

Accepted Manuscript

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PII: S1521-6934(17)30118-9

DOI: [10.1016/j.bpobgyn.2017.08.001](https://doi.org/10.1016/j.bpobgyn.2017.08.001)

Reference: YBEOG 1733

To appear in: *Best Practice & Research Clinical Obstetrics & Gynaecology*

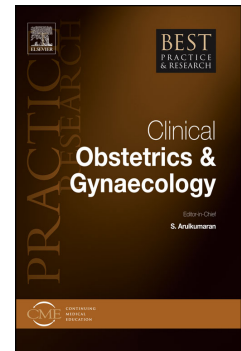
Received Date: 1 August 2017

Revised Date: 1521-6934 1521-6934

Accepted Date: 1 August 2017

Please cite this article as: Doorbar J, Host Control of Human Papillomavirus Infection and Disease, *Best Practice & Research Clinical Obstetrics & Gynaecology* (2017), doi: 10.1016/j.bpobgyn.2017.08.001.

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Host Control of Human Papillomavirus Infection and Disease

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Abstract

Most human papillomaviruses cause inapparent infections, subtly affecting epithelial homeostasis to ensure genome persistence in the epithelial basal layer. As with conspicuous papillomas, these self-limiting lesions shed viral particles to ensure population-level maintenance, and depend on a balance between viral gene expression, immune cell stimulation, and immune surveillance for persistence. The complex immune evasion strategies, characteristic of high-risk HPV types, also allow the deregulated viral gene expression that underlies neoplasia. Neoplasia occurs at particular epithelial sites where vulnerable cells such as the reserve or cuboidal cells of the cervical transformation zone are found. Beta papillomavirus infection can also predispose to the development of cancers in individuals with immune deficiencies. The host control of HPV infections thus involves local interactions between keratinocytes, as well as the adaptive immune response. Effective immune detection and surveillance limits overt disease, leading to HPV persistence as productive microlesions, or in a true latent state.

keywords papillomavirus, epithelial homeostasis, wart, CIN, HPV, infection

Human Papillomavirus Infection and the Host Response

As for many viruses that have coevolved with their human hosts, most papillomavirus infections are either asymptomatic or inapparent, or lead only to the appearance of benign self-limiting lesions that are not life threatening. This balanced relationship ensures survival of the infected host without compromising evolutionary fitness, while allowing virus transmission and persistence within the population as a whole (1, 2). To achieve this, papillomaviruses must be delicately tuned, both with regard to their effects on the infected cell, as well as their interactions with the innate and adaptive immune system – a process that has been driven by virus/host co-evolution over millions of years. In this review, the delicate steady state regulation that allows virus persistence in the host, will be considered alongside the host events that lead eventually to virus ‘clearance’, or which may sometimes allow deregulated viral gene expression and the development of neoplasia.

Sexually-Transmitted HPV types and the Diversity of HPV-Associated Disease

Although the anogenital HPV types attract most attention, much of our papillomavirus understanding can be applied more widely to the 200 or so HPV types that are known to infect humans (Figure 1). Human Papillomaviruses are divided into 5 phylogenically distinct genera, with the majority residing in the Alpha or the Beta/Gamma Genera (3). The Mu HPV types, which include HPV1, contains only 3 members, while only one Nu papillomavirus is known (HPV41). In general, the Beta and Gamma papillomaviruses cause inapparent infections of cutaneous epithelial sites. The Alpha HPV Genera include the well-studied high risk types that can be associated with anogenital and oropharyngeal cancers, but also two additional evolutionarily branches of low risk papillomavirus that cause cancers much more rarely (Figure 1). Amongst these are cutaneous HPV types such as HPV2, that cause common warts in children, and HPV6, which is an important cause of genital warts in young adults (1).

In addition to genital warts, sexually transmitted HPV types (both low and high risk) also cause less obvious inapparent infections. Genital warts are an immediate problem, and generally prompt the infected individual to seek treatment. By contrast, inapparent infections are likely to go unnoticed in the absence of screening programmes, and in many instances will be effectively resolved by a host immune response, and pose no long-term problem. At particular epithelial sites however, infection by high-risk HPV types can be associated with the development of neoplasia and sometimes also cancer. Cervical screening, which is accessible in many developed countries, addresses the unique vulnerability of the cervical transformation zone region to high-risk HPV-associated neoplasia, and that fact that the majority of high-risk HPV-associated cancers occur at this site (4). The general principles that underlie papillomavirus transmission and disease formation, and the way that the basic biology of these viruses has been adapted to suit not only the sexual transmission route, but also their direct and indirect transmission are discussed below.

Epithelial Homeostasis and the Maintenance of a Self-Limiting HPV Infection;

Papillomavirus Disease-Strategy; It is now generally accepted that HPV infection requires virus access to the basal lamina and the epithelial basal cells, and that infected basal cells serve as the reservoir of infection to sustain the lesion. At stratified epithelial sites such as the ectocervix, or at cutaneous penile sites, there remains a debate as to whether infection of a specialised epithelial stem cell is required for lesion formation, or whether papillomaviruses encode particular gene functions that modify the infected basal cell to ensure persistence (2, 5, 6). Clearly papillomaviruses have gene functions that limit the ability of the infected basal cell to commit to differentiation when compared to neighbouring uninfected cells, a process which in itself allows expansion of the infected cell population in the infected basal layer (Figure 2) (7, 8). In the case of the high-risk HPV types, which are contained in the Alpha genus, this is a largely E6-mediated function. HPV16 E6 gene expression is facilitated in the growth factor rich environment of the basal and parabasal epithelial layers, with an increase in E6 abundance driving a corresponding decrease in p53 activity as a result of E6-mediated proteasome degradation (9, 10). In the epithelial basal layer, this p53 reduction leads to a loss of p53 transcriptional activity, which in turn leads to reduced levels of the Notch receptor on the cell surface. Basal cell density and commitment to differentiation are critically dependent on normal Notch function, with minor reductions in Notch activity providing the cell with a competitive advantage over its neighbours (Figure 2) (11). It is likely that some variation on this general theme underlies persistence in lesions caused by both high and low-risk HPV types, although the effects on p53, and indeed other E6 cellular targets are expected to have subtly different mechanisms of regulation. This can be anticipated by comparison with the evolutionarily distinct Beta HPV types, which cause widespread inapparent infections at cutaneous sites, and which similarly target Notch, but at a different point in the pathway (12, 13). Of course, papillomaviruses need not only to control the rate of cell loss through differentiation, but also the replication of their own genomes in the infected basal cell. Not surprisingly, studies using model systems have suggested that HPV genome copy number is linked to the replication of cellular DNA in the epithelial basal layer, with viral episomes persisting at a constant low level (14). Although most of the DNA replication machinery that the virus requires is provided by the basal cell, it is though that HPV genome persistence is critically dependent on the viral E2 protein, which amongst its various roles, is involved in HPV genome partitioning as the infected cell divides (15).

