Postmodern cancer: the role of human immunodeficiency virus in uterine cervical cancer

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The association between cervical cancer and human papillomavirus (HPV) is well known, but its association with human immunodeficiency virus (HIV) is controversial. Coinfection with HPV and HIV is to be expected and recent epidemiological data from Africa show that cervical cancer is the most common AIDS defining neoplasm in women. Unlike other AIDS defining neoplasms, the occurrence of cervical cancer is not dependent on immune compromise. HIV alters the natural history of HPV infection, with decreased regression rates and more rapid progression to high grade and invasive lesions, which are refractory to treatment, requiring more stringent intervention and monitoring. The more aggressive behaviour is mirrored by a different molecular pathway. HIV associated cervical cancers are thought to progress through the microsatellite instability pathway, whereas HIV negative ones progress through loss of heterozygosity. Interaction is probably via viral proteins, with HIV proteins enhancing effectiveness of HPV proteins, and perhaps contributing to cell cycle disruption. Dysregulation of the cellular and humoral arms of the local and systemic immune systems may ensure disease progression. Furthermore, HPV infection may predispose to HIV infection and facilitate its progression.

VIRUSES

HPV

Twenty three of the 80 known types of HPV may infect the genital tract. The types are stratified into low risk (types 6, 11, 42, 43, and 44) and high risk (types 16, 18, 31, 33, 35, 39, 45, 50, 51, 53, 55, 56, 58, 59, 64, and 68) types. The HPV virus targets receptors on the basal epithelial cells, a possible candidate being the α6β4 integrin receptor.

Although viral replication occurs in the basal, less differentiated layers of the epithelium, late protein synthesis and viral packaging occur in the mature epithelial layers. Despite a high rate of HPV infection in the general population, only 2–3% of women develop dysplasia. The term low grade squamous intraepithelial lesion of the Bethesda classification system encompasses mild dysplasia/cervical intraepithelial neoplasia (CIN) I and koilocytic change induced by HPV. High grade squamous intraepithelial lesions include moderate dysplasia/CIN II and severe dysplasia/CIN III.

Low grade squamous intraepithelial lesions are attributable to both high and low risk types of HPV, with 30% containing more than one type of HPV, and less than 10% harbouring only low risk types. Viral DNA is found in its episomal form and there is low expression of the viral oncoproteins E6 and E7. Up to 25% will progress to high grade lesions, with most regressing spontaneously.

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It is postulated that high grade squamous intraepithelial lesions may originate from mild dysplasia or may arise directly from infection by high risk HPV. High grade squamous intraepithelial lesions are usually monoclonal, and harbour high risk HPV genotypes in 90% of cases, with high expression of viral oncoproteins E6 and E7. Integration of the viral genome into the host genome, with subsequent disruption of the E2 reading frame and survival of epithelial cells containing this integrated viral DNA, is the postulated mechanism of development of high grade squamous intraepithelial lesions.

Abbreviations: CDC, Centres for Disease Control; CIN, cervical intraepithelial neoplasia; HIV, human immunodeficiency virus; HPV, human papillomavirus; IFN, interferon; IL, interleukin; LOH, loss of heterozygosity; MMR, mismatch repair; MSI, microsatellite instability; pRb, retinoblastoma protein; Th, T helper cell; TNF, tumour necrosis factor.
The subsequent disruption of cell cycle machinery has been reviewed extensively. E6 binds p53 with loss of tumour suppressor function and enhancement of telomerase activity. E7 binds the retinoblastoma protein (pRb), with untethering of E2F transcription factors. Cyclin D is made redundant, with increased expression of cyclin A, E, and B. p21 is also inhibited by E7. The high risk types of HPV have more efficient E6 and E7 molecules. Integration of the HPV genome denotes irreversibility, with between 33% and 50% eventually progressing to cervical cancer.

