of truly infected individuals classified as such) and the specificity (i.e., the proportion of truly non-infected individuals classified as such) of the different hybridization techniques in clinical practice are unknown. Similarly, the inter-laboratory and inter-test reproducibility is not known. Of particular concern in epidemiological studies is the possibility that the sensitivity and specificity of the hybridization tests in detecting HPV–DNA differ according to whether the sample is from normal tissue, CIN or carcinoma. There exists, then, a need for development of sensitive, specific, reliable and cost-effective biochemical procedures (preferably non-radioactive) for the detection of viral nucleic acids in infected cells or tissues.

Serological procedures

Commercially available antisera, prepared by immunization with purified animal papillomaviruses, are used to detect broadly reactive "group-specific" capsid antigens in cellular smears or tissue sections. However, virus particle production in infected tissues is restricted and the number of detectable antigen-producing cells is usually small. Moreover, these "group-specific" serological reagents cannot distinguish between different HPV types.

In an attempt to develop type-specific serological reagents, several viral proteins are now being produced by genetic engineering in bacterial expression systems and tested for their antigenic characteristics. First results suggest the usefulness of the L1 and E4 proteins (see below) in the identification of virus-infected cells. The same proteins may be useful as antigens for the detection of immune response against HPV-specific types. There exists an urgent need for the further development of type-specific serological reagents and their application for detecting viral antigens and the development of the immune response in infected individuals.

MOLECULAR BIOLOGY

Human papillomaviruses

Papillomaviruses are a group of small DNA viruses which induce squamous epithelial tumours (warts and papillomas). The first papillomavirus described was the cottontail-rabbit (Shope) papillomavirus (CRPV). Subsequently, papillomaviruses have been isolated and characterized from other vertebrate species, including man. Standard virological approaches to the study of the papillomaviruses have been limited by the lack of a tissue culture system for their *in vitro* propagation. HPVs only replicate in terminally differentiating keratinocytes *in vivo*. To date, tissue culture systems for keratinocytes have not permitted the full expression of the papillomavirus life-cycle. Recombinant DNA technology, however, has permitted the molecular cloning of a number of papillomavirus genomes and provided sufficient quantities of viral DNA to begin a systematic study of the papillomaviruses. There are no serological reagents yet available to distinguish the various human papillomaviruses. Different HPV types are, therefore, distinguished on the basis of their DNA. An HPV type is considered a new type if it shares less than 50% DNA homology by stringent hybridization analysis with each of the other HPV types defined. To date, approximately 50 different HPVs have been described, each of which is usually associated with specific pathological entities.

Papillomavirus genomes are double-stranded closed circular molecules of DNA containing approximately 8000 base pairs. The analysis of the nucleotide sequence of a number of human and animal papillomaviruses has shown the presence of several stretches of DNA with the known molecular characteristics of probable structural genes. These stretches of DNA are known as "open reading frames" (ORFs), referring to the possibility of "reading" relatively long segments of the genetic code (400 bases or more) before reaching a termination signal. All of the papillomavirus ORFs are located on one of the two DNA strands and, as a consequence of this, all detectable messenger RNA in transformed as well as in productively infected cells are copied from only one of the virus DNA strands.

As many as ten ORFs have been identified among the known papillomaviruses. Sequence comparisons have shown that all papillomaviruses have a similar genomic organization regarding the general distribution of the different ORFs. Eight of them are called "early" ORFs (E1 to E8), and are expressed in transformed cells and in the basal, non-productive cells of the lesions. The other two are called "late" ORFs (L1 and L2) and are expressed only in the differentiated keratinocytes of the superficial layer of the lesion, where mature virus particles are produced. In addition to the coding regions, the viral genome contains transcription and replication control regions. Most data relating to the functions of the papillomavirus gene products come from the study of bovine papillomavirus type 1 (BPV-1), which has served as the prototype for the genetic analysis of the papillomaviruses.

