Leader

Human papillomavirus infection with particular reference to genital disease

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Papillomaviruses were originally grouped together with the polyomaviruses in a single taxonomic group, the Papovaviridae. The division into two separate groups was based on genomic organisation, virion size (polyomaviruses being smaller (50 nm) than papillomaviruses (55 nm)), and on their differing molecular patterns of replication.¹ Papillomaviruses are epitheliotropic, with the exception of bovine papillomaviruses (BPV) which can induce equine sarcoid and fibropapillomas, and infection across species is uncommon (for example, BPV infection of hamsters and horses).²

Papillomavirus gene function

The molecular organisation of all papillomavirus genomes conform to the same pattern (fig 1). It is still useful to consider viral genes as forming two functional groups, early (E) and late (L), although some workers now consider this outmoded.³ There is an "upstream regulatory region" (URR) between the early and late genes (not shown in the figure) which contains gene sequences responsible for regulating gene expression and DNA replication.

OPEN READING FRAME E1

The E1 open reading frame (ORF) encodes a phosphoprotein of approximately 68 kDa with intrinsic helicase, ATPase, and specific DNA binding ability.⁴ The E1 protein, in association with E2, is essential for viral replication and it is thought that the E1-E2 complex binds through E2 to specific sites in the upstream regulatory region, stabilising E1 in a conformation which recognises the viral origin of replication (ori) for the initial steps in viral DNA replication.⁵

OPEN READING FRAME E2

HPV E2 protein has several functions which include the positive and negative regulation of

gene expression.⁶ It has recently been suggested that E2 can activate the tumour suppressor gene, p53, independently of E6 (see below), and that E2 expression may result in cell apoptosis.⁷

OPEN READING FRAME E3

This is of unknown function and is absent from human papillomaviruses.

OPEN READING FRAME E4

The function of E4 protein is yet to be defined. For some HPV it is transcribed only in terminally differentiated keratinocytes, but for HPV 1 transcription occurs lower down in the epithelium.⁸ The interaction between E4 protein and cytokeratins may induce collapse of the intermediate cellular network, resulting in koilocyte formation.^{9, 10}

OPEN READING FRAME E5

This is lacking from some HPVs and truncated in others. E5 has been shown to have a transforming function in HPV types 1, 6, and 16^{11, 12} and may be capable of acting in cooperation with epidermal growth factor to induce cellular proliferation.¹³

OPEN READING FRAME E6

E6 protein plays a key role in cellular transformation, partly through inactivation of cellular tumour suppressing protein p53. Interestingly, the oncogenic potential of different HPV types correlates with the binding affinity of their E6 proteins. E6 has been reported to act in conjunction with E6 associated protein (E6-AP) to degrade p53. Reduction of E6-AP in HPV infected cells produces increased levels of p53, whereas reduction in normal cells has no effect on p53 levels.¹⁴

E6 protein has also recently been shown to interact with cellular MCM (minichromosome maintenance) proteins,¹⁵ which are believed to play a key role in regulating cellular DNA replication. Other potential transformation related functions of E6 include telomerase activation,¹⁶ paxillin binding,¹⁷ transactivation of the c-myc promoter,¹⁸ and upregulation of epidermal growth factor receptor.¹⁵

OPEN READING FRAME E7

E7 proteins also play an important role in cell transformation, with different domains of the E7 gene sequence appearing to determine

Figure 1 Genome organisation of HPV 16.