**HIV**

HIV is the causative agent of AIDS. It is a retrovirus belonging to the Lentivirus family and consists of a core surrounded by a lipid envelope. Within the viral core are the p24 protein, p7/p9 protein, two copies of genomic RNA, and the viral enzymes protease, reverse transcriptase, and integrase. The HIV 1 provirus encodes the three structural retrovirus proteins—gag, pol, and env—in addition to the six accessory proteins—tat, rev, vif, nef, vpr, and vpu. Currently, the HIV proteins of particular importance with regard to its speculated role in cervical carcinogenesis are tat, rev, and vpr. Tat and rev are two control mechanisms for viral gene expression. The tat protein enhances the transcription of viral genes, whereas rev acts post transcriptionally, shuttling viral mRNA from the nucleus to the cytoplasm. The vpr protein ensures the infection of macrophages and other non-dividing cells and causes G2 phase arrest, thus enhancing viral production.

**EPIDEMIOLOGY OF AIDS RELATED CERVICAL CANCER**

Women represent a growing proportion of the AIDS epidemic. With women being infected at a much higher rate than men, they now represent half of the infected population. Numerous studies have documented a high prevalence of HPV coinfection, with an increase in both latent and symptomatic HPV infection. Some have found this to be dependent on the severity of immune compromise, whereas others have noted HIV infection to be an independent risk factor.

Coinfection with HIV and HPV would be expected in view of similar risk profiles, including multiple sexual partners, early age of first coitus, sex with men who have had multiple partners, low socioeconomic status, and low usage rates of barrier contraceptives. However, an alternative explanation is mutually enhanced transmissibility.

Two large studies have also confirmed an increased incidence of squamous intraepithelial lesions, with HIV emerging as an accurate marker of squamous intraepithelial lesions, independent of the above mentioned risk factors. Both HIV seropositivity and HIV induced immune compromise have emerged as independent risk factors, with dysplasia being evident in patients with a median count of 450/ml, and severity correlating with the progression of immunocompromise. In the setting of HIV, these preinvasive cervical lesions are characterised by multifocality, rapid progression, and high recurrence rates despite treatment. The association between HPV type and degree of squamous intraepithelial lesion in HIV positive patients is still unclear.

Squamous carcinomas at numerous sites including the lung, anogenital region, oral cavity, epiglottis, and cervix have been documented in HIV infected persons. In 1993, the CDC declared invasive cervical cancer an AIDS defining illness in women infected with HIV. In the first year of the expanded definition, 1.3% of all women over 13 years of age with AIDS were reported to have invasive cervical cancer. However, the relation between HIV and cervical cancer is controversial. Data from the Sentinel Hospital surveillance system have noted the incidence of cervical cancer to be only modestly higher in HIV positive women. Rigorous screening programmes in the developed world could account for this. Studies conducted in developing countries have also failed to document an association between invasive cervical cancer and HIV 1 infection, despite the increased incidence of HPV infection and cervical dysplasia among HIV positive women. It was postulated that the progression from dysplasia to invasive cancer might exceed the mean survival time, especially in view of the burden of other opportunistic infections common in the third world. In support of this was the significantly increased rate of such cancers seen in patients infected with the HIV 2 virus—a virus that is less aggressive with a longer incubation period.

In epidemiological studies conducted in South Africa in 1997 and Rwanda in 1995, no excess risk for cervical cancer was seen in the setting of HIV infection. However, subsequent studies conducted last year in South Africa and Uganda did see a significant excess risk. Maiman et al, reviewing rates at an inner city Brooklyn Hospital between 1987 and 1995, found a high rate of HIV cervical cancers, accounting for 55% of the AIDS related malignancies. Women with AIDS related cervical cancer differed from women with other HIV related malignancies in three ways: they had less immune suppression, with CD4 counts more than double those of women with other malignancies; the diagnosis of cancer was more likely to precede the diagnosis of HIV (70% of cases); and the cause of death was more likely to be attributed to cancer than to opportunistic infections. Compared with the general population, these cancers are seen in a younger age group of women. In 1990, Maiman et al found a 19% prevalence of asymptomatic HIV infection in young patients with cervical cancer and an 11% rate of seropositivity in women attending a colposcopy clinic for the evaluation of abnormal pap smears. Interestingly, there was no difference in severity of neoplasia in asymptomatic patients with HIV and those with AIDS. Maiman suggests that latent HIV infection may explain the worse prognosis in younger patients with cervical cancer than older patients.