Open reading frames encode (or carry the genetic information for) the synthesis of corresponding specific proteins. The products of the L1 and L2 ORFs are structural components corresponding to the major (most abundant) and minor (less abundant) proteins that form the icosahedral capsid that encloses the viral DNA. As mentioned, the "early" ORFs are expressed in the absence of virus particle formation and may have important functions related to replication and/or transformation. Two BPV-1 ORFs, E5 and E6, have been shown to encode proteins which are involved in cell transformation *in vitro*. The E5 protein is very small and is localized in nuclear and cytoplasmic membranes. The E6 protein is thought to be a DNA-binding protein and is localized to the nucleus and membranes. Three ORFs are associated wih replication functions: the E6 and E7 ORFs are involved in the control of the number of viral DNA molecules per cell and the E1 ORF encodes functions essential for replication. The product of the E2 ORF is a DNA-binding protein which controls the expression of the E6 and E7 ORFs. The E4 ORF of HPV-1 has been shown to encode the abundant cytoplasmic protein which is also expressed in the late phase of the viral replication cycle. No function has yet been identified for the E3 and E8 ORFs.

Molecular biological evidence for involvement of HPVs in genital squamous-cell carcinomas

Several findings support the involvement of specific HPV types in the development of genital squamous-cell carcinomas. The high frequency of detection of some HPV types (i.e., HPV-16, 18, 31, 33 and 35) in carcinomas of the uterine cervix, vulva, penis and anal regions is particularly noteworthy. Specific viral mechanisms are apparently involved in the progression of genital tumours. HPV-16 and HPV-18 DNA sequences are usually detected as non-integrated, free DNA molecules in benign lesions, i.e., dysplasia and intraepithelial neoplasias. In contrast, they are usually found integrated into the host cell genome of carcinomas and in cell lines derived from cervical carcinomas. The integration usually interrupts the viral genomes in a specific region containing E1 or E2 ORFs. The E6 and E7 ORFs are consistently expressed in the tumours and

the cell lines. It should be mentioned that the predominant viral messenger RNA present in skin carcinomas associated with the Shope CRPV also corresponds to the E6 and E7 sequences.

In vitro experiments carried out with HPV-16 DNA demonstrate the transforming activity of this DNA for murine cells. Moreover, human foreskin keratinocytes and fibroblasts are immortalized by the introduction of HPV-16 DNA into the cells. The immortalized cells contain the viral DNA and express early viral genome functions. They quickly become aneuploid but are non-tumorigenic upon inoculation into athymic nude mice. These studies demonstrate the transforming functions of the HPV-16 type. They also indicate that the mere infection of cells by this virus is not sufficient for the expression of a malignant phenotype, which should require additional changes within the infected cell.

A recent approach used has been the grafting of HPV-11-infected human tissues (cervical, foreskin or laryngeal) beneath the renal capsule of nude mice. Within the epithelial cysts of the grafted cervical tissue, typical koilocytotic dysplasias, or condylomatous proliferations developed. HPV-11 could be recovered from these proliferations and was shown to reinduce the respective changes. This directly supports the previous evidence linking this virus type etiologically to condylomatous lesions.

Role of cofactors

The progression of HPV-associated lesions into carcinomas represents most likely a multistep process involving the activation or inactivation of some unknown genes, possibly under the influence of cofactors such as tobacco smoking, oral contraceptives, other genital infections and other poorly understood factors. Cofactors have been shown to play a role in the malignant conversion of rabbit warts (chemical carcinogens as the cofactor) and HPV–5-associated skin lesions observed in patients with epidermodysplasia verruciformis (sunlight as cofactor).

Research needs

Future research should be aimed at:

— identification of all possible HPV types associated with genital disease;
— development of an *in vitro* system for the propagation of HPVs;
— elucidation of the functions of the non-structural viral proteins and the definition of the role of the E6 and E7 genes in cell transformation;
— determination of the role played by the integration of viral sequences into the cell genome in the progression of genital tumours;
— identification of cellular genes involved in the development of HPV-16 or HPV-18-associated carcinomas;
— interaction between physical and chemical carcinogens and viral or cellular genes in the development of genital tumours.

