Immune response in cervical dysplasia induced by human papillomavirus: the influence of human immunodeficiency virus-1 co-infection - Review

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Human immunodeficiency virus (HIV-1) has become an important risk factor for human papillomavirus (HPV) infection and the development of HPV associated lesions in the female genital tract. HIV-1 may also increase the oncogenicity of high risk HPV types and the activation of low risk types. The Center for Disease Control and Prevention declared invasive cervical cancer an acquired immunodeficience virus (AIDS) defining illness in HIV positive women. Furthermore, cervical cancer happens to be the second most common female cancer worldwide. The host's local immune response plays a critical factor in controlling these conditions, as well as in changes in the number of professional antigen-presenting cells, cytokine, and MHC molecules expression. Also, the production of cytokines may determine which arm of the immune response will be stimulated and may influence the magnitude of immune protection. Although there are many studies describing the inflammatory response in HPV infection, few data are available to demonstrate the influence of the HIV infection and several questions regarding the cervical immune response are still unknown. In this review we present a brief account of the current understanding of HIV/HPV co-infection, emphasizing cervical immune response.

Key words: human papillomavirus - human immunodeficiency virus-1 - cervical intraepithelial neoplasia - cervical immunity response - women

All papillomaviruses have a nonenveloped icosahedral capsid that is 55 nm in diameter and that contains a double-stranded circular DNA genome of about 7900 nucleotide pairs (Howley 1995, Shah & Howley 1995) that codes for at least eight early and two late (capsid) proteins. The expression of viral genes is modulated by an 800-base pair long-control region (LCR) that is epithelial tissue specific and regulated by physiological signals. The products of the early genes E6 and E7 are oncoproteins that destabilize the cellular tumor supressors p53 and pRB (Dyson et al. 1989, Hawley-Nelson et al. 1989).

Human papillomavirus (HPV) is currently detected in a large number of mammals and have been shown highly species-specific. HPV subtypes are defined by DNA sequence analysis and represent genotypes. There are over 100 different types of virus; approximately 130 additional isolates represent only partially characterized putative novel genotypes (de Villiers 1997, zur Hausen 1999). Among them, more than 20 types can infect the genital epithelia. In order to be considered a new HPV type, the E6, E7 and L1 gene sequence in the HPV genome has to differ by more than 10% from those of any previously described HPV types. The viral E6 and E7 oncoproteins are essential components in malignant conversion, although, in spite of being necessary, they are not sufficient for the development of the malignant phenotype (revised by zur Hausen 1999).

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The viral particles in these lesions have been identified by electron microscopy even before the association between the virus infection and the outcome of the disease (Dunn 1968, Oriel & Almeida 1970 revised by Koss 1987). Nowadays, with the advances in molecular biology, the identification of HPV DNA has been attempeted in several cancer like lesions, such as oral and esophageal carcinoma (Giovannelli et al. 2002, Si et al. 2003). The first report to describe the associations of HPV infection and the cervical cancer was published in the late 1970's by Zur Hausen et al. (1975) and Zur Hausen (1976); afterwards Syrjanen (1979) showed the HPV-associated lesions with cervical cancer in the female genital tract. Cervical carcinoma is a major worldwide public health problem, with 500,000 new cases reported annually. It is the most common female malignancy in less developed countries (revised by Waggoner 2003). In Brazil, according Ministry of Health, it is estimated 17,600 cases of cervical cancer and 4005 causes of death in 2002.

The HPV infection of the genital tract may be asymptomatic or may be manifested as a range of genital lesions, from genital warts to mildly dysplastic lesions to invasive carcinomas. Some observers proposed the term cervical intraepithelial abnormalities or CIN (revised by Koss 1987). CIN is graded from I to III depending on the degree of epithelium abnormality, (described as the presence of atypia along the basal layers, a disorderly pattern of maturation, and abnormal mitotic figures). According to the Bethesda classification system, the term low grade squamous intraepithelial lesion (LSIL) encompasses mild dysplasia/cervical intraepithelial neoplasia (CIN I) and koilocytic change induced by HPV. High grade squamous intraepithelial lesions (HSIL) include moderate dysplasia/CIN II and severe dysplasia (CIN III). Genital HPV DNA

has been consistently detected in invasive cervical carcinoma. Some genital HPV are categorized as high-risk type, frequently associated with invasive cervical carcinoma, different from low-risk type associated with benign lesion. Indeed, HPV types 6, 11, and 42 are mainly associated with benign genital warts and low-grade CIN and specific types, most notable 16, 18 and less frequently 31, 33, and 35, have been identified as an infectious agent to cause the majority of cervical cancers and their high-grade precursor lesions (Boshart et al. 1984, Beaudenon et al. 1986, zur Hausen 1999).

However, the relationship between HPV genotypes and the different grades of CIN has just recently been reported by Matsukura and Sugase (2001), where 38 skin and 42 genital genotypes were tested in 386 unfixed cervical biopsies. HPV's 40, 42, 43, 54, 62 or 71 were found in 10 CIN I, while HPVs 18, 30, 39, 51, 56, 59, 66, 68, 69, or 82 was found in 35 CIN I, 20 CIN II or 8 CIN III. HPVs 16, 31, 33, 35, 52, 58 or 67 was identified in 43 CIN I, 74 CIN II and 164 CIN III. These results indicated that most genital HPV have the potency to induce CIN I and a close relationship of HPV 16 and induction of CIN III, followed by HPV 58, 52 and 31. No skin HPV was identified in the cervical lesions. Interestly, the genital HPV 18 high-grade genotype was present in both low and high-grade CIN (2 CIN I, 2 CIN II and 1 CIN III). The detection frequencies of HPVs in CIN may highly reflect the potencies of an individual HPV to induce different grades of CIN. CIN can naturally regress, persist, or progress and do not naturally develop in a stepwise fashion through increasing grades of CIN (Wright et al. 1994).

