The role of inflammation in HPV carcinogenesis

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The role of inflammation in human papillomavirus (HPV) infection and disease is complex since it involves responses capable of preventing initial infections, clearing those ongoing as well as promoting persistence and progression of associated lesions. Avoiding the immune response has been considered a key aspect of HPV persistence which is the main factor leading to HPV-related neoplasia. HPV have evolved different ways of targeting immune signaling pathways. Moreover, host inflammatory response may promote lesion progression and affect tumor fate by diverse mechanisms including the direct participation of inflammatory cells. In this review, we discuss the interplay between HPV oncogenic proteins and an array of inflammatory responses that ultimately may lead to cancer.

Introduction

Tumor-associated inflammation and the inflammatory component present in the tumor microenvironment are recognized as having a role in tumor development (1,2). Distinct pathways, soluble compounds and cell types are involved and their interaction is complex. Infectious agents etiologically linked to different types of cancer are among the several factors that can activate inflammatory pathways. Worldwide, ~20% of cancers are attributed to infections, a considerable proportion of which are linked to human papillomaviruses (HPVs), particularly in developing countries (3,4). HPV-related cancers occur, however, in a small proportion of individuals exposed to these very common infections. In fact, the vast majority of HPV infections and a significant proportion of neoplastic lesions caused by HPV are eliminated by an effective immune response (5,6). Failure to eradicate infected cells increases the risk of developing neoplasia, which is related to the ability of HPV to evade the immune system (7). This process involves a series of inflammatory responses which are been characterized in the last years and described in detail along this review.

HPVs pertain to the Papillomaviridae family, a highly diverse group of viruses that infect the skin and mucosal epithelia of several vertebrate species. To date, >100 different types have been identified (8). HPVs, as other papillomaviruses, are small viruses (~50 nm) composed by a non-enveloped protein capsid surrounding a double-stranded DNA genome of ~8,000 base pairs. The genome of all HPV can be divided in three functional regions. A non-coding region involved in the regulation of both HPV transcription and replication named upstream regulatory region or long control region. Besides, the early and late coding regions carry the early and late viral genes, respectively. Early genes (E1, E2, E4, E5, E6 and E7) regulate the vegetative and productive phases of viral cycle. The late genes, L1 and L2, synthesize the major and minor capsid proteins, respectively (9).

HPVs show a high degree of tissue tropism with different HPV types infecting specific anatomic regions. For instance, HPV types of the β-papillomavirus genus (including HPV5, HPV8 and others) are highly prevalent in cutaneous lesions in humans. These as well as many other types are also known as EV-HPV types due to their close association with the rare dermatological disease epidermodysplasia verruciformis, where they contribute to the development of squamous cell carcinoma in sun-exposed areas. On the other hand, ~40 HPV types belonging to the α-papillomavirus genus have been detected infecting the epithelium and mucosa lining the ano-genital tract. These are collectively known as mucosal HPV types and have been further classified based on their oncogenic potential as low- or high-risk HPV types. Low-risk HPV types (i.e. HPV6 and HPV11) cause common genital warts, benign hyperproliferative lesions with very limited tendency to malignant progression. Infection with high-risk HPV types is associated with the occurrence of high-grade dysplasias in different anatomical locations: in the uterine cervix, they are known as high-grade cervical intraepithelial neoplasias (CIN 2 and 3), which are precursors of invasive cervical carcinoma. This is the second most common cancer among women worldwide and the leading female malignancy in several developing countries (10). HPV16 and 18 are the most prevalent high-risk HPV types accounting for ~50 and 20% of all cervical cancer cases, respectively. Together with other high-risk types, namely HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66, these viruses have been recognized as group 1 carcinogens by the International Agency for Research in Cancer due to their etiological association with cervical cancer. Besides, high-risk HPV types are associated to a significant percentage of anal, vulvar, penile and oropharyngeal carcinomas (8). For the sake of this review, we will focus our discussion on data concerning mucosal HPV types. However, some studies conducted with cutaneous HPV types were included along the text to highlight similarities and differences between these viral groups.