EPIDEMIOLOGY

There is strong epidemiological evidence suggesting that a sexually-transmitted infectious agent may be involved in the causation of cervical cancer (*6*). Both female and male sexual behaviours have been shown to influence the risk. During the last two decades, human (alpha) herpesvirus 2 (formerly called herpes simplex virus type 2) (HSV-2) has been studied intensively. Despite the fact that past exposure can be measured serologically, a possible role of HSV-2 in cervical cancer etiology is uncertain.

During the last decade, attention has been focused on the role of HPV in cervical cancer and more recently also in other anogenital cancers. A large number of observations in humans has been reported. In addition to the possible role of viral infection, other risk factors have also been proposed such as smoking, oral contraceptives and some dietary factors. Specific types of HPV were first identified and associated with cervical cancer in 1982 (HPV-16) and 1983 (HPV-18). HPV-16 and HPV-18 have also been reported to be present in cancer of the penis, vulva and vagina. Recently identified types of HPV have not been included in this review, since no (or only limited) information is available. In view of the time necessary to perform epidemiological studies, no such investigations have been completed and published. A number of epidemiological studies now under way include measurement of specific HPV infections.

Prevalence studies

Prevalence refers to the number of diseased persons in a defined population at a given point in time. The prevalence of infection with various HPV types has been reported for patients with invasive cancer of the cervix, patients with precancerous lesions, and persons with normal cervical cytology. All of the reported studies were conducted in small and selected groups (*7*).

Some 15–92% of all invasive cervical cancers have been reported to contain HPV-16 and 0–54% contain HPV-18 (HPV-31, 33, 35 and additional types have also been reported). Around 40–60% of all cervical

squamous-cell cancers are regularly found to contain HPV-16. In all these series, sensitive methods of detecting HPV–DNA have been employed (Southern and reverse blots). Most of the patient series reported are small (8–53 patients) and the differences reported could be partly due to chance. Variations could also be influenced by patient referral patterns, the age distribution of the patients, and socioeconomic factors. Information on these characteristics is generally not available. HPV-16 and HPV-18 have been found in a similar proportion of a small series of patients with adenocarcinoma of the cervix. This is surprising, since adenocarcinomas appear not to share the same risk factors as squamous-cell carcinomas. This points to the need for study of the epidemiology of adenocarcinoma of the cervix.

Some 14–83% of precancerous cervical lesions, i.e., dysplasia (CIN I–III), contain HPV-16 and 0–3% contain HPV-18 in reported series (7–80 patients). Different techniques have been used for the collection of cellular material (scrapes or biopsy) and diagnostic methods include Southern blot, reverse blot, dot blot and filter *in situ*. In most studies no distinction has been made between different grades of dysplasia (CIN). Specimens from the cervix with morphological characteristics of HPV infection may be misdiagnosed as minor degrees of dysplasia. Such misclassification may pose a problem since it would lead to a spurious overestimate of the prevalence of infection in patients with precancerous lesions.

In the normal cervix the reported prevalence of HPV-16 is in the range 0–39% and of HPV-18 around 0–2%, the number of patients in the series varying from 9 to 229. All groups may be highly selected since they are derived from clinics for family planning, sexually-transmitted diseases and cancer. Demographic information is not available. Reports are restricted to women with normal and inflammatory cervical smears. This may mean that a number of HPV-infected women have been misclassified as minor degrees of dysplasia and have been excluded, thus underestimating the true prevalence of HPV infections in the population of women without neoplastic changes of the cervix.

It is questionable to compare the rates of infection in persons with invasive cancer, precancerous lesions, and normal cervices since different methods of specimen collection and diagnostic and hybridization techniques have been used. In most studies it is unknown whether the assessment of HPV infection has been carried out without prior knowledge of the disease status of the person. Demographic characteristics which may influence HPV infections are not reported and it has been suggested that adjustment for age eliminates differences in prevalence of HPV-16 infection between persons with normal cervices and cancer of the cervix.