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transformation and transactivation. E7 protein interacts with the tumour suppressing retinoblastoma (Rb) gene proteins and promotes Rb protein degradation through a ubiquitin-proteasome pathway.¹⁹ Decreased stability of Rb protein mediated by E7 could have multiple effects on control of cell cycle progression. Numerous cell cycle regulatory protein targets of HPV E7 have now been identified which include p21, the closely related kinase inhibitor p27, cyclin A, TATA binding protein, and members of the AP-1 transcription factor family.^{20, 21}

OPEN READING FRAMES L1 AND L2

The two "late" reading frames extend over almost half of the HPV genome and encode the two viral capsid proteins. The L2 region appears to be important in virus-host cell interactions.³

HPV typing

The current typing system for human papillomaviruses is based on differences demonstrated by DNA hybridisation, in particular differences in the E6/E7 and L1 regions. A new HPV type is, by definition, less than 90% homologous with previously identified types with respect to these regions, as determined following complete viral cloning.¹ A subtype shares 90–98% homology in these regions and a variant more than 98% homology. It is possible that future typing systems may be simplified and rely solely on E6 gene analysis.

Almost 80 different HPV genotypes have now been cloned from clinical material and at least 28 of these affect the genital tract (Stanley M, personal communication).

Pathogenesis of infection

The virus must gain access to the basal epithelial cells for infection to occur, and although epithelial "abrasion" has been proposed as a likely mechanism, evidence for this is lacking and it is indeed difficult to imagine in the case of cervical infection and anal infection in heterosexual men. Although the cellular receptor for HPV has yet to be defined, recent studies suggest that α -6 integrin protein may be a potential candidate.²² The incubation period (that is, from infection to the appearance of clinical lesions) varies from a few weeks to possibly over 18 months. However, in most infected individuals the virus is carried subclinically and never produces clinically apparent lesions.

The transition from latent infection to productive infection is initiated by the differentiation of cells as they proceed upwards to the epithelial surface.³ This is characterised by an increase in viral DNA production secondary to changes in the pattern of transcription of the "early" genes. Nearer the epithelial surface a critical point of cell differentiation occurs and, although E gene expression continues, L1/L2 transcription becomes dominant resulting in viral capsid production and the formation of complete viral particles.³ Individual clinical lesions are thought to represent the clonal expansion of a population of keratinocytes

derived from a single infected basal cell but it is currently uncertain how the vascular core seen in genital warts is derived.

High grade dysplastic and malignant lesions characteristically show evidence of loss of L gene expression but increased E gene expression. Viral DNA is commonly integrated into cellular DNA, with a preference for sites close to cellular oncogenes. The viral E1/E2 regions are often disrupted on integration, resulting in derepression of E6/E7 transcription and subsequent inactivation of p53 and Rb, leading to loss of cell cycle control and cellular transformation.³ Other events and factors are undoubtedly of importance and it would seem likely that HPV infection represents an early event which is insufficient on its own to induce progression of dysplasia.

Immune response to HPV infection

Cell mediated immune responses are crucial to the pathogenesis of HPV infection. Immunosuppressed patients are prone to develop multiple and intractable warts and are at increased risk of developing anogenital dysplasia.^{23–26} The regression of genital warts is characterised by a localised delayed type hypersensitivity response with a pronounced increase in CD4 T lymphocytes and macrophages.²⁷ Various cytokines are secreted, with interleukin (IL)-12 being present in very high levels which may have a direct antiviral effect in addition to inducing further activation of T cells and natural killer cells (Coleman N, personal communication). Although Langerhans cell numbers are not reduced as they are in dysplastic lesions, a loss of Langerhans cell dendritic arborisations has been reported in regressing warts.²⁸

Much interest has focused on potential HPV cytotoxic T lymphocyte (CTL) epitopes. Although naturally occurring CTL responses have been difficult to demonstrate in patients with infection, a recent study has shown that CTL responses to E6/E7 are more commonly detectable in women with cervical HPV 16 infection without evidence of cervical intraepithelial neoplasia (CIN) than in HPV 16 positive women with CIN, suggesting a possible protective role for the CTL response.²⁹ Interestingly, a study in mice has shown a lack of E7 specific CTL responses, suggesting a state of immunological "ignorance."³⁰ The failure of E7 immunogenicity may be because of low levels of expression or possibly an absence of costimulatory factors such as cytokines. The issue of whether the immune system is ignorant or tolerant to HPV is of great current interest.