Given that papillomaviruses are epithelial specialists, it is perhaps not surprising that they interfere with the regulatory pathways that control cell expansion and cell density in the epithelial basal layer, and that they generally do this in a very ordered way that results in a self-limiting infection (16, 17). Once a basal cell has become infected, it is not easily lost from the body, even as genetic errors accumulate. The difficulty in eradicating HPV infections, including verrucas, common warts and genital warts, is likely to stem from the common characteristics of the infected basal cells, and that a lesion can be 'rebuilt' if even a small number of infected basal cells remain (Figure 2) (11). An effective immune response to viral gene products is thus of great importance for effective disease control and clearance.

The Basal Reservoir Maintains a Supply of HPV Infected Cells for the Differentiated Epithelial Layers

Although not necessarily complete, the above description outlines the fundamental strategies on which papillomavirus infections are built. It appears that the high and low-risk Alpha HPV types, and the Beta HPV types exhibit variations on the theme rather than following a completely distinct approach (18). These differences reflect the characteristic disease biology and tropisms of each HPV genus and type.

Completion of the productive stages of the HPV life cycle, depends on the steady flow of infected cells from the basal layer reservoir into the upper epithelial layers (Figure 3). During this process, local growth factor stimulation declines, extracellular signals driving differentiation increase, and the patterns of viral gene expression change, in order to favour viral genome amplification over viral persistence. Although not yet mapped in great detail, this involves changes in viral promoter usage and changes in mRNA splicing, and the expression of additional viral gene products that facilitate life cycle completion. Here, the E7 protein specialises in driving cell cycle re-entry in cells of the parabasal and mid epithelial layers in order to provide the cellular replication machinery required for viral genome amplification (19). To a large extent, this is achieved by association with members of the retinoblastoma family of proteins (particularly p130), which releases the E2F transcription factor to allow progression into S-phase. The broader retinoblastoma specificity of the high risk E7 proteins (which includes p105 and p107) is thought to contribute to their ability to drive cell cycle entry in both the basal and suprabasal layers – a characteristic not shared by low-risk HPV types (20). In these epithelial layers, E6 may still be required to suppress p53 function, which would otherwise be enhanced by E7. Curiously, the elevation of E5 predicted in these epithelial layers, acts to mimic growth factor stimulation by constitutively activating the Epidermal Growth factor receptor despite the absence of ligand (21). This is thought to enhance the expression of full-length E6 protein, as well the post-translational modification of E1, a virally encoded DNA helicase necessary for viral genome amplification. In fact, the timely elevation of E1 and E2 in cells that are driven into S-phase by E6 and E7, is key to increasing viral genome copy number in preparation for packaging (22).

(23)

Genome amplification and the subsequent increase in gene expression, also provides an environment in which the HPV late promoter can be activated, allowing the production of the E1^{E4}, L2 and L1 proteins. The L2 protein associates with E2 transiently during genome packaging, but is also (unlike E2) a minor component of the icosahedral capsid. Papillomavirus particles consist of 360 L1 molecules arranged in 72 pentameric capsomeres, with a variable number of L2 molecules, which are present at the centre of the capsomeres (24). The most abundant viral protein by far is E1^{E4} which assembles into keratin-associated amyloid fibres in the mid to upper epithelial layers. Although not part of the infectious particle, the E1^{E4} amyloid structures are thought to enhance virus release, and may also contribute to virus stability and transmission. Productive infection depends

therefore on the co-ordinated expression of a number of viral proteins during the process of epithelial differentiation. As discussed later, the success of this process depends on the nature of the infecting HPV type, the epithelial site of infection, but also the host response to infection, and in particular whether an adaptive immune response is raised to control infection.

Levels of Human Papillomavirus Control by the Host Immune System

A key suggestion from the above, is that mechanical pressure brought about by cell to cell contact can limit papilloma lesion size, at least in situations where viral gene expression is appropriately regulated. The self-limiting warts and verrucas caused by low risk HPV types are likely to be regulated in part by these mechanisms. It is clear however, that both the adaptive and the innate immune system are also critically important in limiting the extent of new infection and spread, and that immune deficiencies can facilitate deregulated viral gene expression and viral copy number elevation.