Longitudinal studies

Two studies have reported on the progression of dysplasia in relation to type-specific HPV infection. In one, progression of mild dysplasia to severe dysplasia or carcinoma *in situ* (CIN III) in 100 women was reported in a higher proportion of women positive for HPV-16 than in women positive for HPV-6. Follow-up was by cytology, colposcopy, and HPV hybridization only. No biopsies were taken of the initial lesion. In the second study, nearly 500 women with signs of HPV infection in cytological smears are being followed by cytology and colposcopy, with biopsies in some persons. HPV-typing has been carried out in a small proportion only. Preliminary results indicate that progression is higher in women with HPV-16 than for other HPV-types.

Longitudinal studies of patients with a cytological diagnosis of HPV infection have been carried out in Australia, Canada, Italy and France. Although these studies are suggestive of an increased rate of progression, they are difficult to interpret since no specific typing of HPV has been carried out and the end-points are in general ill-defined.

Ongoing and planned investigations

In view of the limited nature of the epidemiological evidence for an increased risk of cervical cancer in association with HPV infection, case–control and cohort studies are under way or are being planned in different parts of the world. Notably, case–control studies are being carried out in Latin America and Europe, in areas with large differences in cervical cancer incidences. Prospective cohort studies are under way or are being planned in Denmark, India, the United Kingdom, and the USA. All of these studies attempt to address the question of HPV-associated risk and the risk attributable to other factors, including male sexual behaviour either alone or in combination with HPV.

Research needs

It is essential that well-designed descriptive and analytical studies of adequate size are performed. Population-based studies are needed to establish the magnitude of the problem of infection with various HPV types in various parts of the world, in populations with contrasting incidence rates of cervical cancer. Descriptive studies of the natural history of the various HPV types are required to plan strategies for prevention. Studies should be encouraged in areas with different incidence rates of cervical cancer and/or with suspected differences in the prevalence of risk factors. Epidemiological

investigations should include the concomitant study of HPV types and other known and suspected risk factors, such as age at first sexual intercourse, number of male and female sexual partners, contraceptive use, smoking, sexually-transmitted infections, and dietary components.

Particular attention should be paid to case–control and cohort studies of well-defined diagnostic groups of invasive and precancerous lesions. There is a need for the use of consistent criteria for the classification of cancerous and precancerous lesions of the cervix uteri, based on published WHO standards.

It is of the utmost importance to develop valid, standardized methods for the diagnosis of type-specific HPV infections, applicable in population-based investigations. The validity, measured by the sensitivity and specificity of the tests, should be similar in groups of persons with normal epithelium, clinically overt warts, defined grades of dysplasia, and *in situ* and invasive carcinoma. Methods should be developed to diagnose type-specific HPV infection in fixed cytological and histological material. This would permit the study of the risk of cancer development in HPV-infected women with negative cytology.

CONTROL OF HPV INFECTION

Health education

The general principles for the control of sexually-transmitted diseases apply to HPV infection (*8*). These are:

—to promote discriminative sexual intercourse by avoiding multiple sex partners and casual sex;
—to encourage the use of barrier contraception, particularly condoms;
—to encourage attendance for medical examination when lesions are noticed;
—to encourage compliance with treatment;
—to promote and facilitate a detailed examination of sex partners. It must be remembered that both male and female partners of individuals with apparently uncomplicated genital condylomas may have not only condylomas but also intraepithelial disease affecting the cervix, vulva, penis and anus.

Professional education and training

It is important for doctors and other health care personnel to understand the potential seriousness of HPV infection, not only in the pathogenesis of lesions that may be uncomfortable and distasteful for the individual, but also in the infection of others via sexual contact. The possible involvement of HPV in the pathogenesis of genital precancer or invasive cancer must therefore be stressed during their training. The presence of such lesions, in association with genital warts, is of great importance. The methods available for their detection vary in different localities; many centres have access to biopsy and cervical cytology while colposcopy is available in some major centres. The use of these diagnostic aids must be taught. The value of contact-tracing in the control of this sexually-transmitted disease should be emphasized.