Thus, the statistical evidence is unclear and more intensive epidemiological studies need to be carried out. In conducting such a study, it must be remembered that these cancers can present in women whose HIV status has no other clinical manifestation. In addition, efficient screening programmes would slant the natural history of this disease.

**Effects of HIV on the natural history of HPV infections**

Adequate prevention and treatment strategies for these patients require knowledge of the natural history of HPV infection and CIN in the setting of HIV infection. The history of HPV infection in HIV positive patients is still uncertain. In immune competent individuals, most HPV infections are self limited, such that only 2–3% of patients develop dysplasia, despite the much greater prevalence of asymptomatic HPV infection. The greatest determinants of disease progression are the genotype of HPV, the viral load, and the persistence of infection. Prospective studies using the polymerase chain reaction for HPV have shown the incidence of HPV infection to be as high as 95% in HIV positive women, compared with 22% in HIV negative women. These infections are persistent and often involve multiple HPV genotypes. Up to 22% harbour high risk types of HPV, with the incidence of oncogenic types increasing with progressive immune suppression.

Of importance is whether there is any alteration in associations between specific types of HPV and the degree of CIN as seen in the general population. Although some have shown a similar association between high grade lesions in HIV positive patients and oncogenic types of HPV, there seems to be an increased incidence of high risk types in low grade CIN lesions.

Some have documented high grade lesions associated with both high and low risk HPV types, leading to speculation that HIV may increase the oncogenicity of the high risk types, and possibly the activity of low risk types also.

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Effects of HIV on the natural history of CIN

There is a 60% regression rate for low grade cervical lesions, and a 20% regression rate for high grade lesions. The progression form CIN to invasive cancer is thought to take about 10 to 20 years. In the setting of HIV infection, regression rates for low grade lesions decrease to 27% and the occurrence of cervical cancer in young patients infected with HIV suggests more rapid progression. These HIV related precursor lesions are also refractory to treatment and are often recurrent despite treatment, requiring closer monitoring and perhaps more aggressive intervention. Recurrence is independent of residual disease. Immunodeficiency and residual disease are the two most important indicators of recurrence, with mode of treatment not being important. Why these dysplasias are more aggressive is still uncertain, and hypotheses ascribe blame between viral interaction and alterations in the local immune responses.

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MOLECULAR PATHOGENESIS

Whereas the development of AIDS related malignancies such as Kaposi’s sarcoma and non-Hodgkin’s lymphoma are attributable to immune deficiency, the relation between HIV and cervical cancer is more complex. Some authors have commented on the lack of a significant difference regarding the severity of neoplasia in asymptomatic HIV positive women and those with AIDS.

Cervical cancer has long been associated with HPV infection. Undermining of the cell cycle checkpoints through the actions of the E6 and E7 proteins interacting with p53 and pRB, respectively, is well known. However, this alone is insufficient to cause cervical cancer, and other carcinogens and further genetic changes are required for disease progression.