The American Cancer Society now recommends that HPV screening be initiated within 3 years of the onset of vaginal intercourse but no later than 21 years of age. Natural history studies of HPV suggesting that there is little risk of a significant precancerous lesion going undetected within the first 3 to 5 years after the onset of sexual activity (Moscicki 2003).

Cervical HPV diagnosis is mostly performed by Papanicolaou-stained smears (Pap-test) which is a cytological screening that detects changes in cellular morphology in cervical cancer screening programme. Although there are several technical limitations, such as low sensitivity, diagnostic errors, and inter-screener variations. Therefore, the confirmatory diagnosis of HPV is done only by molecular hybridization methods, of which the polymerase chain reaction (PCR) is the most sensitive (Das et al. 2000). A recente study (Nonogaki et al. 2004) comparing the performance of HPV DNA detection by PCR and Hybrid Capture II (HCII) found that both tests yielded concordant results in 76.5%. New techniques in primary cervical cancer screening programs have been evaluated. Nieminen et al. (2004) compared the validity of the high risk HPV DNA detection by HCII and conventional Pap smear screening, and found that Pap smear, as a screening test is clearly more specific than HCII, but markedly less sensitive. Due to high relative sensitivity of the HPV, only very few histologically confirmed high grade lesions would be detected among HPV negatives using simultaneous cytology. Interestingly, previous reports, using single detection of high risk types of HPV was more sensitive and less specific than cytologic screening for the identification of subsequent CIN III diagnosis. However, the combination of both techniques did not significantly improve the performance of HPV test (revised by Villa et al. 2002).

The overall prevalence of HPV 16 in invasive cervical cancer, based on the MY09/11 PCR assay, which targets a 450 base pair (bp) fragment within the HPV L1, is around 50%. In 7% of these tests in cervical carcinomas there been a failure of HPV DNA detection due to either the absence of HPV DNA or false-negative HPV results (revised by Bosch et al. 1995). Walboomers and Meijer (1997) suggested that this methodology would lead to the identification of HPV DNA in virtually 100% of cervical carcinomas. In fact further analysis of the tumours, originally thought to be HPV-negative, identified HPV DNA in a prevalence of 99.6% (Walboomers et al. 1999).

This could be the major concern about the HPV identification. Around 10% of these HPV infections lead to warts, papillomas or dysplasia; but the majority is not associated to clinical consequences. About 11% of women with evidence of HPV infection as the only abnormality of cervical cytology will already have CIN by colposcopic biopsy; furthermore 33% will develop CIN, in average, 10 months after the initial screening. In the general population, only 2-3% of women will develop dysplasia, despite a high rate of HPV infection (Ho et al. 1998). Some data are now emerging to suggest that HPV 16 behave differently from all other oncogenic and non oncogenic HPV types. Data from one longitudinal study suggested that over a period of 5 years, the absolute risk of progression of HPV 16 infection to high-grade disease and cancer is very high. Suggesting that HPV 16 has the ability to evade the immune system even in the immune competent individuals (revised by Villa et al. 2002).

HIV/HPV co-infection - Human immunodeficience virus (HIV) and HPV are both sexually transmitted diseases (STDs). Prevalence of HPV infection among HIV-1 seropositive women is so great due to high epidemiological risk factors such as early age sexual intercourse, multiple partners and presence of other STD. Several studies showed high levels (2 to 3 times more) of HPV DNA in cervicovaginal washings and in anal swabs (15 times more) in HIV-1 seropositive women than in seronegative women (Hillemanns et al. 1996, Chiasson et al. 1997, Sun et al. 1997). Furthermore, HIV-1 infection has become an important risk factor for HPV infection and the development of HPV-associated lesions in the female genital tract; in fact, HIV-seropositive women are about five times as likely as HIV seronegative women to have CIN (Laga et al. 1992, Ruche et al. 1998, Ellerbrock et al. 2000). There is a clear association between HIV disease and development of CIN. In 1993b, the CDC declared invasive cervical cancer as an acquirede immunodeficience syndrome (AIDS) defined illness in women infected with HIV; moreover, many authors have reported a high prevalence and severity of genital tract infection in HIV-1 positive woman (Iman et al. 1990, Hankins et al. 1992, Hocke et al. 1994, Duerr et al. 1997, Minkoff et al. 1999).