Little is known about the transmission of genital HPV by fomites, but it certainly can occur with non-genital strains of HPV. For this reason, careful instruction must be given to medical staff about the possibility of cross-infection by the careless use of towels and dressing materials. The correct sterilization of all instruments used for the examination and treatment of patients with HPV infection is of great importance. Viral genomes have been detected on imperfectly sterilized instruments and in containers of acetic acid used repeatedly for colposcopy. Thorough cleaning with a detergent followed by autoclaving or, failing this, boiling for 20 minutes is recommended.

In the past, HPV infection, in the form of genital warts, was regarded as a tiresome but essentially unimportant disease. Its association with neoplasia has necessitated a complete reappraisal of attitudes, which must now be reflected in the training of medical and paramedical staff.

Treatment

Anogenital HPV lesions may be uncomplicated or associated with intraepithelial neoplasia in the same or other parts of the genital tract. The aim of treatment of uncomplicated lesions is to cure an unsightly infectious condition, while the treatment of those associated with intraepithelial neoplasia is intended to prevent invasive cancer. The available treatment modalities consist of locally destructive techniques such as cautery, electrofulguration/desiccation, cryosurgery, diathermy (under general anaesthesia), CO_2 laser and surgical excision. Non-invasive techniques involve the use of caustics (trichloroacetic acid), antimitotics (podophyllin, fluorouracil), immune modulators (isoprinosine) and interferons. Interferons also have antiviral and antiproliferative activity. Antiviral drugs (idoxuridine, acyclovir) have given disappointing results.

There are few well-controlled studies of the results of treatment of anogenital HPV-induced lesions, but the available data indicate that between 15% and 60% of lesions recur within 3 months of therapy. If possible, the patient should be kept under observation

for at least 3 months after treatment. Since some women develop neoplastic genital lesions months or even years after treatment, cytology should be performed at intervals of every two years indefinitely.

Dysplastic cervical lesions (CIN I–III) should be treated by local destructive methods (*9*). Cryosurgery gives cure rates of 80–95%, depending on the size of the lesion, while other modalities (diathermy, cold coagulation or CO_2 laser evaporation) achieve rates of 90–97%. The destruction of normal tissue surrounding the dysplastic lesion to a distance of about 2–3 cm will remove much virus-infected material which may exist in seemingly benign tissue. Cone biopsy is indicated in lesions that cannot be completely visualized by colposcopy or where cytological evidence suggests endocervical neoplasia. The removal of mild dysplasia (CIN I lesions) is controversial, but a recent study suggests a 30% progression rate to severe dysplastic carcinoma *in situ* (CIN III) over 2½ years; in nearly 90% of the lesions that progressed, HPV-16 DNA was recovered. In biopsies that show only evidence of HPV, a problem in management exists. Recent studies suggest that there is a risk of progression to CIN but more evidence is needed before definitive recommendations can be given. Obvious condylomatous lesions should be treated.

Vaginal intraepithelial neoplasia (VAIN) may occur independently but usually presents in conjunction with similar disease in either the cervix or vulva. An examination of these areas with the colposcope and directed biopsy is mandatory before any treatment is undertaken. If no evidence of invasion exists they can be treated by either local surgical removal or destruction with either diathermy or CO_2 laser. Concomitant usage of fluorouracil as a vaginal cream is suggested by some because of a significant recurrence rate.

Vulvar intraepithelial neoplasia (VIN) is an increasingly common disease which is associated in many cases with clinical evidence of HPV, usually in younger women, and causes therapeutic problems (*10*). The malignant potential of such lesions is uncertain, especially the minor grades which may be no more than a HPV infection. However, a consensus of opinion seems to indicate that the VIN-III (*in situ* carcinoma) lesion should be removed. Many are associated with obvious condylomatous lesions or are symptomatic (i.e., pruritic) and so the decision to treat is made easier. The treatment of VIN is either surgical or non-surgical. In the former modality either *excision* employing vulvectomy, skinning vulvectomy with split-skin graft or wide local excision can be employed, or *destruction* using either CO_2 laser evaporation or rarely other modalities such as diathermy are used. The use of CO_2 laser for treating VIN in non hair-bearing areas is associated with minimal morbidity, surgical excision being reserved for disease in hair-bearing areas. Recurrences are not infrequent, with recurrence rates of 20–40%. Non-surgical methods of therapy involve the use of topical fluorouracil cream and interferons. The presence of other intraepithelial lesions in the anogenital area must be looked for and treated as outlined above.