Pathways of tumour progression

Two pathways of tumorigenesis have been postulated. The first is the loss of tumour suppressor genes, referred to as the loss of heterozygosity (LOH) pathway, and the other involves genetic instability at the microsatellite loci. This instability is believed to arise as a result of defects in the mismatch repair (MMR) genes, making them unable to repair slippage errors that occur during replication. However, loss of cell cycle checkpoints could also cause this instability. Lazo reviewed the studies conducted on this and states that of 344 cases, only 28 displayed microsatellite instability (MSI) (8%). Thus, in cervical cancer, the loss of tumour suppressor genes seems to be the usual pathway of progression. LOH has also consistently been detected at specific loci such as 3p14.1–p22, 4p16, 6p21, 3p25, and 17p13.3. This has led to rampant speculation as to candidate tumour suppressor genes. An increased rate of MSI has been described in several HPV related malignancies including Kaposi’s sarcoma, non-Hodgkin’s lymphoma, anal intraepithelial neoplasia, and HPV associated lung cancers. A significantly higher frequency of MSI has also been observed in HIV related CIN lesions, and these changes were found to be independent of CD4 counts. Similar HPV subtypes were found in both HIV positive and sporadic CIN lesions, and there was no difference in the frequency of high risk HPV positive and high risk HPV negative CIN lesions. Thus, these changes appear to be independent of HPV type and HIV induced immune suppression. In addition, the pathogenesis of the increasing incidence and more aggressive behaviour of cervical lesions in HIV positive patients seem to be mirrored by a different tumour pathway. There are several possible explanations. It has been suggested that HIV may target the MMR genes or other repair pathways. Alternatively, a viral protein may specifically bind to the DNA or cellular proteins, corrupting the replication process. However, the HIV genome has not been detected in cervical tumour samples and, in those studies where it has been detected in low copy numbers, it has been attributed to HIV in background inflammatory cells. The hypothesis that HIV might use a “hit and run” mechanism, modifying the cell without detection of the viral genome, should also be considered. Graham et al postulated that the pathway of tumour progression is determined by a specific cell cycle checkpoint aberration. Loss of control at the G1/S checkpoint allows the accumulation of numerous small genetic changes, leading to microsatellite instability, with unchecked progression through G2/M allowing loss of larger segments of DNA code, and thus causing LOH.

To account for this difference in both clinical and molecular behaviour, there are several schools of thought, each implicating different biological aspects. As mentioned, some favour HIV targeting of specific genes. Others have suggested local immune dysregulation involving both alteration in cervical cell profile and alteration of cytokine profiles. Aberration of systemic immunity has also been implicated, whereas some favour direct viral–viral interactions.

IMMUNE FACTORS

Local immune dysregulation

HPV does not disseminate and thus the local cervical immune response is a crucial factor in HPV replication. Components of the local immune system include the lymphocytes, both CD4 and CD8 cytotoxic cells, and the Langerhans antigen presenting cells. Spontaneous or treatment induced regression of cervical dysplasia is probably mediated by the cell mediated immune system, particularly T helper type 1 (Th1) lymphocytes and macrophages. Thus, it seems likely that suppression of the immune response would worsen the natural course. Both HIV and HPV affect the immune system.

Lymphocytes and cytokine production

CD4 : CD8 ratio

Cell mediated immunity is important in controlling HPV infection. CD8 cells are increased in CIN lesions. Bell et al documented a significantly greater number of lymphocytes in CIN lesions of HIV positive patients, none of whom had AIDS. Not only was there an increased number of lymphocytes, but these were within the epithelial squamous cells, as opposed to their normal subepithelial location. Despite increased numbers, there was an inverse CD4 : CD8 ratio, and it was hypothesised that the CD4 T cells may be ineffective in activating the recruited CD8 cytotoxic T cells. Olaïan et al analysed both systemic and cervical lymphocytes in HIV positive and negative controls, and found that the immunocompromised patients had decreased plasma and Langerhans cells in the cervix, but increased T cells. In addition, there was inversion of the CD4 : CD8 ratio independent of the systemic CD4 counts. Infiltrating CD4+ (T helper cells) and CD8+ (cytotoxic/suppressor T cells) are found in regressing warts. It is postulated that loss of the CD4+ T helper cells may mean that the local CD8 T cells are ineffective in bringing about regression.

Th cell profile

T helper cells can be classified as Th1 or Th2, a distinction based on the different cytokines that they secrete. Th1 cells secrete interleukin 2 (IL-2) and interferon γ (IFN-γ). Th2 cells secrete IL-4, IL-5, IL-10, and IL-13. Lymphocyte protection against viral (HPV) infection and tumours is mediated by Th1 cells and impaired by Th2 cells. Thus, Th1 cells represent the main component of cell mediated immunity against HPV infection and HPV associated neoplasms with impairment of this response by Th2 cells. IL-2 and IFN-γ are most likely to be important. IL-2 production has been shown to be decreased and the IL-4 and IL-10 response increased in proportion to the extent of HPV
infection. This Th2 profile has also been observed in Hodgkin’s disease, renal cell carcinoma, and gliomas, and it has been suggested that the Th2 profile is associated with the persistence of viral infection and the development of neoplasms. Similar observations were made in asymptomatic HIV positive women. It is yet to be determined whether the Th2–Th1 shift allows progression of the neoplasia, or whether it is induced secondarily by the tumour cells or the persistent viral infection. Evidence suggests that HPV infected keratinocytes can modify local cytokine profile. Concomitant HIV infection would thus worsen the immune profile.