The exact role of humoral immunity in HPV infection is uncertain. The presence of HPV 16 E7 antibodies has been associated with clearance of HPV 16 infection but further studies are needed before firm conclusions can be drawn.³¹ Genetically engineered virus-like particles can now reliably be used as antigen substrates in enzyme linked immunosorbent assays (ELISAs) for measuring HPV directed antibodies. Studies using HPV 16 L1/L2 virus-like particle ELISAs have suggested that current or recent exposure to HPV generates a good antibody response, but with time the response

declines.³² Seroconversion occurs at a median of approximately eight months after detection of HPV DNA at the cervix and antibody persists for at least 40 months, long after cervical samples become HPV DNA negative.³³ Between 50% and 94% of HPV 16 infected women develop antibodies, and those failing to seroconvert may have less persistent infection.³³ HPV 16 seropositivity is associated with a three to six times increased risk of developing CIN.^{34, 35} However, it is currently uncertain whether naturally occurring anticapsid antibodies play any role in delaying the onset or progression of HPV associated dysplasia.

Vaccination

There are two main objectives for an HPV vaccination programme—first, to prevent initial infection, and second, to eliminate an already established infection or infection associated carcinoma. Recent animal studies have shown that virus-like particles are capable of eliciting neutralising antibodies which protect against virus challenge, and of inducing regression of existing papillomavirus associated tumours.^{36, 37} In humans, recombinant vaccinia virus expressing HPV 16 and 18 E6 and E7 proteins has been reported to induce E6/E7 CTL mediated immune responses in women with cervical carcinoma.³⁸ Escalating doses of vaccine may be required to elicit a maximal CTL response and the suggestion from ongoing phase 1 vaccine trials is that disease outcome in some vaccinees may be less severe than without immunological intervention. Vaccination of patients with established severe dysplasia or malignancy would probably be considered as an adjunct to other treatments in an attempt to prevent recurrence.

Diagnosis of HPV infection

Most clinically apparent lesions are diagnosed by appearance, biopsy being performed only if there is clinical uncertainty. The cytological and histological features of HPV infection will be familiar to all readers and include koilocytosis, hyperkeratosis, parakeratosis, and dyskeratosis.³⁹ Electron microscopy may identify virus particles in clinical material but this is a costly and time consuming procedure which is rarely used for routine diagnosis. One of the major problems in the field of HPV research is the inability to propagate the virus with ease in conventional cell cultures and, although there has been some recent success with primary keratinocytes transfected with HPV 18 DNA producing infectious HPV virions,⁴⁰ molecular biological techniques have to be used for most research studies. HPV DNA or RNA detection methods are most often used for the diagnosis and typing of HPV infection but these have yet to find a place in the routine diagnostic laboratory. Southern blotting is a highly sensitive and specific method and can determine whether viral DNA is integrated or episomal. It is, however, labour intensive, particularly when attempting to identify numerous genotypes.³⁹ Dot blot assays are rapid and relatively inexpensive but false positives can sometimes be a problem.³⁹ Tissue in situ hybridisation is

useful for determining exactly where HPV is localised in a histological specimen and also whether the virus is latent or replicating.³⁹ Polymerase chain reaction (PCR) and nested PCR are the most sensitive methods for detecting HPV sequences in clinical samples, with typing performed by Southern blotting or restriction fragment analysis of the amplified products.³⁹ Numerous PCR primers have been described. Consensus primers may target L1/L2 or E6/E7 ORF and are useful in routine testing or epidemiological studies as they can rapidly screen for many HPV types. The technique of PCR enzyme immunoassay has recently been reported as a simple and rapid method of detecting multiple HPV types in cervical smear material and may prove useful for large epidemiological studies.^{41, 42} Hybrid capture, in which hybrids formed between HPV DNA and a RNA probe are detected by antibody binding and subsequent chemiluminescence, is of similar sensitivity to PCR.⁴³ The method uses a "two probe" mixture comprising "low risk" and "high risk" HPV types and may be used to quantitate HPV DNA in a clinical sample.