Preventing Infection; Early studies using model systems of disease have clearly shown that prior-immunisation with papillomavirus particles or papillomavirus 'virus-like particles' (VLP) can protect against subsequent challenge (25, 26). This protective effect is mediated by neutralising antibodies to the viral capsid proteins, with inflammation and local antibody levels rising at sites of tissue damage. In most cases, disruption of the epithelial barrier is necessary to allow papillomavirus access to the basal layer, a situation that can also facilitate virus neutralisation in vaccinated individuals. In fact, a key limitation of HPV VLP vaccination, arises from the antigenic diversity apparent amongst the structural proteins of different HPV types, with HPV genotype divisions resembling the divisions that might also be expected on the basis of serology. In order to protect the 8kb viral genome, and to assemble into pentameric capsomeres and infectious virus particles, much of the structure of the L1 molecule must however be conserved. The regions of L1 that are displayed on the virus surface can vary greatly between HPV types however, with the L1 protein having 5 hypervariable surface loops, which act to limit the production of cross-neutralising antibodies following HPV immunisation or natural infection (23). The rapid evolution of coat protein diversity is well characterised amongst RNA viruses such as Influenza A and Norovirus, where the error-prone replication of RNA genomes maintains a pool of antigenically diverse viruses in the population that can infect the same host repeatedly. For papillomaviruses, which have a slower mutating double stranded DNA genome, it appears that the host immune system has driven the appearance of multiple co-existing HPV types, that have diverged in their immunogenicity, but which have similar tropisms and disease-associations (3, 27). HPV2, 57 and 27, which cause common warts in children, are an example of this from the low-risk cutaneous Alpha papillomaviruses, with HPV16, 31, 33 and 35 forming a similar group amongst high-risk 'mucosal' types. At a population level, it appears that the host immune responses that occur in individuals during virus exposure and infection, contribute to the maintenance of HPV diversity in the wider general population, allowing individuals who have already cleared one HPV type to be infected with another.

As outlined above, vaccine studies have clearly shown us that a potent antibody response can protect against infection. During natural infection however, only low virus titres are required at the basal lamina, and because newly assembled HPV virions are in general produced only in the upper epithelial layers, the humoral immune response to viral capsid during natural infection can be highly variable (28). Vaccination, which involves the intramuscular injection of VLP and adjuvant, always produces a more potent immune response. In the case of persistent ongoing infections, the extent to which further infection by the same HPV types is inhibited, will depend on the extent of the anti-viral antibody response. Once an infection has been cleared as a result of a cell-mediated immune response, we would expect protection against new lesion formation, or at the very least, effective control of such infections as described below.

Controlling the Spread of Disease Following Infection; Once infection has occurred, the process of lesion formation can begin. Our current knowledge of how viral gene expression is regulated during this process is at present quite limited. In animal models of disease, lesion formation takes four weeks or so depending on infectious titre, with microlesions being apparent by histology after two weeks, and the first signs of L1 and L2 capsid protein expression being seen at three weeks post-infection. Our current hypothesis suggests that papillomaviruses have a limited ability to increase the growth rate of infected cells during the initial stages of wound healing, and act instead to prevent cell cycle exit and G0-progression as cell density increases (11, 16). In an immune competent host, both low and high-risk HPV types can persist for months or years, causing chronic productive lesions that shed virus from their surface layers over a prolonged period of time. To achieve this, papillomaviruses have a number of key adaptations (described below), which allow them to persist in infected epithelial cells, even in the face of an active adaptive immune system.

One of the key ways in which papillomaviruses avoid immune detection, is by limiting viral gene expression in the epithelial basal and parabasal layers to very low levels. This is possible because only low-levels of just a small number of viral proteins are required for basal cell genome maintenance, a situation that restricts the presentation of viral antigens on MHC class 1 and the stimulation of adaptive cell-mediated immunity. In fact, for many low-risk HPV types, viral gene expression in the epithelial basal cell has been extremely difficult to detect. The elevated viral gene expression that is essential for viral genome amplification and virus synthesis, is typically delayed until the infected cell reaches the mid or upper epithelial layers where T-cells and dendritic cells are less abundant, and where immune surveillance is thus less efficient. In addition, some but not all HPV types contain a short E5 gene between the early and late regions of the viral genome (21). E5 can contribute to viral genome amplification through the constitutive activation of MAPK signalling, but E5 also inhibits the presentation of viral peptides on MHC class 1 as a result of MHC down-regulation (29). Although this model is well accepted, and applies to the medically important Alpha HPV types and also to some animal papillomaviruses (e.g. BPV), not all HPV types encode an E5 protein, with E5 being conspicuously absent in the Beta and Gamma HPV Genera (18). Furthermore, in lesions caused by a few HPV types, including the plantar/palmar warts caused by HPV1 (Mu Genus) and HPV65 (Gamma Genus), the viral E4 protein can become abundantly expressed in the first parabasal cell layers of the epithelium, and can

sometimes be additionally detected in a subset of cells in the basal layer itself, presumably in cells that have committed to differentiation (30). These observations almost certainly reflect the different life-cycle strategies used by different HPV types, with some such as HPV16, and probably also other Alpha HPV types such as HPV11 and HPV2, actively inhibiting MHC antigen-presentation to produce the more conspicuous lesions. Others, including the Beta and Gamma types that typically cause wide-spread, chronic, but 'generally asymptomatic' lesions in immunocompetent individuals, appear to be better served when a controlled immune response is mounted. This allows long-term virus shedding, but not the clearance of viral genomes from the infected basal layer (31). Curiously, Mu papillomaviruses and a subset of Gamma HPV types, cause visible lesions only at particular epithelial body sites. The most notable of these are the soles of the feet, with individual HPV1 verrucas containing as many as 1×10^{12} virus particles, and accumulating E4 to levels as high as 30% of total lesional protein content (32).

In addition to down-regulating canonical MHC class 1 levels, an approach that is also employed by other viruses(33), papillomaviruses may also retard the adaptive immune response by inhibiting the retention of Langerhans cells at the site of infection. HPV-infected tissue typically has a lower Langerhan cell density than the surrounding uninfected epithelium (34, 35). Langerhans cells are epithelial-specific dendritic cell, that can display foreign peptides on their surface-MHC class 2, and facilitate the activation of a T-cell response following lymph node migration. Because papillomaviruses do not stimulate a lytic infection however, and because they shed virus particles only from the epithelial surface, the opportunity for Langerhans cells to sample and appropriately present viral antigens is severely restricted. Even so, lesion regression, when it eventually occurs, appears to depend on the cross-priming of epithelial-specific dendritic cell with viral antigens, and the subsequent activation of a T cell response in the draining lymph node (36). It is generally thought that these virus-specific defences, by stalling the adaptive immune response, act to prolong the duration of infection.