Langerhans cells
Langerhans cells are the antigen presenting cells of the cervical squamous epithelium. Studies have shown their number and distribution to be of prognostic significance in lung, thyroid, gastric, and cervical cancers. These cells may be detected by a variety of methods, including thymocyte antigen (T6), adenosine triphosphatase (AT-Pase), major histocompatibility complex class II antigen, S-100 protein, and anti-CD1a. Furthermore, studies using different techniques have yielded contradictory findings of decreased, normal, and even increased numbers of Langerhans cells. Both HIV and HPV may affect these cells. A negative correlation was found between HPV replication and HPV E7 values and Langerhans cells. The S100 subset of Langerhans cells has been found to be decreased in CIN and HPV infection. In a comparative study between an HIV positive cohort and a matched control group, Spinillo found a significant reduction in Langerhans cell counts in CIN lesions in the HIV positive group. There was also an inverse correlation between HPV infection and the severity of CIN. Thus, impairment of local immunity may arise through a variety of interactions between HPV and HIV. HIV may directly deplete or affect the function of these cells. The reduction in numbers of Langerhans cells in CIN lesions devoid of HPV supports a direct role for HIV, and HIV is also known to target cells of the monocyte–macrophage lineage. HPV itself may depress the number of Langerhans cells, with HIV merely facilitating local cytokine profile. Concomitant HIV infection would thus worsen the immune profile.

Antibody production: local and systemic
Although HPV does not disseminate, and it is the local immune response that is of primary importance, current studies seem to implicate the systemic immune response. In a study by Maraís et al comparing two cohorts of sex workers, one being positive for HIV, the other negative, more HIV positive women were found to mount an anti-VLP-16 (virus-like particle) IgG and IgA response in the cervix. This increased production of antibodies could result from the systemic polyclonal B cell proliferation characteristic of HIV infection. Despite this increased antibody response, the HIV positive patients were still unable to clear the HPV infection. They postulate that the local HPV antibody response does not seem to be relevant to their increased susceptibility to HPV infection. The HIV negative women who were able to clear the HPV infection had higher serum concentrations of anti-VLP-16 IgA. Thus, it is the serum values that are important in preventing persistent infection.

VIRAL–VIRAL INTERACTIONS
Whereas some scientists favour the hypothesis of indirect immune based interactions between the viruses, others favour a theory of direct molecular interaction. This is a reciprocal interaction, with a mutual increase in transmissibility as a result of either local immune compromise or compromise of mucosal integrity and mutual alteration of gene expression. Interaction between herpes simplex virus and HIV has been demonstrated and is understandable because they both express tropism for the same cell types. However, HPV and HIV seem to infect different cell types. HIV is postulated to infect CD4 T cells, macrophages, and Langerhans cells, M cells, dendritic cells of the human genital tract, and colonic, vaginal, laryngeal, adrenal, and renal epithelial cells. HPV infects the squamous epithelial cells of the cervical mucosa. Some have postulated that HIV can infect cervical cancer cell lines lacking CD4 receptors; however, Nuovo et al found no HIV-1 DNA or RNA in squamous epithelium. The detection of HIV virus in the macrophages marks these as an important reservoir and a potential mechanism for HPV–HIV interaction. In addition soluble factors and viral proteins may interact.