HPV cycle initiates when virus gains access to undifferentiated cells from the basal layers of stratified epithelia presumably through micro-wounds in the superficial cells strata. However, HPV genome amplification, late gene expression and virion mounting take place in differentiated squamous epithelial cells that have withdrawn from the cell cycle. Since HPV rely on the cellular machinery to replicate their genome, viruses have to induce de novo DNA synthesis in otherwise quiescent cells. This is achieved by the concerted action of viral proteins expressed from specific HPV early genes.

High-risk HPV types express two proteins, E6 and E7, with proved oncogenic potential. These are the two viral products always present in HPV-associated cervical tumor samples and derived cell lines. Their central role in HPV-mediated carcinogenesis is underscored by the fact that their repression impairs tumor cells growth and revert the malignant phenotype in vitro (11,12). Besides, their combined expression is sufficient to efficiently immortalize primary human keratinocytes (13,14). E6 is known for promoting the degradation of the tumor suppressor p53 and activating telomerase expression and activity, whereas the best-described function of oncogenic E7 is the binding and inactivation of members of the retinoblastoma susceptibility protein (pRb) family. These interactions are considered central to the transforming capacity of these proteins (9). Besides, molecular studies show that these proteins operate by targeting a growing number of other cellular factors. The interaction of E6 with pRB35, paxillin,
tuberin, E6-AP, E6-BP, E6TP1, Bak, TNFR-1, FADD, caspase-8, p300/CBP, MCM7, XRCC1, MGMT, NFX1-91, hADA3, Gsp2 and at least eight PDZ proteins has been documented (15,16). These interactions affect keratinocytes transcription and differentiation, activate telomerase and inhibit apoptosis, extending the life span of the infected cell and allowing maximal HPV amplification. Similarly, the interaction of E7 with several cell cycle regulators including cyclins, cyclin-dependent kinases, CDKs-inhibitors, cullin 2, histone deacetylase, AP-1, E2F1 and E2F6 favors cell cycle progression. Therefore, infected cells are capable of reentering S phase and sustain DNA synthesis to serve the replicative interests of the virus (15,17).

The high-risk HPV E6 and E7 oncoproteins also play a key role in the deregulation of innate immunity by interfering with the expression of Toll-like receptors. These receptors recognize pathogen-associated molecular patterns to activate antigen-presenting cells and phagocytes, enhancing innate and adaptive responses against pathogens (18). Specifically, Toll-like receptors 9 transcription is inhibited in cells expressing HPV16 E6 and E7 (19). Impairment of these responses is considered an important step in HPV carcinogenesis.

Furthermore, cervical cancer progression has been recently associated to upregulation of matrix metalloproteinases, which play a significant role in tumor invasion and metastases in different cancers (20). Finally, other interactions have been reported that may determine the fate of HPV-infected cells by interfering with critical aspects of the antiviral mechanisms of the host favoring HPV persistence and progression of associated lesions.

**HPV infection and innate host antiviral mechanisms**

The several immune mechanisms operating in an immunocompetent individual play a critical role in preventing the onset and determining the duration and outcome of active or subclinical HPV infections. These multistep lines of defense assure that the great majority of HPV infections are cleared after a relatively short period of time with little or no sequel to the host. Different lines of evidence indicate that regression of HPV-induced lesions is the consequence of the cell-mediated immune response. For instance, infiltration by T lymphocytes and macrophages is characteristic of regressing warts. On the other hand, immunosuppressed individuals exhibit a higher prevalence of extensive HPV-induced lesions and are at a higher risk of developing HPV-related tumors (21).