The Immune Response Controls Chronic Infection and Latency, and can lead to Clearance; Most high risk HPV infections eventually clear as a result of a host cell-mediated immune response in under 18 months, with effective immune recognition leading to T-cell homing and T-cell infiltration at the site of infection (Figure 4). The limited availability of animal models in which to study PV biology, has meant that the process of immune regression is still not well understood. According to current thinking, lesion infiltration by HPV-specific CD4/CD8-positive T-cells does not necessarily control disease through cytotoxic killing of virus-infected cells. Instead, it has been suggested that viral gene expression, and thus the visibility of infected cells to the immune system, becomes actively suppressed as infiltration occurs, a process that is mediated by changes in the cytokine milieu that accompanies the regression process (Figure 4). HPV-specific helper CD4 T cells that are able to recognise epitopes on the HPV E2 and E6 proteins have been reported to be important in the 'clearance' of low-grade HPV-induced disease (37, 38), with a CD4 response to E7 being more important in the control of high-grade neoplasia (39). Clearly, the precise nature of the host response to infection depends on which viral antigens are seen by the immune system, as well as the time-period between infection and eventual immune-detection. Importantly, the very low levels of viral antigen expressed in the basal epithelial cells, particularly in productive low-grade

disease, is thought to render these cells effectively 'invisible' to the immune system, even after a successful antiviral response has been mounted and visible disease has been cleared. The concept of genome maintenance in the absence of significant viral gene expression underlies our concept of viral latency, and the immunological mechanisms that allow chronic inapparent active HPV infection and the long-term shedding of virus particles from apparently normal epithelial tissue (40). Indeed, chronic inapparent infection is the life cycle strategy favoured by the Beta and Gamma HPV genera that are ubiquitously present at many epithelial surfaces. It is though that a comparable immune control can also operate after the immune regression of productive lesions caused by high-risk Alpha HPV types such as HPV16, or the conspicuous genital and common warts caused by HPV 11 or 2.

To understand the process of immunosurveillance further, it is worth noting that in addition to Langerhans cells, there is also a significant population of skin-resident T cells in the basal and parabasal epithelial layers, with the total number of T-cells at this location being actually higher than in the blood (41, 42). In humans, these are predominantly α/β TCR+ memory T cells, that can undergo immediate clonal expansion when viral antigens are seen (43). Studies on other viruses (notably HSV) have shown that dendritic cell-mediated antigen presentation to memory CD8+ T cells, in the presence of CD4+ T cells in the skin, leads to rapid T-cell proliferation in the absence of lymphatic organ involvement (44). Infection with vaccinia virus can similarly stimulate the generation of protective long-lived non-recirculating CD8+ memory cells that can be found throughout the skin (45), and it appears the skin-resident memory T cells play an important role in limiting the extent of HPV infection also. In fact, in many cases, we should regard visible productive papillomas, inapparent infections, microlesions, and true papillomavirus latency, as variations on a theme rather than distinct entities. Conspicuous productive infections can thus persist in the absence of an effective immune response, a situation that is further compounded by the immune evasion capabilities of E6, E7 (see below) and E5 (see above). Following immune detection however, such active infection is suppressed, with the level of control being dependent on the ability of skin resident memory T cells to recognise the occasional infected basal cell that manage to support genome amplification and virus synthesis. Clearly there is a balance between the potency of the host immune response, and the viruses ability to stimulate host immunity at low level over a prolonged period of time, while still producing sufficient virus into the environment. Any reduction in the level of immunosurveillance, such as may occur during aging or following treatment with immune suppressive drugs, can allow more extensive viral gene expression, and even the appearance/reappearance of papillomas or neoplasia (40, 46, 47).

In the context of the above, there is an ongoing discussion as to whether immune regression leads predominantly to latency, a situation where the viral genome may persist in the epithelial basal cell without being seen by the immune system, or whether true clearance of viral genomes from the basal layer can also occur. Clearly, viral genome maintenance is sensitive to the basal cell environment, with viral genome copy number declining in response to type 1 interferons, which suggests that basal cell copy number may be affected indirectly during the process of regression (36). Indeed, keratinocytes themselves can secrete IFN κ , a type 1 IFN (48), as well as a range of proinflammatory cytokines and chemokines in response to virus infection, which is expected to recruit immune cells, including NK cells to the

site of infection. In many cases, it appears that HPV gene products can however render the infected cell less sensitive to these local changes, and can also inhibit innate immune responses within the cell. The E6 and E7 proteins, at least in the case of high-risk HPV types, disrupt the type 1 Interferon response by inhibiting both STAT phosphorylation and their nuclear accumulation(49). In addition, high risk E6 can disrupt the function of Tyk2, an immediate downstream component of the interferon signalling pathway, with E7 inhibiting IRF 1 (Interferon response factor 1), a transcription factor directly involved in the induction of interferon stimulated genes (49). In addition, E7 inhibits MHC presentation by repressing LMP2 and TAP1, and reduces the levels of MHC class 1 heavy chain, which along with E5, contributes to the general loss of MHC class 1 on the cell surface. NK killing is avoided because non-canonical MHC levels are thought to be unaffected, and because the E5 protein can also down regulate at least some of the signals required for NK activation (50). In most cases, these functions are only established for the high risk Alpha HPV types, although we also expect some functional conservation amongst the low risk Alpha papillomaviruses. Importantly, immune evasion is unlikely to be required solely in the infected basal cell, where viral gene expression is low. Similarly, the intracellular detection of viral DNA by the pattern recognition receptors may be less important in the epithelial basal layer, where only low copy numbers of double-stranded nuclear HPV episomes are present. A more conspicuous requirement is expected during viral genome amplification, where copy number per cell can rise 10,000 fold. Even so, it has been suggested that the HPV genome can activate the RIG-1-like (RLR) and STING/cGAS pathways that detect cytoplasmic dsDNA, as well as endosomal toll-like receptor (TLR) pathways (51), and that these innate defences against viral genomes are countered by the activity of HPV encoded genes (E6, E7 and E2). The question as to whether host immune responses can clear viral genomes from the epithelial basal layer still remains the subject of speculation, but it is reasonable to expect this in some instances. Indeed, in the ROPV (Rabbit Oral Papillomavirus) model of PV latency, viral genomes are cleared slowly from the epithelial basal layer following regression, which could be explained by stochastic process of cell division and cell loss through differentiation. Interestingly, it may be the precise nature of the adaptive immune response, and the relative predominance of CD4+ helper T cells vs. CD8+ cytotoxic T cells that determine the precise outcome of infection, as has been suggested from studies on MmuPV (52).