Clinical manifestations of genital HPV infection

Genital HPV infection may be clinically obvious on examination or be subclinical.

CLINICALLY APPARENT INFECTION

The commonest manifestation of clinically apparent genital HPV infection is genital warts (condylomata acuminata). Between 1981 and 1994 there was a 300% increase in attendances for new and recurrent cases of genital warts at genitourinary medicine clinics in England and Wales⁴⁴ and we now recognise HPV as the commonest sexually transmitted viral infection in the United Kingdom.

Papular warts and flat lesions are less common than condylomata acuminata and may require examination under magnification, such as with a colposcope, for adequate identification. Some workers classify such small lesions as subclinical disease, whereas this term should strictly be reserved for lesions which require means other than magnification for identification. Most genital warts are caused by HPV types 6, 11, or 42, irrespective of their site, whereas flat or macular lesions are more often associated with types 16, 33, and 42. Women with genital warts have been reported to be at increased risk for developing vulval, anal, and cervical cancer and CIN III, with the risk of vulval cancer being particularly high (standard incidence ratio 40.1; 95% confidence interval 20.0 to 71.7).⁴⁵

Dysplastic lesions

Dysplastic lesions ("intraepithelial neoplasia") have been described at all anogenital sites and may be apparent to the naked eye or on colposcopic examination or require the application of acetic acid for identification (see below). Low grade dysplasia is associated with HPV types 6 or 11, whereas moderate and high grade lesions (that is, intraepithelial neoplasia

II/III) are associated with HPV types 16 and 18, and less commonly types 31, 33, and 35. High grade vulval dysplasia (VIN III) appears to be increasing in prevalence,⁴⁶ particularly in women under 35 years of age. Lesions may be flat or papular, white or off-white in colour, and are most commonly found on the labia and perineum. In approximately 70% of cases the lesions are multifocal.⁴⁷ Recent studies have suggested that the annual progression rate from VIN III to invasive disease may be in excess of 10%.⁴⁸ A history of smoking or immunosuppression increases the risk of progression.⁴⁸

Bowen's disease and the related but more benign self limiting condition, Bowenoid papulosis, are probably now better classified under the term intraepithelial neoplasia.

Buschke-Lowenstein tumour or "giant condyloma" was first described in detail in 1925 and is a very uncommon manifestation of genital HPV infection.⁴⁹⁻⁵⁰ Lesions usually present as large, foul smelling, cauliflower-like masses which are slow growing and prone to recur. They are histologically similar to condylomata acuminata but show both downward and upward growth and thus appear locally invasive. Verrucous carcinoma is considered a low grade and well differentiated variant of squamous cell carcinoma.⁵¹ Bogomoletz *et al* questioned the need to consider Buschke-Lowenstein tumour and verrucous carcinoma as separate entities and suggested they be considered as part of the continuous spectrum of HPV infection, along with condylomata acuminata.⁵²

SUBCLINICAL INFECTION

As mentioned previously, subclinical infection is more common than clinically obvious disease and may conveniently be divided into three categories.

Aceto-white lesions

An HPV infected epithelium will often appear normal on examination but may whiten after the application of a 3–5% acetic acid solution. This is thought to be the result of coagulation of epithelial cytokeratins, in particular cytokeratin 10.⁵³ The time required for aceto-whitening to occur varies from between 20 and 60 seconds for the cervix and anal canal to about three to five minutes for the penis, vulva, and perianal epithelium. Aceto-whitening is not specific for HPV infection and a biopsy should be performed to make a definitive diagnosis.

Cytological and histological evidence of infection

Cytological or histological evidence of HPV infection may be found in a normal appearing epithelium which fails to whiten after the application of acetic acid. Koilocytosis is less often seen at sites other than the cervix and in these cases the specificity of cytology is less well defined.⁵⁴

Presence of HPV DNA

The polymerase chain reaction will frequently detect the presence of HPV DNA in clinical

specimens which are normal on macroscopic and microscopic examination. This is often termed "latent" infection, although it is also appropriately classified as a subdivision of sub-clinical infection. This may represent HPV during its incubation period, after regression of clinical lesions or, more contentiously, gene sequences which have been transmitted silently from generation to generation.