HPV infections elicit the production and release of several inflammatory cytokines from keratinocytes, their main target cell type, from skin fibroblasts and from different components of the innate and adaptive immune response including macrophages, natural killer cells and lymphocytes. The relevance of this response to infection outcome is highlighted by the observation that tumor necrosis factor-α (TNF), interleukin (IL)-1 and interferon (IFN)-α and -β (IFNα/β) inhibit HPV oncogenes transcription in vitro and inhibit the growth of cell lines harboring the viral genome (22–27). However, in a small proportion of cases, HPV succeeds to evade the immune response and establish persistent infections which, when caused by certain oncogenic HPV types, constitute the main risk factor for the development of cervical carcinoma and its precursor lesions (28). Persistent virus infection, as in the case of cancer-causing viruses as HPV, has been linked to chronic inflammation, an important cofactor for cancer development (29).

Avoiding rather than actively inhibiting the immune response has been considered a key aspect of HPV persistence (30). This is achieved by a combination of factors including the restriction of viral infection and replication to specific anatomic sites, the lack of associated cell lysis and reduced expression levels of viral proteins. However, although useful to reduce exposure to the immune system, intracellular location triggers potent antiviral and immunomodulatory mechanisms including the activation of IFN-pathway and the production of TNF and IL-1. Therefore, HPV has evolved different ways of targeting cytokine and chemokine signaling as well as antigen-presenting pathways to impair the cross talk between infected and immune effector cells.

**HPV and the IFN pathway**

IFNs are antiviral effector molecules that collectively induce a state of resistance to viral replication both in infected and uninfected neighbor cells and upregulate the cellular immune response against these agents. Such effects are exerted by the binding of type I (IFNα/β) and type II (IFNγ) IFNs to specific cellular receptors, which activate Janus kinase/signal transducers and activators of transcription signal transduction pathways leading to the transcriptional induction of IFN-stimulated genes. The antiviral effect of IFN on HPV-infected cells has been supported by the observation that cells transfected with bovine papillomavirus or HPV-31 episomes as well as the W12 cell line, derived from a HPV16-positive cervical intraepithelial neoplasia I (CIN I), undergo gradual loss of viral episomes upon treatment with type I IFN (31–33) (Figure 1). Moreover, the rapid loss of episomal HPV DNA after long-term culture of W12 cells has been associated to the upregulation of endogenous antiviral response genes normally induced by type I IFN pathway (34). However, clinical results accumulated during the last three decades show that IFN treatment of HPV genital infections induces only marginal effects in terms of virological cure indicating that HPV may have developed resistance to IFN. In *vitro* observations indicate that the response to these factors by HPV-infected cells varies according to IFN type, the HPV type infecting the cell, the cell type analyzed and HPV transcription profile. For instance, IFNγ inhibited the colony formation capacity of HPV16-immortalized cells or cervical carcinoma-derived lines, whereas IFNα did not (35). On the other hand, downregulation of IFNγ expression in both cervical intraepithelial neoplasia and cervical cancer tissues as compared with normal cervix has been reported (36,37). Importantly, the response rate of individuals with lower genital tract infections to IFNγ was higher in those infected by low-risk HPV types when compared with those harboring high-risk HPV types (38). Finally, response to IFN treatment of patients with condyloma was associated to higher L1 gene expression, whereas unresponsiveness was paralleled by E7 messenger RNA (mRNAs) expression (39).