Failure to Control Infection allows Chronic Deregulated Gene expression, and the Development of Neoplasia and Cancer

Disease persistence corresponds with a failure of the immune system to properly detect infection, and is generally characterised by only low numbers of circulating antigen-specific T cells and an abundance of CD25-positive T-regulatory cells, which produce an intraepithelial cytokine milieu that restricts T cell trafficking. Interferon gamma levels are low, and anti-inflammatory cytokines such as IL10 and TGF- β are elevated, inhibiting the local proliferation and differentiation of the CD4 and CD8-positive T cells that might otherwise control infection (36). In this immune-tolerant environment, disease persistence is facilitated, and deregulated viral gene expression can go unchecked. For the high risk Alpha HPV types, the loss of control of key HPV genes, particularly the E6 and E7 genes that regulate cell cycle

entry, cell proliferation, and differentiation leads to a dramatic change in lesion phenotype (53). During the ordered productive life cycle, these genes are carefully regulated, and act to subtly control the basal cells commitment to differentiation, and the suprabasal cells ability to enter the cell cycle for genome amplification. As their expression increases in the basal layer, normal cellular controls are progressively compromised, leading to neoplasia (e.g. CIN1, 2 and 3), with the ability of the virus to complete its life cycle and to produce infectious virions at the epithelial surface being progressively lost. Progression to high grade neoplasia it seems, is advantageous for neither the virus nor the host. In fact the situation is exacerbated, because the viral proteins that drive neoplastic progression (i.e. E6 and E7), at least amongst high risk HPV types, also contribute to immune evasion (36). As a result, increased high risk viral gene expression does not result in increased immune visibility. For the low risk Alpha HPV types, the regulation of E6 and E7 is differently controlled and involves two separate promoters. The effects on p53 and Rb are differently regulated, and their contribution to immune evasion less clearly defined. As a result, the propensity of low risk HPV types to cause neoplasia and cancers appears to be very low, and is often considered to be negligible in the general population. However, the concept of persistent deregulated viral gene expression as a general cause of HPV-induced cancers extends beyond just the extensively studied high risk Alpha group. The ubiquitous Beta and Gamma HPV types can cause non melanoma skin cancer in individuals who are immune compromised, and in these situations, it is clear that deregulated viral gene expression can be seen. Even the low risk Alpha HPV types such as HPV11 can cause cancers in individuals who are persistently infected and who cannot properly control their active infections. The cancers associated in individuals suffering from recurrent respiratory papillomatosis clearly attest to this (54). In each case, cancer development takes time, and is associated with the host's inability to keep a check on viral gene expression. The unique organisation of the high risk HPV genome, coupled with the curious additional functions encoded by their E6 and E7 genes, means that it is these HPV types that are responsible for most of the HPV-associated cancer burden.

HPV Induced Cancers Occur at Vulnerable Epithelial Sites

Although high risk HPV types can cause cancers at various epithelial sites, including the penis, vagina and vulva, the cervical and anal transformation zone, and the oropharynx are very clearly hot spots. Of the 600,000 invasive cancers caused by HPV in 2012, invasive cervical cancer constitutes 500,000 or so cases, with other anogenital cancers accounting for the bulk of the remainder (data from GLOBOCAN 2012: <http://globocan.iarc.fr/>). Oropharyngeal cancers and cancers of the oral cavity constitute around 40,000 cases/year. Cancers caused by HPV are lower in men (~1%) than in women (~8.6%), primarily because of the unique vulnerability of the cervix uteri to HPV carcinogenesis (55, 56) (Figure 5). Although a full molecular understanding of site-specific vulnerability is not yet available, our current thinking is that these epithelial sites lack the protective layers of differentiating cells that characterise the external epithelium, but that in addition, these particular sites contain specialised epithelial cells that are not able to support the productive HPV life cycle. At the tonsillar crypts, it is the loosely structured

reticular epithelium that is the site of high risk HPV-associated neoplasias, rather than the more differentiated epithelium that lines the outer tonsillar surface. The unique characteristics of the tonsillar crypt can facilitate pathogen recognition by the immune system, but also enhanced virus infection in situations where the infected cells are not effectively recognised. A similar situation occurs at the squamocolumnar junction of the cervical and anal transformation zones, where stratified epithelial cells abut a columnar epithelium that is more vulnerable to infection. For the cervical transformation zone, the particular vulnerability for the development of high risk HPV-associated neoplasia is thought to result from the presence of a unique type of epithelial cell known as the cervical reserve cell, that normally gives rise to either the columnar epithelium of the endocervix and cervical glands, but also under some circumstances, can give rise to the stratified cells of the cervical transformation zone (Figure 5) (57, 58). The epithelial changes that allow reserve cell to form a stratified transformation zone typically occur at puberty as the endocervical cells become exposed to the more acid environment of the vagina. Such metaplastic changes can however occur throughout a woman's life whenever the conversion of columnar endocervical epithelial cells to a multi-layered epithelium is required. A second group of vulnerable cuboidal cells have also been identified more precisely at the squamocolumnar junction (59). According to these models, viral gene expression is deregulated at these specific sites following infection, with an abortive rather than productive viral infection ensuing. It is clear that future work needs to consider not just viral protein function, but function and gene expression in the context of particular epithelial sites.

