Involvement of innate immunity in Human Papilloma Virus infection

Envolvimento da imunidade inata na infecção do Papilomavírus Humano

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Abstract

Innate immunity is the first defense in the host and is crucial to prevent Human Papillomavirus (HPV) infection and subsequent development to neoplasia. HPV is involved in the squamous intraepithelial lesions and cervical cancer development. Fortunately, not everyone infected with HPV develop cervical cancer as the immune system can control the virus infection. Innate immunity is the first defense in the host and is crucial to prevent HPV infection and subsequent development to neoplasia. This type of immunity has different antiviral defense pathways, among which are: physical barriers (skin and mucous membranes), keratinocytes and immune cells such as Langerhans cells, dendritic cells, and natural killer cells, which may secrete interferons and cytokines that activate signaling cascades that may prevent HPV infection. However, HPV has also developed various strategies to evade this immune response, which could allow the squamous intraepithelial lesions and cervical cancer development. The aim of this review was to describe the innate immunity involvement in HPV infection.

Keywords: Cervical cancer; Human papillomavirus; Immunology.

INTRODUTION

Among sexually transmitted infections, human papilloma virus (HPV) is one of the most prevalent diseases, but the immune system may be involved in controlling the virus, allowing a remission in some cases. If the virus evades the immune response, the infection persists and the infected cells could transform into cancer cells[1].

Worldwide, the HPV infection prevalence is in the range of 1.4-44%[2,3], this becomes important because of its association with cervical cancer (CC). In Mexico, it has been reported a HPV infection prevalence of 37.5% in pregnant women[4] and 4.8 % in women seeking cervical Papanicolaou cytology[5].

Due to the progression to cancer, in 2000, in Mexico, an estimated of 6,650 women died of CC[6] and in 2008, approximately 5,061 deaths were reported with a prevalence of 20.5%[7]. The CC is related to at least 20 genotypes, of the 100 identified for HPV. For example, in England it has been estimated that 70% of CC cases are associated with HPV genotypes 16 and 18[8].

Fortunately, not all women with HPV infection develop a neoplastic process, phenomenon in which the immune system may be involved. Immunity is the set of innate and adaptive responses. The first one has different functions that act against pathogens at the moment of the first contact with them, being this immediate response not specific and without memory[9,10,11]. Usually, in the cervix there are dendritic cells (DCs) and macrophages, typical cells of the innate immune response. However, in the presence of HPV, the immune system triggers nonspecific responses, such as, neutrophil chemoattraction and activation of macrophages, natural killer cells (NK), DC and Langerhans cells (LC)[12,13]. Therefore, the aim of this review is to describe the innate immunity involvement in HPV infection.

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HUMAN PAPILLOMA VIRUS

HPV is a virus of the Papillomaviridae family, sized 50 nm. This virus has no envelope and its genome consists of a double strand DNA, divided into three regions: the large control region (LCR), the early region (E), and the late region (L). The LCR contains the promoters and the initiation sites. The E region includes genes for nonstructural proteins (E1-E7). E1 and E2 are involved in viral replication and transcription, E5 acts in the early stages of infection, E5 plus E6 and E7 have main role in oncogenesis, particularly E6 and E7 which are involved in viral transformation process by binding to the cell cycle proteins p53 and Rb, respectively, inhibiting apoptosis and allowing the cells to proliferate without control. It is in these proteins where the immune system must act to prevent a neoplastic process. Finally, the L region contains the L1 and L2, two genes expressed in late stages of infection in differentiated epithelial cells that codify capsid structural proteins[14,15].

INNATE IMMUNITY

Physical barriers

To produce infection, HPV must pass through the skin and mucous membranes, translocation facilitated if the tissues are damaged. Mucus is a major barrier due to its viscosity and substances therein are collaborating in reducing pathogens[16].

Furthermore, the mucosa-associated lymphoid tissue (MALT) consists of several populations of lymphoid cells[16,17], bearing pattern recognition receptors (PPR's) that recognize HPV pathogen-associated molecular patterns (PAMP's). Particularly, in high grade squamous intraepithelial lesions (SIL), it has been observed that PAMP's virus interact with PPR's inducing the secretion of interferons (IFNs) and other cytokines for the activation and recruitment of DCs and macrophages in MALT[17]. However, although MALT in the genital mucosa is an important barrier against the virus infection, HPV has developed mechanisms to evade this response.