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Tat and rev
It seems that the natural course of HPV infection is altered in the presence of HIV infection. HPV gene transcription is linked to keratinocyte differentiation. Early gene products are detected in all cell layers, but late factors, L1 and L2 capsid proteins, and infective viruses are exclusive to the terminally differentiated superficial keratinocytes. Late transcripts, although present in the less differentiated cells, are prevented from leaving the nucleus owing to blockage of RNA processing by the AU rich, cis acting inhibitory sequence. The activation of both early and late HPV genes that has been observed experimentally may account for the increased virulence of HPV in the milieu of HIV infection. Such interaction is feasible because some hosting of HIV replication has been observed in epithelial cells, both in vivo and in vitro. HIV products such as tat and cytokines may be accountable. Two mechanisms control HIV expression. Tat/tar (tat activation response) involves tat activated transcription of viral and cellular genes by interacting with tar RNA elements and cellular proteins. In vivo, tat can be released into body fluids and can bind to integrin receptors on uninfected cells and activate cellular promoters. Rev/rev (rev responsive elements) acts post transcriptionally on mRNAs, preventing nuclear degradation of mRNA and transporting them into the cytoplasm. HIV also causes the release of several cytokines, including IL-6 and tumour necrosis factor (TNF). In patients with HPV, the HPV infected epithelial cells are exposed to HIV virions and soluble factors. Dolei et al reported a dose dependent increased expression of E1 and L1 genes on exposure to tat protein. Vernon et al showed increased E2 dependent HPV-16 transcription; others have shown long controlled region transactivation and increased HPV-18 E7 expression.

It has been shown in vitro that rev expression in epithelial cells allows expression of L1 protein in undifferentiated basal keratinocytes, bypassing the effect of AU rich negative element in HPV mRNAs.

Viral protein R
Viral protein R (vpr) is a 96 amino acid structure. It facilitates the HIV infection of macrophages, which are important reservoirs and possible sources of soluble viral factors and proteins involved in viral interaction. Vpr disrupts the G2/M checkpoint with induction of apoptosis. This effect is potentiated by HPV E6. The results from a study by Toy et al suggest that Vpr might be useful as a cytostatic agent in treatment. However, their experiment was conducted in isolation and more studies involving all cell factors will have to be performed. The delineation of cell cycle checkpoint abberation is also intriguing because it might be the basis for the different tumorigenic pathways.
HIV and uterine cervical cancer

OTHER FACTORS

Adhesion molecules
Impairment of catheter and CD44 mediated adhesion has been noted in invasive and metastatic cervical cancers, and could account for the advanced stage of most HIV related cervical cancers at the time of diagnosis. However, no difference in CD44 and E catheter expression was noted between HIV positive and negative patients with cervical cancer.17

Effects of HPV infection on HIV gene expression
Not only may HPV increase susceptibility to HIV infection, but progression of HIV infection is facilitated by concomitant HPV infection. Luque et al correlated active HPV infection with high HIV plasma RNA values.18 Cell culture studies have shown increased positive regulation of HIV expression in HPV infected cells.19 Possible mediators of this enhanced expression are the HPV induced inflammatory cytokines, particularly IL-6 and TNF. IL-6 may induce HIV p24 expression in monocytes by binding to the CAAT/enhancer binding protein B, with subsequent activation of a cascade of acute phase reactants and cytokines. These can either act individually or in collusion, with IL-6 and IL-10 potentiating TNF, and minimal doses of IL-6 being aided by other chemical mediators (IL-10), or even HPV gene products.

Cytology and screening in HIV positive patients
The CDC advocate cytological screening for cervical disease in HIV positive women. The current recommendation is that two Papanicolaou smears should be performed within the first 12 months of the diagnosis of HIV. If these are normal, then yearly follow up is adequate. However, any cytological abnormality in an HIV positive patient warrants colposcopy.

CONCLUSION

The morphological progression of cervical cancer provides an ideal opportunity for the study of carcinogenesis. Although profound insights into its molecular pathogenesis have been achieved, much still needs to be defined. The relation between HIV and cervical cancer, and between HIV and HPV, is complex and requires observation from many perspectives. Although at times this may seem bewildering, further study could greatly advance our understanding of viral cancers.

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