Current studies have been correlating the plasma level of HIV-1 as a predictor for HIV as well as HPV infection (Mellors et al. 1996, Luque et al. 1999, Davis et al. 2001). Although high levels of HIV-1 RNA were found in patients plasma associated with cervical infection by onco-

genic HPVs (Luque et al. 1999), most HPV infections are self limited in immune competent individuals, such that only 2-3% of the patients develop dysplasia, despite a much greater prevalence of asymptomatic HPV infection. The determining factors of disease progression are mainly the HPV genotype, the viral load, and the persistence of infection (revised by Clarke & Chetty 2002). Some studies have shown high grade lesions associated with both high and low risk HPV types, suggesting that HIV may increase the oncogenicity of the high risk types, as well as activation in low risk types (Tweddel 1994, revised by Clark & Chetty 2002). One study, investigating the impact of HIV infection on prevalence, incidence, and short-term prognosis of cervical intraepithelial lesions, found that HIV was associated with high prevalence and persistence/progression of CIN (Six et al. 1998). However, other epidemiological studies in South Africa in 1997 and Rwanda in 1995 could not find excess risk for cervical cancer in the setting of HIV infection (revised by Clarke & Chetty 2002, Sitas et al. 1997, 2000). Studies among HIV-positive and HIV-negative groups indicated that the relative increase in HPV 16 prevalence in HIV-positive compared with HIV-negative women is much smaller than that for other types and indirectly indicates that HPV 16 has the ability to evade the immune system (revised by Villa et al. 2002).

The natural history of HPV infection and the association of HIV-infection were reported recently in a longitudinal study using adolescent girls. The authors claimed that when type-specific loss of HPV was examined, HIV-uninfected girls had a shorter mean time to loss infection than did HIV-infected girls and both CD4 immunossuppression and the presence of mutiple HPV-types were found to be associated with persistence of HPV (Moscicki et al. 2004)

The host cervical immune response - Cell-mediated immune response plays an important role in controlling HPV-associated neoplasias. HPV-associated lesions are usually transient, and presumably regress as a result of a cellular immune response (Coleman et al. 1994). HPV undergoes a period of clinical latency but frequently reappears, especially in HIV seropositive women (Maiman et al. 1993, Fruchter et al. 1996). Furthermore premalignant lesions can regress spontaneously, and if progression to cervical tumors occurs, a cellular infiltrate is seen consisting of $CD4^+$ and $CD8^+$ T cells, monocytes, macrophages, and granulocytes (Hilders et al. 1993). There is clear evidence that the HPV/HIV co-infection may influence the progression of AIDS by two potential mechanisms: (1) by recruitment of HIV target cells, such as $CD4^+$ T-cells and macrophages, into the site of active HPV infection, and (2) by inducing the production of inflammatory cytokines, including IL-6, TNF-α and IL-1β. These inflammatory cytokines have shown to induce the replication and reverse transcription of HIV (Poli et al. 1990, Gage et al. 2000).

An eosinophilic infiltrate and an increase in IL-4 expression in cervical squamous carcinoma, reflecting an imbalance of type 1 and type 2 responses are also showed in HPV infected women (Driel et al. 1999).

The mechanisms whereby HPV infected cells escape immune surveillance, ultimately leading to an invasive cervical carcinoma, may involve changes in local cytokine production and/or loss of responsiveness to cytokines. Local production of cytokines may determine which arm of the immune response is stimulated and may influence the magnitude of immune protection. It is unknown, in the female genital tract, whether co-infection with HIV affects the synthesis of type 1 and type 2 cytokines, in women infected with HPV, to the extent that it contributes to the development of cervical intraepithelial lesions.

De Gruijl et al. (1999) studying the expression of cytokine mRNA transcripts at the site of HPV infection in relation to development of cervical neoplasia found a reduced type 1 immunity correlating with HPV-induced invasive cervical carcinoma. In addition several studies have shown qualitative and quantitative differences in host inflammatory response both in epithelium and in stromal tissue in HPV infected women (Tay et al. 1987, Viac et al. 1990, Coleman et al. 1994, Davidson et al. 1997), but few studies have been addressed to demonstrate this difference in HIV and HPV co-infected patients. Thus, many cells and immune mechanisms are involved in the HPV/HIV-1 co-infection, as will be discussed

Dendritic cells - Dendritic cells (DC) play a role in immune surveillance, their low densities might be a key feature responsible for low local cytokine production (Arrese et al. 2001). Also, Langerhans cells (LC) are specialized cells in epithelial tissues that show dendritic morphology. These DC, residing in the epithelial tissues of various mucosae and the skin, are characterized by the unique ability to capture antigens and migrate to draining lymph nodes, where they can activate native and memory T cells. These cells reside in pluristratified epithelia of the cervix and are referred to as "immature DC" which correspond to prototypical epidermal LC (Kaiserlian & Dubois 2001). Although DC play a critical role in inducing protective immunity to viral infection, they can also be exploited by viruses to evade the host immune response, induce immune suppression or serve as latent viral reservoirs. It is known that HIV-1 infected patients with CIN lesions have a decreased number of these cells. This finishing fact is supported by many studies, where a reduction of DC in HPV infected patients or Langerhans cells in infections caused by a contagious mollusk and HPV were identified, suggesting that the depletion of these cells could be a result of the citotoxic effect of the virus, or it could occur by migration of epidermal LC from the epithelium to the regional lymph node (Morris et al. 1983, Tay et al. 1987, Drijkoningen et al. 1988). There is evidence that could explain this DC absence, one possibility is that T lymphocytes' cytotoxicity is mediated by HIV-infected DC which effectively stimulate a primary T cell response, then become targets of the cytotoxic T lymphocytes (CTL) (Racz et al. 1989); furthermore, it was also observed that the destruction of follicular DC happens in association with the high viral load on the surface of the DC. This fact could be observed in patients that underwent antiretroviral treatment, which showed rebuilding of follicular DC associated to the viral load decrease (Zhang et al. 1999). Interestingly, Nicol et al. (1997) also observed that in lymph node tuberculosis from HIV seroposive patients, there was a decreased number of DC, denoting alterations in the granuloma formatin. An increased infiltration of tu-