Microbiota

Other important issue in the response against pathogens, is the microbiota present in the cervix and vagina, which consists mainly of *Lactobacillus acidophilus* or Döderlein bacillus (which maintains vaginal acidi-

ty), *Streptococcus*, *Staphylococcus*, *Peptococcus*, *Peptostreptococcus*, *Bacteroides speciae*, *Mycoplasma* and *Candida*. Microbiota is affected by temperature, hormones, pH and oxygen tension, allowing installation of pathogens causing bacterial vaginitis, sexually transmitted infections and cervical lesions. Common microorganisms associated with these pathologies are: *Gardnerella vaginalis*, *Candida sp*, *Staphylococcus aureus*, and *Escherichia coli*. In some cases there is an association of two or more pathogens[18,19,20].

Beyond the above mentioned concepts, it is accepted that people with bacterial vaginosis have increased susceptibility to viral infections[21]. Furthermore, it has been found that HPV coinfection with *Chlamydia trachomatis* or *Mycoplasma hominis* is associated with low-grade SIL[22,23].

Cell receptors

If physical barriers and microbiota fail to prevent HPV infection, then the virus, using different receptors, penetrate epithelial cells and proliferate[16]. These receptors are the glycosaminoglycans (GAGs), specifically heparan sulfate proteoglycans (HSPG) found in the extracellular matrix and the cell surface (Figure 1). Other receptors reported for HPV internalization are clathrin, caveolin, tetraspanin and laminin-5[14,24,25].

α6β1 and α6β4 integrins, expressed on the surface of basal epithelial cells, have been suggested as secondary receptors, involved in internalization, preceded by HSPG recognition[14,17,25]. However, it has been found that α6β1 integrin serves as a HPV6 receptor, but is not necessary for other genotypes[24], so that recognition can be genotype specific.

Due to the fact that human and viral nucleic acids are structurally similar, it is necessary to get the viral discrimination between species. This problem is solved by Toll-like receptors (TLR)[26,27], expressed in DC, LC, macrophages, monocytes, neutrophils and keratinocytes (KCs). Several types of TLRs, such as TLR3, TLR7, TLR8, and TLR9 are involved in specific viral recognition in endosomal compartments[26]. Moreover, it has been found, on injured cervical stroma, that IFN--1 may induce increased expression of TLR's 1, 3, 5 and 7[28]. Molecules such as myeloid differentiation factor 88 (MyD88), nuclear factor kB (NF-kB) and the family of interferon regulatory factor (IRF) are necessary to activate the TLR's, thereby allowing the activation of cells that secrete reactive oxygen species (ROS), inflammatory cytokines, interferons, and chemokines[26]. If infected human KCs express E6 and E7, then the
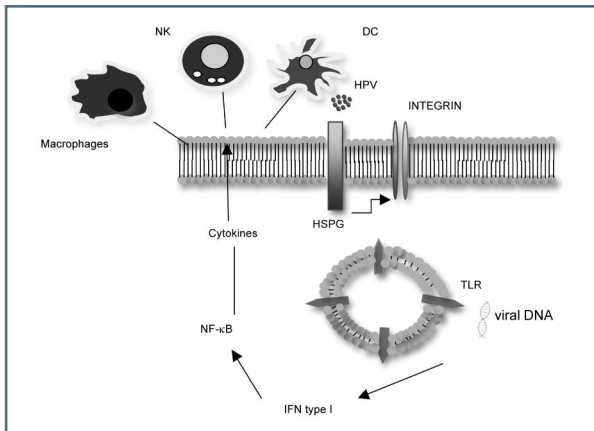
FIGURE 1. HPV infection model and activation of the innate immune response. NK (natural Killer), DC (Dendritic Cell), HPV (Human Papilloma Virus), HSPG (heparin sulfate proteoglycans), TLRs (Toll like receptors). The HPV virus is recognized first by the HSPG receptor and then by the secondary receiver integrin, finally its DNA is released, being recognized by TLRs in the endosomes, leading to IFNs production and NF-κB stimulation along the production of cytokines, activating the innate immune cells such (macrophages, NK and DC)

TLR9 mRNA decreases, especially if the HPV 16 is the infecting virus, leading to the infection persistence[29].

Keratinocytes

These cells predominate in epithelium; they differentiate from the basal layer to the *stratum corneum*. Undifferentiated KCs of the basal layer are targets of viral infection and expression of viral genes is detectable only in these cells. The rapid replication of KCs allows cells to divide, one cell migrates to the next suprabasal layer and the other is maintained in the basal layer allowing the infection to be continued[30,31]. In the epithelial dysplasia the KCs exhibit loss of epidermal differentiation plus an increment of nuclear polymorphisms and cell proliferation. These disorders are characterized by changes in the expression of keratins[32], deregulation of genes of the components of the cytoskeleton and extracellular matrix as vimentin, osteonectin and fibronectin[30].

In the epithelial basal layer, the oxygen partial pressure is low, and the keratinocyte differentiation is higher when the oxygen increases. In HPV infected basal cells, E6 produces an increment of ROS concentration and consequently DNA damage is produced, allowing the integration of HPV and the occurrence of cancer[10].