Several groups have addressed the effects of isolated high-risk HPV genes as well as complete genomes on the IFN signaling pathway (Figure 1). These studies showed that a number of IFN-responsive genes are downregulated by HPV16 and HPV31 in different cell lines (40,41). Impairment of IFN pathway has been associated to E6 and E7 expression from mucosal and cutaneous HPV types (42–46). It has been observed that E7 prevents the translocation into the nucleus of p48, a member of the IFN-stimulated gene factor 3 that is a positive regulator of IFNα-stimulated transcription (42). Besides, this oncoprotein physically interacts with interferon regulatory factor-1 (IRF-1), an IFNβ promoter-binding transcription factor, and interferes with its transactivation function through a mechanism involving the recruitment of histone deacetylase to the promoter (47,48). The negative effect of this oncoprotein on IRF-2 promoter in monolayer cultures and the downregulation of IFN pathway-related genes IFI44, IFI44L and IFNGR1 in E7-expressing epithelial organotypic cultures has been described recently (49,50). Similarly, E6 of HPV16 interacts with IRF-3 and weakens its transactivating effect on IFNβ promoter (46). Ectopic expression of this oncoprotein has also been associated with the inhibition of IFNα, IFNβ and IFN-inducible genes expression as well as to decreased signal transducers and activators of transcription-1 expression and binding to IFN-stimulated response element (44,45). Moreover, downregulation of IFNα mRNA has been observed in cells expressing E6/E7 genes from HPV16 and from cutaneous HPV38 (51).

Although the exact inhibition mechanism is not fully understood, there is evidence that HPV16 E6 is involved in the epigenetic silencing of IFNα in immortalized human keratinocytes. Importantly, IFNα was downregulated in cervical cancer samples when compared with normal patient tissue (52). Given that one of the functions of IFN production by infected cells is to induce an antiviral state in neighbor cells, inhibition of IFNs expression by HPV represents a very effective evasion mechanism. Collectively, these observations show that HPVs have evolved several and sometimes redundant mechanisms to circumvent antiviral effects exerted by IFNs.
Despite the body of data discussed above, the importance of IFN response in controlling HPV infections is undisputed. Therefore, it may seem paradoxical that, in some circumstances, IFN response may foster disease progression. However, recent results studies show that this may be the case. For instance, upregulation of antiviral type I IFN-responsive genes has been associated with accelerated HPV spontaneous episomal loss in W12 cells (53). Moreover, IFNβ treatment of these cells promotes a rapid reduction in viral load (32). In both cases, loss of episomes and consequent decline of E2 expression led to the emergence of clones harboring integrated HPV16 genomes, which exhibit high E6 and E7 and, probably, enhanced growth potential. Moreover, Lace et al. (54) reported that IFNβ activates HPV16 and HPV11 transcription through the binding of IRF-1 to IFN response element present in the viral promoters. Besides, the authors observed that IFNβ treatment induced HPV11, HPV16 and HPV31 replication in transfected keratinocytes. Finally, it has been shown that IFNα2b induces the proliferation of HeLa, a HPV18-positive cervical adenocarcinoma-derived cell line. Cytokine treatment also accelerates p21cip1/waf1 protein degradation and overcomes G1 and G2 phase arrest imposed by 5-fluorouracil and paclitaxel, respectively, in these cells (55). All in all, these observations underscore the need of a more profound understanding of the mechanisms underlying the balance between viral clearance and persistence in order to implement more effective therapeutic strategies.

**HPV and other inflammation mediators**

Pro-inflammatory cytokines, including several ILs and TNF, are important mediators of skin and mucosa inflammation. In this context, keratinocytes are major contributors to epidermal cytokine production in response to diverse stimuli including viral infection. Production of these molecules has important autocrine effects on keratinocyte proliferation, differentiation and cytokine secretory pattern. Besides, their paracrine activity may influence other skin cell types and induce inflammatory cell migration (56). One of these cytokines, TNF, is a major regulator of inflammation in response to injury and infection (57,58). This cytokine binds two cell surface receptors, tumor necrosis factor receptor 1 and tumor necrosis factor receptor 2, activating multiple signaling pathways involved in antiviral activities, growth arrest, cell proliferation, differentiation or apoptosis, depending on the cell type and growth factor availability (57). TNF exerts a potent cytostatic effect on normal human keratinocytes where it upregulates the cyclin-dependent kinase inhibitor p21cip1/waf1 through the activation of nuclear factor-kappaB and downregulates the mitotic regulatory proteins cyclin A, cyclin B and p34cdc2 (26,27,59–61).