mors by DC has been shown to be correlated with better prognosis (Tazi et al. 1993). In the uterine cervix, most SIL are characterized by a lower density of LC compared with the normal exocervical epithelium; this fact was noted by Mota et al. (1999), who observed a decreased number of LC (marked by CD1a) with increasing severity of neoplasia. Interestingly, our previous study also showed a decreased number of DC, marked by RFD1 in the uterine cervix (Nicol et al. 1999). These observations suggest that a quantitative and/or qualitative disturbance of LC could potentially interfere with the immune surveillance of HPV-associated cervical (pre) neoplastic lesions (revised by Hubert 2001).

$CD4^+$ T cells - The human female tract contains all essential elements for mounting an effective immune response against genital pathogens. Givan et al. (1997) estimated that leukocytes represent 6-20% of the total number of cells in fallopian tubes, endometrium, cervix, and vaginal mucosa. T cells acounted for about 50% of all leucocytes, with $CD8^+$ cells predominating over $CD4^+$ cells. Johansson et al. (1999) confirmed this observation, in addition they showed a characteristic histological distribution in the vaginal and ecto-cervical epithelium and submucosa of antigen-presenting cells, T cells and plasma cells. They found that T cells, both $CD4^+$ and $CD8^+$, were concentrated in a band directly beneath the epithelium in both ecto-cervix and vagina.

Immunohistological studies indicate clearly that regression of both animal and human papillomavirus infected lesions is associated with a type 1 response but the viral antigens which provoke this response are not known. From that understanding, one might be able to design immunotherapies based on T-cell intervention at one stage or another of the disease process. Evidence for incresead presence in T-cell- immunossupressed patients strongly suggests that $CD4^+$ and/or $CD8^+$ T cell responses play a vital role in controlling infection with HPV (Benton et al. 1992). This is supported by histological evidence of T-cell infiltration in both cutaneous (Thivolet et al. 1982, Iwatsuki et al. 1986) and mucosal (Coleman & Stanley 1994) lesions during the spontaneous regression of papillomas, but the nature of these immune responses and the mechanism of their initiation are not fully understood.

$CD4^+$ T cell response is regulated by major histocompatibility complex (MHC) class II molecules, which are also expressed on professional antigen presenting cells (APC). Several authors (Benton et al. 1992, Al-Saleh et al. 1998), suggested that the $CD4^+$ T cells are involved in the prevention or limitation of lesions associated with HPV from observing the frequency of those lesions in cervical neoplasias in patients with AIDS. In the same way, significant number of positive cells were observed in the uterine cervix of HIV and HPV co-infected woman (Nicol et al. 2002). However, controversial results can be seen in the peripheral blood, where there is a serial depletion of total $CD4^+$ T cells in the HIV-infection. This selective depletion could be caused directly by HIV infection or by immunological mechanisms. These aspects can be used for AIDS prognosis, although before the cell depletion, there is a functional deficiency with low expression of IL-2, IFN-γ and IL-4 after to the stimulation by antigens and mitogens, as a result, the T helper function can be compromised (Manetti et al. 1996).

$CD\,8^+$ T cells - HPV may evade host immune surveillance in a cervical microenviroment of type 2 cytokines (Arany & Tyring 1996, Al-Saleh et al. 1998) that are capable of down-regulating the expression of MHC-1 antigens and β_2-microglobulin (Torres et al. 1993, Cromme et al. 1993) and diminishing the function of intraepithelial antigen-presenting cells (Rosini et al. 1996). While HIV-specific peripheral CTL in HIV-infected patients may be functional, high levels of type 2 cytokines in cervicovaginal secretions (Belec et al. 1995) can down-regulate the activity of cervical CTL (Olaitan et al. 1996) and lead to persistent HPV infection (Schafer et al. 1991, Garzetti et al. 1995).

Since HIV-specific perpheral and cervical CTL of HIV-infected patients recognize the same epitopes (Musey et al. 1997), it is reasonable to suggest that cervical CTL are recruited from the peripheral circulation (Arany & Tyring 1996, Al-Saleh et al. 1998). Hence, one might hypothesize that the competence of the peripheral blood T cell is a reliable measure of the type and quality of the immune responses that are present at the cervical lesion. What is unclear from these studies is whether co-infection with HIV affects the synthesis of type 1 and type 2 cytokines in HPV infected women to the extend that it contributes to the development of cervical squamous intraepithelial lesions. Lee et al. (1999) showed that HIV infection adversely affects the synthesis oh Th1 cytokines by $CD4^+$, but not IFN-γ synthesis by $CD8^+$ T cells of women with active HPV infection. The increase in IFN-γ^+ $CD8^+$ T cells of women is less likely to be HPV-specific as there was a higher incidence oh HPV-related cervical SIL in HIV+/HPV+ women compared with HPV women.