Treatment of penile intraepithelial neoplasia is usually done in respect of the adjacent condylomatous area and may be in the form of either local application of fluorouracil cream or CO_2 laser evaporation.

Vaccine development

The high prevalence of papillomavirus infections in the general population and their association with cancer make the papillomaviruses a significant health problem that might be eased by the development of an effective vaccine. The most pressing research need in the development of a papillomavirus vaccine is the elucidation of the roles played by humoral immunity and cell-mediated immunity in the protection against papillomavirus infection and the regression of papillomas. In particular, it should be determined if humoral immunity can protect against papillomavirus infection, since vaccine using purified structural proteins will be the most straightforward to develop and get approved. In addition, since secreted IgA will most likely be more effective than circulating IgG in the neutralization of the anogenital HPVs, it should be determined if immunity against HPVs leads to the production of IgA. It may be necessary to try atypical vaccination methods to get an IgA response, such as application of the vaccine to the genital tract for vaccination against genital HPVs.

The development of a papillomavirus vaccine which induces humoral immunity may prevent the transmission of papillomaviruses with the eventual eradication of papillomavirus-related diseases, but will have little effect on the large number of people who currently have papillomavirus infections and who may be at risk of developing papillomavirus-associated cancers. Regression of HPV-induced lesions is likely to be due to cell-mediated immune reactions but the viral proteins which are the target have not yet been identified. It needs to be determined if a vaccine which induces cell-mediated immunity targeted at the papillomavirus-containing cell of a papilloma or carcinoma can have a therapeutic role as well as a preventive role. In addition, it should be determined if cell-mediated immunity can prevent reactivation of latent papillomavirus infections. Biochemical characterization of papillomavirus gene

products indicates which proteins are associated with the plasma membrane and homology comparisons between HPV protein sequences point out the antigenic determinants (epitopes) which are shared between HPVs. Ultimately, however, it will be necessary to determine which antigens can act as targets of cell-mediated immunity.

Since there is no *in vitro* culture system for the propagation of papillomaviruses, the classical live, attenuated and killed virus vaccines are not currently an alternative for the development of a papillomavirus vaccine. Even if such culture systems were available, the use of a live vaccine would not be feasible because of the oncogenic potential of the papillomaviruses. Recombinant DNA technology makes it possible to express any of the viral genes from prokaryotic and eukaryotic vectors. Large amounts of fusion proteins can now be produced in bacteria or yeast and used to make vaccines. Several groups have shown that fusion proteins for either "early" or "late" HPV proteins are immunogenic in animals but it still remains to be shown that these proteins can give protective immunity against papillomaviruses. Another approach will be the identification of potential shared epitopes and the synthesis of short peptides containing these antigenic determinants. Synthetic peptides have been successfully used to generate antibodies that react with native proteins encoded by the papillomaviruses. However, it has not been demonstrated that synthetic peptides can be used to induce protective immunity to papillomaviruses.

There are two general strategies for vaccination against papillomaviruses. The first is to induce humoral immunity against structural capsid proteins of the viruses and both L1 and L2 induce antibodies which react with intact viral particles. The goal of this approach is the production of neutralizing antibodies and the prevention of infection. However, no group-specific epitopes appear to be present on intact viral particles and, therefore, vaccines based on structural proteins will need to be polyvalent. Moreover, humoral immunity may not be adequate for the prevention of infection since it probably occurs by direct inoculation of the epithelium. Thus, for cutaneous exposure the virus would never come into contact with circulating antibodies prior to infection. In support of this, people who develop a humoral response to papillomaviruses are still susceptible to reinfection.