Several lines of evidence point to the involvement of TNF in the immune response against HPV-infected cells and in the natural history of HPV-associated diseases. For instance, expression of this cytokine by infiltrating mononuclear cells correlates with spontaneous regression of papillomas (62). We have shown that HPV-positive cervical cancer-derived cell lines are resistant to the anti-proliferative effect of this cytokine. Interestingly, it was also observed that HPV16-immortalized keratinocytes were sensitive to TNF, whereas HPV18-immortalized cells remained resistant to this drug (26,27). This disparity in the response to TNF treatment is reflected in the different gene expression profiles and nuclear factor-kappaB activation levels exhibited by these two cell lines upon cytokine treatment (63).
cytokine also inhibits viral transcription in HPV16-immortalized human keratinocytes (25) and HPV18 expression in non-tumorigenic HeLa/fibroblast hybrids (64,65). In addition, tumorigenicity of HeLa/ fibroblasts hybrids and HPV16-transformed human keratinocytes has been correlated with TNF resistance (66,67). Different studies show that HPV oncogenes E6 and E7 play a role in overcoming TNF effect. While E6 expression has mainly been associated with resistance to TNF-mediated apoptosis (67–69), the presence of E7 has been related to resistance to TNF anti-proliferative effect (49,59,61). Altogether, these observations suggest that the acquisition of TNF resistance may constitute an important step in HPV-mediated carcinogenesis. Importantly, there is increasing evidence that in some conditions, TNF can act as tumor promoter (70). In the case of HPV-associated diseases, this is supported by the observation that TNF stimulates the proliferation of HPV-immortalized and HPV-transformed cervical keratinocytes through an autocrine pathway involving the induction of the epidermal growth factor receptor ligand amphiregulin and ras pathway activation (71,72). Despite all the accumulated data, most of the molecular events underlying the biological role and the clinical relevance of TNF in HPV-related diseases outcome remain elusive, warranting further studies.

The effect of other pro-inflammatory cytokines in HPV-related diseases has been addressed by different groups. For instance, it was observed that IL-1α negatively regulates the proliferation of HPV-positive cells (71), whereas IL-1β and IL-1β exert opposite effects on the promoter of different cutaneous HPV types (73). Besides, the study of the sensitivity of HPV-expressing cells to transforming growth factor (TGF)-β1 shed conflicting results (74,75). In addition, it was reported that HPV16 oncoproteins can inhibit the expression of TGF-β2 isoform (76) and that TGF-β1 isoform may promote chemo- mosomal instability in cells expressing HPV16 E6 and E7 proteins (see below) (77). At this point, it is important to consider that local concentration of pro-inflammatory cytokines may have a deep impact on the adaptive immune response to HPV. While high TGF concentration may induce naive T cells to develop a regulatory phenotype (regulatory T cells), low TGF levels together with IL-6 may induce Th17 cells, which are involved in pathogens clearance, particularly at mucosal surfaces (78). On the other hand, a role of IL-6 in cervical carcinogenesis is supported by the detection of higher levels of this protein in invasive cervical carcinoma as compared with precursor lesions and normal tissue and by studies showing that IL-6 promotes growth of cervical cancer cells in vitro (79,80). A study by Hess et al. (81) showed that although HPV-positive carcinoma cell lines express high levels of IL-6, they exhibit limited autocrine responsiveness to it as revealed by low constitutive STAT3-binding activity. In these cells, STAT3-binding activity is not enhanced by exogenous IL-6 due to the lack of the gp80 subunit of the IL-6 receptor. Addition of soluble gp80 was sufficient to restore IL-6 responsiveness in carcinoma cells and to induce the expression of the chemokine monocyte chemotactrant protein-1. Interestingly, expression of E6 and E7 oncoproteins of HPV16 has been associated to the selective suppression of monocyte chemotactrant protein-1 in human cervical and epithelial keratino- cytes (82). Therefore, IL-6 production and monocyte chemotactrant protein-1 silencing might contribute to local immunosuppression allowing HPV-infected cells to escape the immune system.