$CD8^+$ T cells play an important role in the riddance of virally infected cells (Zinkernagel 1996), which are often referred to as CTL regardless of their function. There has been an increasing interest in $CD8^+$ T cells (CTL) as vehicles for immunotherapy in human cancers, using either vaccines capable of inducing CTL or adoptive therapy protocols. Some authors studying the immunocompetent cells in cervical HPV associated lesions, described a depletion of intraepithelial lymphocytes, specially $CD8^+$ T cells, in both HPV infection and CIN, suggesting that there is a local intraepithelial immune deficiency associated with HPV infection and the resulting CIN (Tay et al. 1987). However, Olaitan et al. (1996) observed the distribution of immunocompetent cells in the female lower genital tract of HIV-positive women, and they found an increased number of $CD8^+$ T cells, but with reduced cytolytic ability which was showed by the absence of perforin and reduction in TIA-1 expression. In the same way, Appay et al. (2000) showed that HIV-specific $CD8^+$ T cells are functional with regard to antiviral cytokine production (IFN-γ and MIP-1α) in the asymptomatic phase of HIV infection, and therefore could contribute to the control of HIV replication to a certain extent. However, the defect in cytolytic function of these T cells, which are apparently unable to mature into genuine cytotoxic effector cells, could render them unable to eliminate the virus. In fact, our current study also verified a great number of $CD8^+$ T cells in the HIV/HPV co-infected patients compared with only HPV women. These cells migrated through the stroma

to the epithelium, where HPV infection takes place (Nicol et al. 2002).

Kobayashi et al. (2002), studying the organization of inflammatory cells in normal cervix or, during high grade CIN from both HIV seropositive and seronegative women, found a novel type of lymphoid aggregation consisting predominantly of $CD8^+$ T cells. This lymphoid aggregation was seen predominantly in HIV seropositive high-grade CIN and was associated with the worst clinical outcome in these patients. In addition, other studies found differences in the numbers of T cells in the areas of cervix, vagina, and vulva. The transformation zone of the cervix showed the greatest number, suggesting that this area is unique for enhanced immunological activity in terms of antigen uptake, lymphocyte recruitment and differentiation (Edwards & Morris 1985).

A dysfunction of the T-cell response thus may facilitate the progression of HPV-associated cancers. In fact, several studies have demonstrated that there is little or no CTL response to the E7 gene product of HPV-16 and a $CD4^+$ T cell response to HPV-associated proteins can be readily detected in many of them (Stern 1996). The mechanisms for the T-cell unresponsiveness are unclear.

Although there is ample evidence that a CTL response can be elicited against transforming gene products in both animal and human cancer (Chen et al. 1991, Evans et al. 1996), it is unclear whether such a response exists during the early stages of tumorigenesis. Melero et al. (1997) demonstrated that mice transgenic (Tg) for the E6 and E7 oncogenes of HPV-16 do not mount any detectable CTL response against E7, although the expression of the transforming genes in Tg mice results in a progressive phenotype. Futhermore, the Tg mice did not mount any detectable immune response to the HPV-16-encoded antigen in their skin. Their results suggest that the E6 and E7 oncoproteins are ignored by the immune system at a point of time when they are inducing a premalignant state.

The low immunogenecity of tumor cells has been regarded as one mechanism which enables tumor cells to escape host immune survaillance. Although tumor cells may express tumor-associated antigens, they fail to induce an efficient immune response. This may be due to a lack of co-estimulatory molecules such as CD80. It has been shown that transfection of tumor cells with CD80 can lead to the induction of strong anti-tumor T-cell responses in animals (Chen et al. 1992, Townsend & Allison 1993). Kaufmann et al. (1996) have extented these findings to human tumors, showing that cervical carcinomas cells become strong stimulators for allogeneic T lymphocytes when transfected with CD80. They have shown that the activated T cells can lyse the CD80-tranfected as well as the parental tumor cells. This provides a basis for the development of a therapeutic vaccine for patients with cervical cancer.

HPV oncogenicity and cytotoxic mechanisms - The oncogenic E6 and E7 effects are mediated by the binding of E6 and E7 proteins to the products of the tumor suppressor genes p53 and retinoblatoma (rb), respectively. Once formed, the E6-p53 and E7-pRB protein complexes are translocated into endosplasmatic reticulum. There they may bind MHC class I, prior to being transported to the cell surface (Nijenhuis et al. 1996, Yang et al. 1996, Ressing et al. 1996, Tanaka et al. 1997). CTL recognize these viral peptides when they are bound to the surface of MHC class I molecules. As a result of the association of HPV with cervical carcinoma and the intracellular processing and presentation of viral peptides to the cell surface, E6 and E7 related peptides represent unique tumor antigens and so are attractive antigenic targets for specific cervical cancer immunotherapy.

The E5 protein is traditionally known to interact with the *trans*-membrane domain of the EGF receptor and to modulate its concentration and phosphorylation. E5 mutants were found to continue to up-regulate the EGF receptor and the cloned E5 gene of HPV-16, as that of BPV-1 and 4, reduces the expression of MHC-I, thereby contributing to the poor immune response to papillomavirus lesions (revised by Villa et al. 2002).

The CTL granules and their constituent proteins are synthesized 24 to 48 h after stimulation via T cell receptor; perforin, granzymes, and other constituent granule proteins are targeted to cytotoxic granules (Griffiths 1997). After the activated CTL regonizes these peptides, a tight junction is formed between the effector and target cells and the CTL granules vectorially stream towards the site of contact (Yannelli et al. 1986). At this site, the granules are thought to fuse with the effector cell plasma membrane, and the granule contents are exposed directly to the target cell membrane. After entering the target cell, the granzymes are thought to pass into the cytoplasm of the cell, where they may act on specific substrates involved with the ultimate death of the cell, and/or they are transported to the nucleus, where they may directly cleave and induce death.