Summary

It is apparent from the above, that the majority of human papillomaviruses persist quietly within the human population, a situation that fits very well with their coevolution with their human hosts over millions of years. To achieve this, they delicately modify epithelial homeostasis upon infection, and set up a regulatory balance that allows persistent chronic infection without clearance. As part of their life cycle however, papillomaviruses require genes that drive cell cycle entry, in order to allow efficient viral genome amplification and virus particle production. In most cases, these genes are carefully regulated and their effects on cell proliferation and differentiation limited. In humans, an evolutionary branch within the Alpha Genus has led to the appearance of a 'high risk' subgroup, that can more dramatically affect epithelial basal cell function, even in immune competent hosts. This is a consequence of changes in the way that viral gene expression is regulated in high risk types, and from the acquisition of viral protein functions that impact on epithelial differentiation, the regulation of basal cell proliferation and cell density, and the ability to evade immune detection. Even so, cancers associated with high risk types are focused on particular epithelial sites, including the oropharynx and the anal and cervical transformation zones. At present, cervical cancer accounts for the vast majority of HPV-associated cancers, prompting the drive to better understand the target epithelium as well as the virus.

Acknowledgments/Conflict of Interest Declaration

John Doorbar is Professor of Viral Pathogenesis in the Department of Pathology, University of Cambridge, UK. No Conflict of interest is reported. The support of the Medical Research Council (programme grant U117584278) has allowed formulation of many of the ideas outlined in this review.

Research Agenda

- To understand how cell type and cell characteristics determine disease outcome.
- To characterise the molecular events that control lesion formation and lesion size.
- To understand the molecular processes underlying the development of neoplasia at vulnerable epithelial sites.
- To explain how the adaptive immune system drives lesion-regression and allows persistence as a latent infection.
- To establish the minimal level of viral gene expression required for basal cell persistence, and how viral gene expression changes during reactivation.

Figure Legends

Figure 1. Papillomavirus Diversity

- A.** The HPV types found in humans fall into five genera, with the Alpha and the Beta/Gamma genera representing the largest groups. HPV types from the Alpha genus are often classified as low-risk cutaneous (grey), low-risk mucosal (orange) or high-risk (pink). The high-risk types identified using red text are confirmed as 'human carcinogens' on the basis of epidemiological data. The evolutionary tree is based on alignment of the E1, E2, L1 and L2 genes (60).
- B.** Typical genome organisation of the high-risk Alpha, Mu and Beta HPV genomes. While all share a common genetic organisation, the size and position of the major ORFs can vary. The positions of the major promoters are marked with arrows with early and late polyadenylation sites being marked as PAL (late) and PAE (early).

Figure 2. Papillomaviruses, Epithelial Homeostasis and Self-Limiting Disease

- A.** In the uninfected epithelium, proliferating progenitor cells increase basal cell density. Increasing cell density triggers a subset of basal cells to commit to differentiate. These cells are lost from the basal layer, so maintaining basal cell numbers.
- B.** Both uninfected and low risk HPV-infected progenitor cells are thought to behave similarly during cell division. For low risk HPV types and high risk HPV types at some epithelial sites, viral gene expression is thought to result in infected cells that are more resistant to cell-cell contact and cell differentiation signals. As a result, HPV infected cells persist in the epithelial basal layer as cell density increases.
- C.** Delayed commitment to differentiate and sustained cell proliferation can result from deregulated high risk HPV gene expression, and is manifest clinically as a neoplasia.

Figure 3. Patterns of Viral Gene expression in the Infected Cervix

The different patterns of viral gene expression associated with different grades of neoplasia are illustrated in the cartoons. Although the diagrams indicate the patterns seen in cervical neoplasia, it is thought that similar patterns of deregulated gene expression underlie neoplasias at other differentiating epithelial sites.

Figure 4. Role of the Adaptive Immune System in Controlling infection

- 1. Active Infection.** Active papillomavirus infection involves the regulated expression of viral proteins as cells containing viral genomes migrate towards the epithelial surface. Resting T-cells (brown circular cells) and Langerhans cells (orange) can be found in the lower layers of the epithelium and in the dermis.

2. **Immune Regression.** Immune regression involves the presentation of viral antigens to the immune system, and the subsequent accumulation of activated CD4+ and CD8+ T-cells (light blue circular cells) in and around the lesion. During regression, activated T-cells accumulate within and beneath the lesion.
3. **Latency.** Lesion clearance involves the suppression of viral gene expression as lymphocytes infiltrate, and may involve changes in cytokine activity and cytokine signaling at the site of regression.
4. **Reactivation from Latency.** The presence of memory T-cells circulating in the epithelium prevent extensive viral gene expression and keep the viral genomes in the basal layer in a latent state. Changes in immune status would allow local rises in viral copy-number.

Figure 5. Vulnerable Epithelial Sites and HPV Cancer Risk

- A. Cervical Intraepithelial Neoplasia (CIN) of different grades are thought to be associated with different patterns of viral gene expression. The viral E4 protein is shown in green. The cellular MCM protein is regarded as a surrogate of E6/E7 expression in such lesions and is shown in red.
- B. The cervix is thought to contain a number of different cell types that are vulnerable to infection. Infection outcome is thought to be influenced according to the characteristics of the infected cell and the local epithelial environment. Infection of the reserve and cuboidal cells is connected to the development of high grade disease.
- C. Of all the epithelial sites that high risk HPV types can infect, the cervix appears uniquely vulnerable, with the vast majority of HPV-associated cancers occurring at this site. Of the infectious agents associated with human cancers, HPVs are the most significant.