Besides promoting unscheduled cell proliferation, HPV infection also exerts a direct effect on pro-inflammatory gene expression. This may contribute to suppression of host immune responses against in- fected epithelial cells favoring viral persistence. A study comparing the lymphokine secretion pattern of normal cervical keratinocytes and HPV16- or 18-positive cervical cell lines showed that normal cells produced higher amounts of IL-1β, IL-1β, IL-1 receptor agonist, IL-6, IL-8, TNF and granulocyte-monocyte colony-stimulating factor than HPV-immortalized or cervical carcinoma cells (83). However, the study of pro-inflammatory modulators expression has produced conflicting results depending on the HPV type analyzed and the experimental approach used. For instance, De Andrea et al. (84) re- ported that HPV5 E6/E7 proteins enhanced IL-8 release by primary epithelial keratinocytes, whereas HPV16 E6/E7 proteins decreased IL-8 production and HPV38 proteins had no effect on this IL pro- duction by these cells. On the other hand, a recent study showed that E6 protein of HPV5 and 8 downregulate IL-8 secretion in primary human keratinocytes (85). Moreover, HPV38-immortalized kerato- cytes and HaCaT cells expressing HPV38 E6/E7 proteins exhibited reduced IL-8 secretion when compared with normal cells (51,84). Altogether, these results highlight the functional differences between HPV types and underscore the complex interaction existent be- tween HPVs and members of the different families of inflammatory cytokines.

**HPV infection: the role of antimicrobial peptides**

Other antiviral and pro-inflammatory molecules that are part of the innate immune response may affect the growth of HPV-infected cells. For instance, defensins and cathelicidins are small antimicrobial pep- tides involved in the innate defense against viral infections. In mammals, defensins are produced by a number of cell types including neutrophils, Paneth cells, monocytes, macrophages and epithelial cells from the skin and mucosa (86). Their expression and secretion may be induced by several stimuli including the action of pro-inflammatory cytokines. These molecules are classified in α-, β- and θ-defensins based on the connectivity pattern of six highly conserved cysteine residues and sequence homology. Human α-defensins HNP-1 (human neutrophil peptide 1) and HD-5 (human α-defensin 5) have been shown to inhibit HPV16 pseudovirions (PsV) transduction in a variety of cell types. Moreover, these defensins diminished HeLa cells trans- duction by other mucosal and cutaneous HPV types as well as by cottontail rabbit papillomavirus and bovine papillomavirus type 1 although to different extent (87). Interestingly, HD-5 has been de- tected in the stratified squamous epithelium of the vagina and ecto-cervix and in cervicovaginal lavages indicating that it may play a role in preventing HPV infection (88). A recent study has shown that human β-defensin (hBD)-2 mRNA and protein expression is down- regulated in high-grade squamous intraepithelial lesion and squamous cell carcinoma of the uterine cervix as compared with normal cervical epithelium (89). These authors also observed that hBD-2 and the α-defensin HNP-2 recruit dendritic cells (DC) *in vitro* and enhance their infiltration into organotypic cultures of HPV16-transformed cells. Furthermore, inoculation of HNP-2 in HPV-positive cervi- cal carcinoma cells xenografts grafted onto non-obese diabetic–severe combined immune deficiency mice promoted DC recruitment comparable with that obtained by granulocyte-monocyte colony- stimulating factor administration (89). The functional importance of this observation is highlighted by the fact that infiltration by DC leads to apoptosis of HPV-transformed keratinocytes in organotypic cul- tures (90). Furthermore, Le Poole et al. (91) showed that DC can be cytotoxic toward E6- and E7-expressing cells in a murine model of cervix carcinoma. In an independent study, hBD-2 and hBD-3 ex- pression was shown to be significantly upregulated in HPV-associated anal intraepithelial neoplasia and anal condylomata acuminata of both HIV-positive and -negative men (92).