CERVICAL HPV INFECTION

Most cervical HPV infection is subclinical, condylomata acuminata being less common on the cervix than on the external genitalia. Young, sexually active women show the highest prevalence rates for cervical HPV infection, ranging from about 15% to 30% using PCR based data.⁵⁵ Infection with multiple types is seen in 20–30% of all infected women and most of the still uncharacterised types of HPV are found among healthy women.⁵⁵ Between 1% and 4% of cytologically normal women harbour "high oncogenic risk" types, predominantly HPV 16.⁵⁵ Interestingly, most cervical HPV infection is transient, with 50% of infections clearing by eight months and most by two years.⁵⁶ However, continued unprotected sexual activity may lead to reinfection, often with a different HPV type. Women harbouring HPV 16 with normal cervical cytology have over a 100 times increased risk of developing CIN III compared with HPV negative women, and progression to CIN III and invasive disease is strongly associated with persistence of "high risk" viral types, in particular certain HPV 16 variants containing E6 mutations.⁵⁷ Various cofactors for progression have been identified, which may act in conjunction with HPV. Smoking is strongly associated with risk of cervical HPV infection but in case-control studies of smoking and cervical cancer in which HPV infection has been taken into account no independent role of smoking has been found.⁵⁵ Nevertheless, prolonged heavy smoking may be of importance, as has been suggested for progression of VIN.⁴⁸⁻⁵⁸ Immunosuppression is associated with the development of anogenital intraepithelial neoplasia (CIN, VIN, and AIN) but its exact role in progression to invasion is currently uncertain.⁵⁹ Oral contraceptive use has been reported to increase the risk of invasive cervical cancer among HPV infected women⁶⁰⁻⁶¹ and several studies have now shown a direct effect of sex hormones on HPV transcription in addition to an indirect effect leading to increased levels of HPV oncogene expression.⁶² There is currently much interest in the reported positive association between certain human leucocyte antigen (HLA) types, in particular the HLA DQ3 family, and CIN and cervical cancer.⁶³ Although this association is usually explained in terms of a reduced ability to clear HPV infection, the precise immunological mechanism is uncertain.

HPV typing and cervical cytology

The prime objective of cervical screening is to prevent cervical cancer by detecting and treating precancerous lesions which are likely to progress. The use of HPV typing in

conjunction with cytology has been advocated as a means of fine tuning both the screening process and the triage of women with minor cytological abnormalities.⁶⁴ For example, women with normal cervical cytology and negative for HPV DNA may be rescreened at more than five yearly intervals, whereas those with negative cytology but who are positive for the most prevalent high risk HPV types (that is, 16, 18, 31, and 33) may be advised to retest at one year and proceed to colposcopy if HPV positivity persists. For women with persistent mildly abnormal cytology (that is, borderline changes and mild dyskaryosis), colposcopy may be deferred if HPV testing proves negative or shows only the presence of a low risk HPV type. Interestingly, some workers have suggested that for women over 35 years, primary screening for HPV every five years may be adequate, and only women positive by this test should proceed to cervical cytology.⁶⁵ More studies will undoubtedly be forthcoming, looking particularly at the potential cost implications of HPV testing.

Summary

HPV is the commonest sexually transmitted viral infection in the United Kingdom and as such poses a major public health problem. In addition to the potential physical morbidity associated with genital warts, abnormal cervical cytology, and anogenital dysplasia and neoplasia, the associated psychological morbidity should not be forgotten. Although our knowledge of viral function and disease pathogenesis has advanced appreciably in recent years, we are still some way from developing an *in vitro* method of viral propagation. Vaccination against HPV infection will hopefully be achieved within the next 10 years, but a prevention and treatment strategy which is appropriate for both developed and developing nations must be our major long term goal.

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