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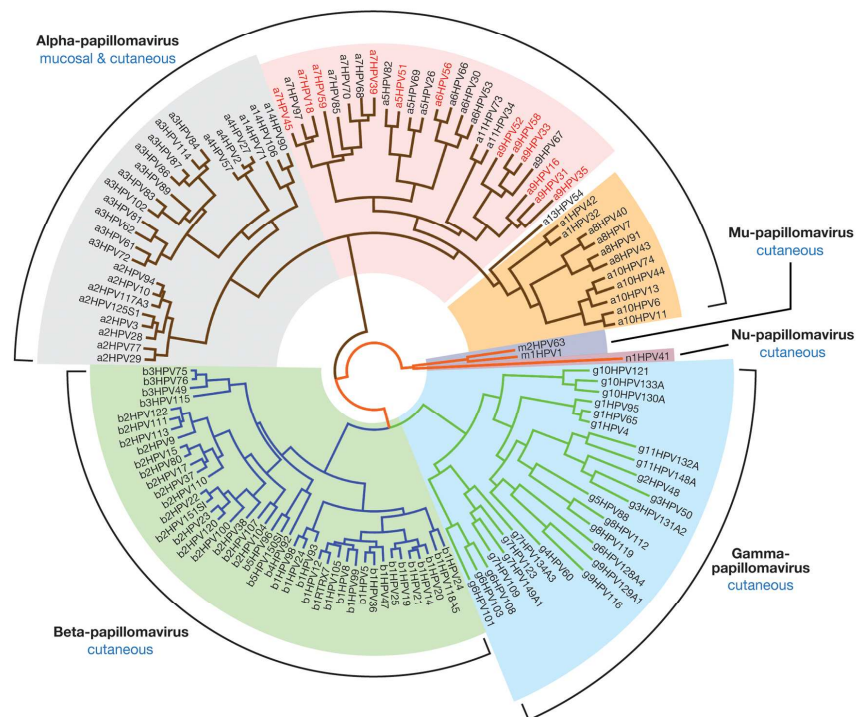
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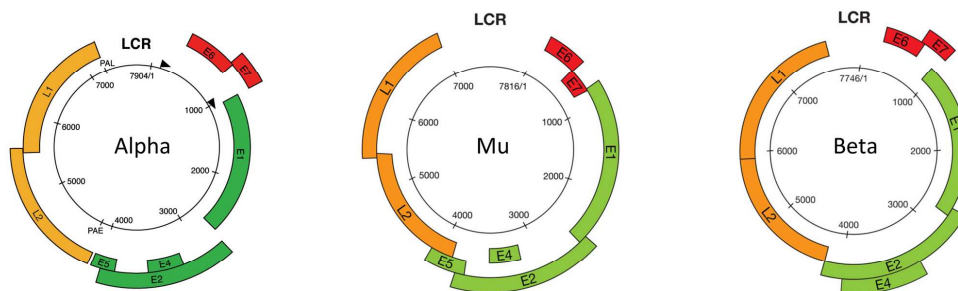
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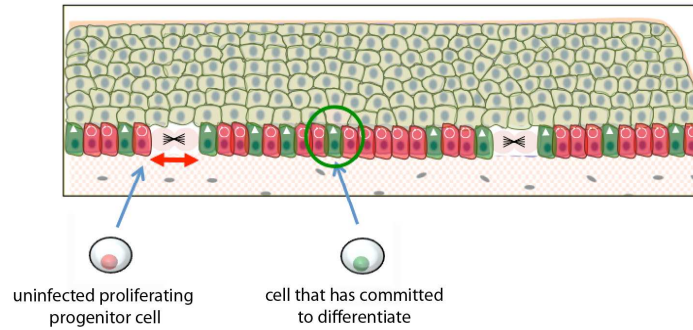
(A)



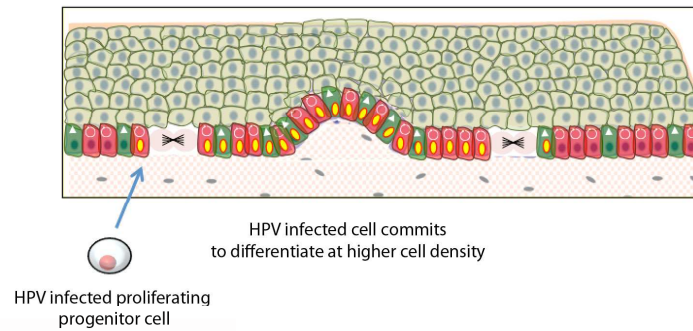
(B)



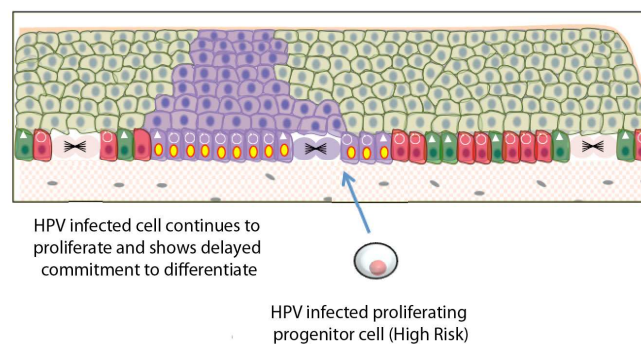
(A) Cell division increases cell density and commitment to differentiate