Another antimicrobial peptide, the α-helical antimicrobial peptide human cathelicidin antimicrobial peptide-18 (CAMP; also known as LL-37), also showed inhibitory effect on HPV16 PsV transduction in HeLa cells. Conner et al. (93) observed that the levels of this peptide detected in the epidermis increase during the development of HPV- associated common warts. On the other hand, expression of LL-37 mRNA is downregulated in organotypic cultures of human keratino- cytes expressing HPV16 E7 (49). Altogether, these observations suggest that small antimicrobial peptides might play a significant role in the innate immunity against HPV. However, their biological re- levanance in the natural history of papillomavirus infections remains to be elucidated.

**Role of inflammatory cells in HPV disease**

Different reports have demonstrated that an increase in inflammatory cell infiltrate correlates with HPV-associated high-grade lesions. The specific contribution of each of cell population present in the tumor
environment, in particular tumor-associated macrophages and immature myeloid cells to disease outcome is matter of debate (94–96). Evidences of the involvement of both cell types in promoting tumor growth by impairing T lymphocytes activity have been obtained using mice models of HPV-associated tumors (97,98). In HPV16 transgenic mice, macrophage recruitment to HPV-associated lesion sites is dependent on CC chemokine ligand-2 (CCL2) and its receptor CCR2 (CCL2/CCR2) (99). Furthermore, there has been shown that tumor-associated macrophages may modulate angiogenesis in the K14HPV16 tumor model through matrix metalloproteinase 9 production (100). In this same model, it has been suggested that B cell responses against extracellular matrix deposited by E6/E7-expressing epithelial cells recruit inflammatory cells, leading to chronic inflammation and tumor progression (101).

The inflammatory response may further contribute to the progression of HPV-associated lesions by inducing DNA damage in infected tissues. Accumulation of the mutagenic DNA lesion 8-nitroguanine, which is caused by nitric oxide and reactive oxygen species produced by inflammatory cells, has been observed in high-grade cervical lesions positive for high-risk HPV types (102). Besides, treatment of cervical keratinocytes immortalized by HPV16 oncogenes with TGF-\(\beta\) resulted in the downregulation of telomerase activity followed by telomere erosion and progressive appearance of chromosome structural aberrations (77). Finally, a recent report describes that high physiological concentrations of nitric oxide induce DNA double-strand breaks and upregulate E6 and E7 mRNA expression in cervical keratinocytes harboring episomal HPV16 genomes (103). However, another study detected a progressive decrease in iNOS expression as cervical lesion progressed from low to high grade (96).

DC, the professional antigen-presenting cells, link innate and adaptive responses. These cells mature in response to local signals and travel to and stimulate lymphocytes in lymphoid peripheral organs. The decrease in numbers of Langerhans cells, the main DC population of the skin, in HPV-associated lesions has been described in the literature (104). There is evidence that this loss is caused by down-regulation in E-cadherin expression in keratinocytes mediated by E6 (105). Moreover, Langerhans cells exposed to chimeric HPV16 virus-like particles are unable to initiate effective T-cell responses, in a mechanism dependent of phosphatidylinositol kinase-3 (106,107).

Besides, the DC and stromal myeloid cells found in HPV-associated lesions interact with HPV-infected epithelial cells. The recruitment of macrophages to HPV-associated lesions is dependent on CCL2 and its receptor CCR2 (CCL2/CCR2) (99). Furthermore, there has been shown that tumor-associated macrophages may modulate angiogenesis in the K14HPV16 tumor model through matrix metalloproteinase 9 production (100). In this same model, it has been suggested that B cell responses against extracellular matrix deposited by E6/E7-expressing epithelial cells recruit inflammatory cells, leading to chronic inflammation and tumor progression (101).