The second approach to papillomavirus vaccination is to induce cell-mediated immunity against non-structural viral proteins (coded by the "early" ORFs) that are exposed in the plasma membrane of infected cells, which could prevent the growth of early lesions and induce the regression of existing benign and neoplastic lesions. An added advantage to this approach is that the vaccine may be both preventive and therapeutic. A vaccine based on E6 and E7 proteins will potentially have the greatest efficacy because these proteins are consistently expressed in cancer cells. It is not known, however, if the E6 protein is present in the plasma membrane. On the other hand, comparison of putative HPV E6 proteins shows only scattered homology which would be insufficient to yield epitopes shared among all HPVs. In addition, it would probably be unwise to vaccinate with a known oncogene, as E5 and E6 have been shown to be. The E5 protein is known to be exposed on the external cell surface but the predicted E5 gene products of different HPVs show very little homology at the primary sequence level, making it unlikely that vaccination with E5 protein or peptide from one papillomavirus would yield immunity to other papillomaviruses. Like the E6 ORF, the E7 ORF is expressed in both benign papillomas and carcinomas. It is the most abundant viral protein in the cervical carcinoma cell lines containing HPV-16 or HPV-18 genomes and appears to be cytoplasmic in location. Comparison of HPV E7 proteins shows only scattered homology.

E2 proteins have not been detected in transformed cells or in cervical carcinoma-derived cell lines. Since integration of the HPV genomes in cervical carcinomas generally disrupts the E2 ORF, the E2 protein is unlikely to be expressed in carcinomas. The E4 ORF is probably expressed as both an early protein (E6/E4 fusion) and by itself as a late protein. The E4 protein is a very abundant cytoplasmic protein in papillomas and is synthesized in those cells which are also producing the capsid proteins. The function of this protein is unknown. Comparison of the HPV E4 proteins shows only weak homology insufficient to give any shared epitopes.

Finally, it is possible that HPV infection induces cellular genes which are expressed on the cell membrane and are unique to the papilloma. These surface antigens might provide a target for cell-mediated immunity. Such antigens would have to be identified and cloned to be useful for vaccination purposes. The potential advantage of this approach is that a common set of tumour antigens may be induced by all HPVs. A single monovalent vaccine could then be produced which would give immunity against all papillomaviruses. The expression of the tumour antigens in normal tissues will then have to be ruled out to prevent destruction of normal tissues by immune response.

One of the newest vaccine approaches is the use of vaccinia virus vectors for the expression of viral proteins *in vivo*. It should be stressed however, that the utilization of a recombinant vaccinia vaccine as a

live vaccine containing genes encoding for potentially oncogenic proteins (such as E5 or E6 proteins) could be dangerous and should be discouraged.

Since initial vaccine studies will not be possible in humans, it will be necessary to have an animal model. A good model system should have papillomavirus-induced true papillomas which progress on to malignant tumours. The cottontail-rabbit papillomavirus system is probably the best at this time. Phase I trials in humans, to test the immunotherapeutic value of vaccines, could then be conducted after being properly tested for safety in animals.

RECOMMENDATIONS

There is a substantial body of evidence linking specific types of HPV infection to human genital cancer. However, the specific role of these viruses in the development of these tumours remains to be clarified. While routine screening for HPV in the general population is not indicated at present, in view of the global magnitude of cervical cancer as a health problem the following recommendations are strongly endorsed.

(1) Studies to define specifically the role of HPV types in anogenital cancer at the epidemiological, clinical and experimental level should be encouraged.

(2) Concerted efforts should be made to develop vaccines to prevent or control HPV infections related to the development of anogenital cancer.

(3) Diagnostic procedures should be developed to permit the rapid, sensitive and reliable detection of type-specific HPV infection.

(4) The prevention and management of HPV infections by health education and professional training should be improved.

(5) Regional reference centres for the diagnosis and study of HPV infections should be established for research purposes.

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