Another mechanism of citotoxicity is the interaction between the receptor Fas ligand of the cytotoxic T cells and the receptor Fas of the target cells, that prompt apoptosis of these cells (Smyth & Sedgwick 1998). The Fas system employs the Fas receptor (a member of the TNF family of death receptors) and Fas ligand, a membrane-associated ligand which is synthesized within several hours after T cell receptor stimulation. Display of the Fas ligand on the T cell surface makes it possible for the Fas ligand to interact with Fas receptors on the surface of target cells, thus engagement of the Fas receptor results in the aggregation of its intracelluar death domains, leading to the recruitment of Fas-associated death domain and procaspase-8 to form a death-inducing signaling complex. It is observed in the peripheral deletion of mature T cells, in the death of infected or cancerous cells by cytotoxic T lymphocytes and in inflammatory cells (Ashkenazi & Dixit 1998). In the immune system the Fas receptor is constitutively expressed in the thymus; so regarding the Fas-L, its expression can be prompted in $CD8^+$ and $CD4^+$ T cells (Th0 and Th1), although in pathologic situations Fas-L expression can be induced by other cells. In the attempt of escape from the immune system, tumour cells present in the uterine cervix express Fas-L, thus not leading to Fas-mediated apoptosis (O'Connell et al. 1996).

Vascular adhesion molecules - In order for leukocytes to enter the stroma and epithelium of tissues such as the cervix, they first have to leave the blood. This process involves a number of stages, including margination to the

periphery of the blood vessels, adhesion to the endothelial cell lining, migration through the vessel wall, and movement within extra vascular structures to the point at which leukocyte activity is required (Butcher 1990). So the regression, persistence and evolution of HPV associated lesions in the uterine cervix are directly dependent to host immunity. Cervical intraepithelial neoplasia is associated with changes in the local immune cell populations, although the functional role of vascular adhesion molecules in mediating such changes is not well known, particularly that of adhesion molecules controlling the traffic of mononuclear cells to the cervix.

Some authors (Coleman et al. 1994) analyzing the role expression of ICAM-1 (intercellular adhesion molecule – CD54), VCAM-1 (vascular adhesion cellular molecule – CD 106) and E-Selectin (CD62E) in the uterine cervix of healthy women and those with different grades of CIN, did not observe any difference in normal cervix and CIN-I (low grade of neoplasia), however all three molecules investigated were significantly up-regulated in CIN II/III (high grade), suggesting that the enhanced expression of these molecules in high grade CIN appears to be functionally important in enabling the local recruitment of immunocompetent cells. In the same way a molecule known as ICAM-1 has also been identified on the surface of keratinocytes in dermatoses with T cell infiltration (Singer et al. 1989). This molecule plays an important role in leukocyte adhesion by acting as a ligand for the lymphocyte function associated antigen LFA-1, expressed by leukocytes.

Cytokines - Dissemination and progressive growth of HPV induced lesions, may be, at least, partially related to escape from local cytokine mediated surveillance. It's well known that type 1 cytokines, IL-2 and IFN-γ, enhance cellular immunity and stimulate humoral immunity. Patients with HPV-associated neoplasms with a type 1 cytokine profile have shown a better clinical outcome compared with others exhibiting a type 2 cytokine profile. Nevertheless, type 2 cytokines are more predominant, either locally or in the peripheral circulation, which may promote the development of cervical SIL and neoplastic change.

Recent studies found that HIV/HPV co-infection predicted the highest IL-10 concentrations and co-infection with HIV, HPV and other STD predicted the highest IL-12 concentrations. The data also suggested that concomitant infection of the genital tract with HIV and other STD might influence the local concentrations of some immunoregulatory cytokines (Crowley-Nowick et al. 2000).

Several cytokines are responsible for cellular activation and consequently increase of HIV-1 viral replication. However there is disagreement about the role of some cytokines known as regulatory such as type 1 (IL-2, IFN-γ) or type 2 (IL-4, IL-10, IL-6) as prognostic to AIDS evolution during HIV-1 infection (Manetti et al. 1996). It is known that HPV infected patients had significantly increased expression of IL-6 and TNF-α. In fact, IL-6 has been shown to play an important role in the immunopathology of several diseases. Many authors have also found elevated levels of plasma IL-6 and IL-6 production in HIV-infected donors (Yarchoan et al.1986, Martinez-Maza et al. 1987, Breen et al. 1990, Poli et al. 1990). In vitro studies also suggest that IL-6 may contribute to elevated HIV burden and to immunological abnormalities in HIV-infected patients (Breen et al. 1990, Poli et al. 1990), although another study showed in vivo that effects of endogenous IL-6, on both viral and immunological parameters in HIV-infected patients in late stage infection, may not stimulate HIV replication but may represents a key mechanism contributing to the metabolic and immunological imbalance of the disease in vitro experiments (Yarchoan et al. 1986, Martinez-Maza et al. 1987, Breen et al. 1990, Poli et al. 1990, Marfaing-Koka et al. 1996), which show that IL-6 increases HIV replication.