![Diagram of the inflammatory response in HPV-induced carcinogenesis](image)

**Fig. 2.** Major inflammatory responses in HPV-induced carcinogenesis. Epithelial cells infection by HPV (yellow hexagon) may trigger an antiviral response with production of IFN-\(\beta\). Blocking of type I IFN pathways is part of the evasion mechanism displayed by HPV proteins (see Figure 1). However, type I IFN production and signaling promotes HPV episome loss (purple nuclei with yellow rings), favoring the emergence of cells with integrated HPV genomes (gray nuclei). These cells may display enhanced E6 and E7 oncogenes expression and consequent competitive growth advantage. In parallel, TGF-\(\beta\) promotes genome alterations (magnified gray nucleus), favoring cell transformation. HPV-infected cells also downregulate E-cadherin expression impairing the adhesion of Langerhans cells to the epithelium. Moreover, Langerhans cells loaded with HPV oncoprotein fail to mature and do express indoleamine 2, 3-dioxygenase (IDO). Eventually, these cells reach peripheral lymphoid organs, where they fail to stimulate effector T cells responses against HPV. TGF-\(\beta\) produced by HPV-infected cells also may influence the fate of myeloid cells recruited to the HPV-associated lesion (Mo CCR2\(^{+}\), blue nuclei). As lesions progress to invasive carcinoma (orange to red cells), there is an increase in the number of infiltrating macrophages. There is evidence that infiltrating macrophages have an M2-like phenotype (M2 M\(\phi\)) expressing TGF-\(\beta\), IL-10 and matrix metalloproteinase (MMP) 9, but not iNOS. MMP9 mediates angiogenesis, whereas IL-10 and possibly TGF-\(\beta\) contribute to the stimulation of specific regulatory T cells ([Treg] lymphocytes) that can suppress the activity of antitumor effector cells (CD8 CTL lymphocytes). Indeed, the ratio between infiltrating regulatory and CTL T cells in primary tumors correlates with lymph node invasion in patients. BV, blood vessel; BM, basement membrane; LC, Langerhans cells; Mo, monocytes; M\(\phi\), macrophages; CTL, cytotoxic lymphocytes.
lesions express the enzyme indoleamine 2, 3-dioxygenase, known for its role in the induction of regulatory phenotype on T cells (95,108). A schematic model of the different soluble factors and inflammatory and immune cell types playing a role in HPV carcinogenesis is presented in Figure 2.

Cervical inflammation and risk of HPV-related cancer
From extensive natural history studies of HPV infections at the uterine cervix conducted in the last two decades, additional risk factors were described which in association with a persistent high-risk HPV infection contribute to the development of cervical neoplasia (109,110). Among such risk factors, common genital tract infections and cervical inflammation have been considered as cofactors in cervical carcinogenesis (111).

Sexually transmitted infections are common causes of cervical inflammation ( cervicitis ), among which Chlamydia trachomatis and Herpes simplex-2 have been associated to an increased risk of cervical neoplasia (112–114). Moreover, an association between degree of cervical inflammation caused by bacterial vaginosis and high-grade cervical lesions has been described (115). In fact, cervical cells coinfected with C.trachomatis and HPV secrete large amounts of pro-inflammatory cytokines, which may be the consequence of a more profound inflammatory state (116). Additional epidemiological studies with large series are needed to determine if these infections play a role in HPV-related diseases.

Conclusions
By targeting different innate and adaptive immune signaling pathways, as well as interfering with cell cycle and death signals, high-risk HPVs are strong carcinogens to humans. These viruses have evolved different ways of evading the immune system. Although HPV infections do not cause cell lysis and clear tissue damage as other infections do, accumulating evidence shows that inflammation plays a role in Papillomavirus carcinogenesis. Cellular, innate and adaptive responses, in which several cytokines are secreted, play a role in HPV-associated disease progression. Understanding the orchestration of HPV interference on different inflammation pathways, as presented in this review, shall contribute ultimately to develop new strategies to treat HPV lesions before progressing into invasive tumors.

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