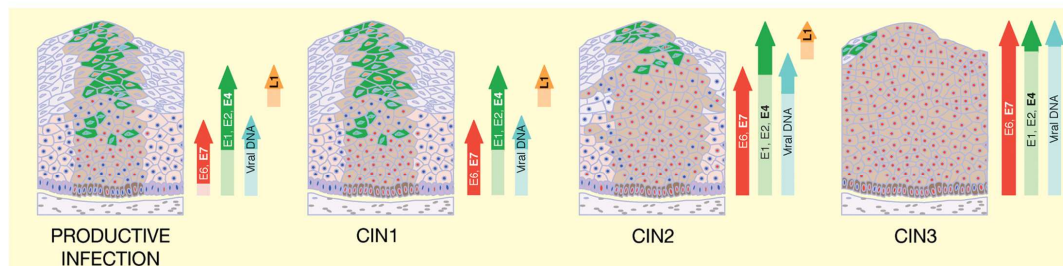


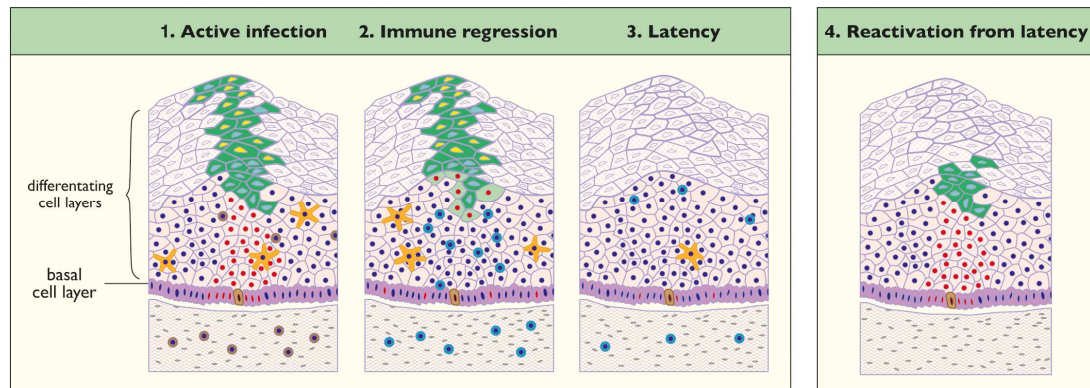
(B) Delayed commitment to differentiate can lead to papilloma formation

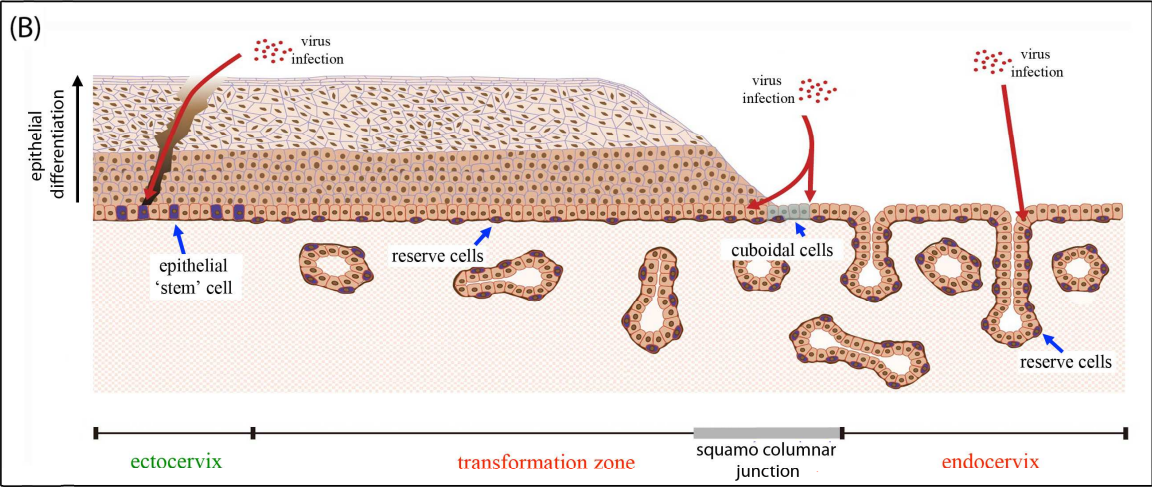
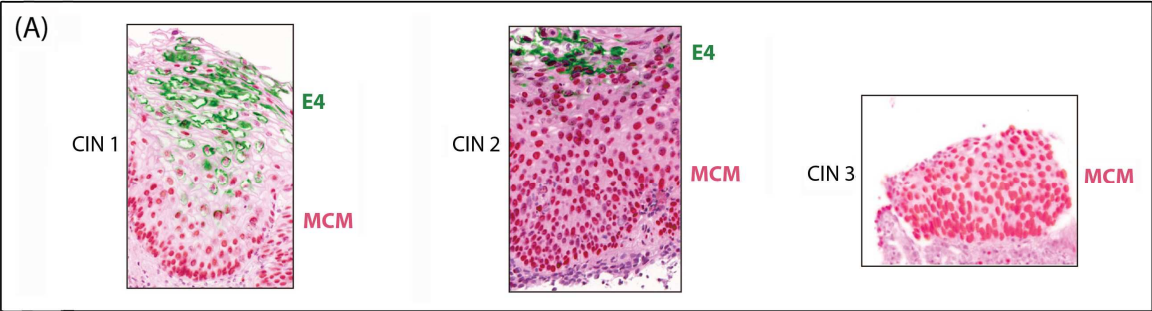


(C) Delayed commitment to differentiate and sustained proliferation can lead to neoplasia









(C)

cancer site	new cases	attributable to HPV	attrib. fraction	men	women
Cervix uteri	528 000	528 000	100%	-	528 000
Anus	40 000	35 000	88%	17 000	18 000
Vagina/vulva	49 000	20 000	41%	-	20 000
Penis	26 000	13 000	51%	13 000	-
Oropharynx	96 000	29 000	31%	24 000	6 000
Oral cavity/larynx	358 000	9000	2.4%	7 000	2 000
total	1 097 000	634 000	58%	61 000	574 000

infectious agent	cancer site/cancer	number of cancer cases	% of cancers worldwide
<i>H. pylori</i>	stomach	490 000	5.4%
HPV	cervix/other sites	550 000	6.1%
HBV, HCV	liver	390 000	4.3%
EBV	Lymphoma/NPC	99 000	1.1%
HHV-8	KS	54 000	0.6%
<i>S. haematobium</i>	Bladder	9000	0.1%
HTLV-1	Leukaemia	2700	0.1%
Liver fluke	biliary system	800	
total infection-related cancer		1 600 000	17.7%
total cancer (1995)		9 000 000	100%

- Most papillomaviruses are associated with asymptomatic infections, or self-limiting benign papillomas.
- The papillomavirus E6 protein restricts the infected basal cells ability to commit to differentiation.
- Viral gene expression is required at very low level in the infected basal layer.
- The viral E6/E7 proteins are deregulated in high grade neoplasia, and are involved in immune evasion.
- Papillomavirus disease is controlled by immune surveillance by skin-resident T cells