Recently our group showed in vitro that epithelial cells and keratinocytes containing HPV were clearly more potent positive regulators for HIV activation than HPV negative cells in human macrophages. This up-regulation is due mainly to induction of pro-inflammatory cytokines, such as IL-6 and TNF-α (Gage et al. 2000). Cell lines of normal keratinocytes were transfected and immortalized with HPV. Then, the ability of these cells to induce HIV replication was tested determining the HIV p24 levels in the supernatants and in mononuclear cell lines which were latently infected with HIV-1 (monocytic line U1 and the T-cell line ACH-2). The HPV-transfected keratinocytes were able to induce HIV-1 p-24 production in the U1 monocytic cell line but not in the T-cell line. Also, using neutralizing antibodies for IL-6, TNF-α and IL-1RA, we demonstrated that the induction of HIV-1 replication in U1 cells was partially due to the inflammatory cytokine expression, mainly IL-6. This study also showed that in vitro, culture of cervical biopsies from patients with HPV infection induced HIV-1 replication in U1 cells (monocytic) but not in ACH-2 cells (lymphocytic).

An important cytokine IL-12, which is produced by DC and monocytes, plays an important role in the feedback systems. IL-12 drives helper T cells type 0 into a Th1 pathway (Crowley-Nowick et al. 2000). It is a potent activator of cellular immunity and has been shown to have antitumor as well as antimetastatic activity against murine tumors.

IL-10 (type 2 cytokine) has been shown to be associated with enhanced tumor growth (Bost et al. 1995). An interesting study (Clerici et al. 1997), which found high levels of IL-10 in HPV-associated CIN, suggested that the increased IL-10 production was supported by immunocompetent cells such as tumor-infiltrating lymphocytes in some cases. IL-10 down-modulates expression of major histocompatibility complex (MHC) class I expression, preventing tumor antigen presentation to $CD8^+$ CTLs (Matsuda et al. 1994, Beissert et al. 1995).

Our previous study (Nicol et al. 2002) showed that the presence of high grade CIN in HIV/HPV co-infected patients had high statistical significance and correlation with IL-4, IL-10, IL-8 and IFN-γ expression. We also demonstrated a significant correlation between IL-4 and IL-10 production in the HIV-positive patients, suggesting a switch to a type 2 cytokine profiles even in the presence of significant levels of IFN-γ (type 1 response).

IL-8 is a neutrophil chemotactic factor; it plays an important role in acute inflammation, being produced by various types of cells including endothelial cells, lymphocytes, and epithelial cells (Spear et al. 1998). Some authors (Tjiong et al. 1999) studying cervical lavage

samples from HIV-1 seropositive woman, found that levels of IL-8 were significantly higher in women with genital tract dysplasia related to HPV infection than in women without. Lee et al. (1999) reported an increase in IFN-γ $CD8^+$ T cells in the peripheral blood of HPV/HIV positive woman compared to woman with HPV infection alone, great number of CD8 T cells produced IFN-γ and TNF-α but not IL-2; they also found a higher incidence of HPV related cervical SIL in woman with both viruses, suggesting that these IFN-γ $CD8^+$ T cells are less effective at controlling the pathogenesis associated with HPV disease. In accordance with this study we also found significant levels of $CD8^+$ T cells, mainly producing IFN-γ in the cervical HPV infection, much less so in the HIV co-infection (Nicol et al. 2004).

Recently it was demonstrated by cervical cytobrush samplings that IFN-γ production by $CD4^+$ and $CD8^+$ T cells is only detectable in women currently or previously exposed to HPV-16 (Passmore et al. 2002). However another study (Cintorino et al. 2002) showed that the expression of specific types of IFN in HPV associated cervical lesions had a decreased production of some specific classes of IFN and is associated with high-risk type HPV lesions.

Hazelbag et al. (2001) showed experimentally that malignant transformed cervical epithelial cells have a decreased ability to express TNF-α, GM-CSF, IL-5, IL-10 and RANTES mRNA. They suggested that the loss of expression of these cytokines by cervical cancer cells may modulate the local tumor environment, thus supporting tumor growth.

Cervical cancer vaccines - Multiple approaches are being tried in the quest for an effective vaccine. Currently there are two major alternatives to make a vaccine against HPV: (1) prophylactic – which are simpler in that they need only to raise an immune response sufficient to limit infection and prevent clinical disease and (2) therapeutic vaccine – which must elicit an immune response that can clear an already established infection. Primary target antigens for a therapeutic vaccine are the oncoproteins E6 and E7 because they are expressed throughout the life cycle of HPV as well as in cancer cells (Kotecha et al. 2003). Noninfectious virus like proteins (VLP), composed of the L1 major capside protein, are current candidate vaccines for prevention of HPV infection (Pinto et al. 2003). The development of a prophylactic vaccine to protect against HPV infection may reduce the incidence of this cancer worldwide. Since multiple different HPV types are linked to cancer, implying a need for a vaccine with a large combination of different HPV components for preventive purposes and that no effective therapeutic vaccine for whatever disease is presently available. Reasons for the latter include an insufficient knowledge about the immune system and the existence of tumor immune escape mechanisms (revised by Villa et al. 2002).

Therapeutic vaccine trials comprised of peptides, fusion proteins, encapsulated plasmid DNA and recombinant vaccinia virus have demonstrated safety and immunogenicity but limited efficacy data are available, the overall vaccines appear to be safe as well tolerated and preliminary data indicates that most are clinically effective. Multiple trials are in progress and more mature data are expected within the next few years (Berry & Palefsky 2003). The main vaccine assayed are:

Virus-like particles (VLP) - Cellular and humoral responses are induced by VLP in clinical vaccine trials, and a strong protective effect against persistent HPV infection has been demonstrated (Koutsky et al. 2002, Pinto et al. 2003). Moreover immunization with a VLP vaccine can induce high titers of antibodies and seroconversion in all vaccinated subjects (Harro et al. 2001, Schiler et al. 2001). The L1 major capsid protein can be expressed in yeast or Sf9 insect cells, and VLP (Rose et al. 1993, Neeper et al. 1996) and both of them are capable of inducing neutralizing antibodies and cellular immunity leading to prevention of infection in animal models (Lowy & Frazer 2003). Moreover studies of HPV16 L1 VLP vaccines have shown that they are well tolerated and generate high levels of anti-HPV16 antibodies (Harro et al. 2001, Koutsky et al. 2002, Brown et al. 2004, Ho et al. 2004).

DNA immunization - Injecting the gene for an antigen in the context of a plasmid, could raise a cellular and humoral response against the antigen (revised by Jansen & Shaw 2004). DNA immunization is a low cost vaccination strategy that might permit a broadening of the cellular responses (Tobery et al. 2003). Furthermore naked DNA was examined as a therapeutic as well as prophylactic vaccine by several groups and showed promise in preclinical models, however results in humans and nonhuman primates were disappointing; multiple very high doses of naked DNA vaccines are required to elicit immune responses (Donnelly et al. 1996, 1997).

Vectored gene delivery - The transforming proteins E6 and E7 of HPV types 16 and 18 were one of the first vaccines into the clinic. Delivering the antigen coding sequence in a viral vector allows the antigen gene to enter cells more efficiently and it permits targeting to a particular cell types, since different virus vectors have different cell tropisms. Although the group of Tobery et al. (2003) had found a strong cell-mediated immune responses using the recombinant adenovirus, it induced a weaker neutralizing antibody response than VLP did.

RECENT FINDINGS

Several studies have shown the successful expression of L1 VLPs from a number of HPV types (revised by Koutsky et al. 2002, Pinto et al. 2003, Jansen & Shaw 2004). For therapeutic vaccines, mouse models that use tumor cells expressing viral antigens have also shown success (revised by Jansen & Shaw 2004). However, mouse and other animal models do not necessarily predict whether a particular approach will work in humans.

With respect to antibody responses to HPV L1, longitudinal studies have permitted an evaluation of whether natural levels of anti-HPV antibodies generated in response to infection protect against subsequent re-infection by the same or related HPV types. However data presented at the 19th International Papillomavirus conference-HPV (Villa et al. 2002) suggested that HPV 16 IgG antibodies do not protect against subsequent re-infection with the same viral type (revised by Villa et al. 2002).

The immunopathology of HPV infection, a novel type

of lymphoid aggregation consisting predominantly of $CD8^+$ T cells was described in HPV infection (Kobayashi et al. 2002). This lymphoid aggregation was seen predominantly in HIV seropositive high-grade CIN and was associated with the worse clinical outcome in these patients. Since the proportion of high risk HPV genotypes increased with more severe cytological abnormalities, one more sensitive primer set (SPF 10 primer) has been developed (Perrons et al. 2002) than the usually used (MY 09/11). HPV DNA detection and genotyping is therefore a useful tool in the clinic colposcopy, used with cytology.

It is suggested that although viral load alone did not appear to be an independent predictor of cervical dysplasia; monitoring viral load in conjunction with $CD4^+$ counts, one may be able to better predict HIV infected women at increased risk for cervical dysplasia and consequently cervical carcinoma (Davis et al. 2001). Moreover, knowing how the cellular cytotoxicity mechanisms occur during HPV infection, associated or not to HIV co-infection, enables us to understand the advancement of malign intraepithelial lesions and in a near future to design possible vaccines against the HPV, utilizing E6 and E7 proteins.

Concerning about the cervical immune response, the cytokines could provide a microenvironment that is favorable for accelerated HPV transcription and/or cellular proliferation and thus, might also contribute to the accelerated progression of intraepithelial lesions in HIV-1 infection (Arany et al. 2001). HIV-positive patients with high grade CIN showed high statistical significance and correlation with IL-4, IL-10, IL-8 and IFN-γ suggesting a switch to a type 2 cytokines profile even in the presence of significant levels of IFN-γ (type 1 response).

Many HIV-1 infected cells were detected in the uterine cervix of HIV/HPV co-infected women. The virus was found mainly in the transformation zone near the junction of the epithelium and stroma as well as around small vessels. This is the same distribution of the activated endocervical macrophage (Nuovo et al. 1993, Nicol et al. 2004) suggesting that HPV infection may either trigger the migration of HIV-1 reservoir cells being an important mechanism contributing to HIV replication in co-infected women (Nicol et al. 2004) and that macrophage-tropic strains tend to dominate early in HIV-1 infection acquired through unprotected sexual relations.

In conclusion, most prophylactic vaccines are VLP composed of the L1 structural protein appears promising. However many practical issues must be addressed before these vaccines can be licensed in clinical practice and public health programs.

This review supports the idea that the immune response in cervical dysplasia is critical in the HPV infection. A marked increase in T cell, macrophage activation and type 2 cytokine profile may facilitate infection by HIV-1 leading in the progression of CIN to cancer.

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