TLR9 and TLR3 of the KCs recognize HPV activating NF-kB, resulting in the production of proinflammatory cytokines and activation of signaling pathways, particularly type I interferons (Figure 1) and transcription factors IRF7 and IRF3[33,34]. DeCarlo *et al* have reported that normal ectocervix KCs express abundantly TLRs 1, 2, 3, 5, and 6 and express low levels of TLRs 4, 7, 8 and 9, while in KCs from pre-malignant epithelium, TLR3 is highly expressed and TLR1 is expressed at low levels. In epithelial carcinoma, RTTs receptors are decreased except TLR8[28].

In addition, KCs also have antimicrobial defensins, involved in the innate response[31]. The α-defensins (a group of arginine and cysteine-rich antimicrobial peptides) are innate compounds effective in inhibiting HPV infection in humans and are expressed in leukocytes and epithelial tissue. It has been observed high concentration of defensin HD-5 in differentiated KCs in various genitourinary sites of the woman, and the testicles and the urethra in men. This defensin could be absent in KCs that become the target of HPV infection. It has been suggested that women who express high levels of α-defensins may be resistant to initial infection and persistence of HPV, also, this defensin has a postcoital microbicide property, proving a natural barrier to the sexual transmission of HPV[35].

Dendritic cells (DCs)

The DCs represent a group of antigen-presenting cells involved as a link between the innate and adaptive response. They can be classified into myeloid and plasmacytoid lineages. The former has two types: the interstitial cells (ICs) which are found in peripheral tissues and in the dermis and LCs, located in the epithelium where they express TLR4. The plasmacytoid lineage cells (PDCs) are found into lymphoid organs and abnormal skin, expressing TLR7 and TLR9. The PDCs can be activated through TLR9, by CpG domains of HPV, allowing the production of high levels of IFN-α and stimulation of macrophages and NK[36]. Other activation pathway is through TLR2/TLR6, which regulates IL-10 and TGF-β secretion, instead of pro-inflammatory cytokines (IL-6, IL-12 or TNF--α)[27]. Furthermore, LCs called "immature DC", are specialized cells that reside in the cervical epithelium[37]. After HPV recognition by LCs, signaling cascades are activated by mitogen-activated protein kinases (MAPK), NF-kB, phosphatidylinositol 3-kinases (P13-K), and Protein Kinase B (PKB). These proteins play a crucial role in regulating multiple signaling

channels of viral clearance[38]. It is well known that Th1, NK and DCs cells produce IFN-γ which could activate DCs and NK cells receptors triggering antiviral effect[39,40].

Macrophages

As well as dendritic cells (DCs), macrophages are antigen presenting cells involved as the link between the innate and the adaptive response. Tumor associated macrophages (TAM) are important components of precancerous lesions and cancer caused by HPV, with direct effects on the growth, vascularization and modulation in the tumors of stroma[41,42].

There are two types of macrophages: type 1 (M1) and type 2 (M2). M1 are characterized for the production of inducible nitric oxide synthase (iNOS), ROS, and IL-12. This cytokine stimulates the production of Th1 and NK cells. M1 can phagocyte HPV, and are predominantly found in HPV infected tissue and squamous intraepithelial lesions (SIL). M2 may develop in response to IL-4 or IL-13[43], they are found predominantly in cancer[41].

In CC, the increased number of macrophages correlates with disease progression and bad prognosis. Some authors have shown that macrophages increment in SIL is correlated with progression to invasive cancer, both in the epithelium and cervical stroma, being the largest macrophage infiltration in persistent SIL. Specifically CD68+ macrophages are associated with invasive cervical carcinogenesis[41-43].

Two more points are important. First, CC cells also express macrophage attractants, including colony stimulating factor (CSF-1) then serum levels of CSF-1 are increased in patients with HPV infection and SIL[42]. Second, the macrophages phagocytize, *in vitro*, the C33A and C114 human tumor cell lines that express the HPV 16 E6 protein. In turn, these lines are not capable of activating the perforin secretion of NK cells[44].

Natural Killer cells

NK are cells of lymphoid origin, involved in defense against infected cells and are essential components of innate immunity against viruses that respond via direct destruction and inflammatory cytokine secretion[9]. Viral infection stimulates the production of IFNs by macrophages and DCs that are responsible for the activation of NK cells, which in turn performs lysis of infected cells. These cells can activate themselves and other cells of the adaptive immune system[45]. Cell lines

of human epithelium infected with HPV and low-grade SIL tissue are resistant to cytolytic activity of NK cells[41,46].

A variant of NK cells expressing the T cell receptor (TCR), named NKT cells recognize CD1d, a glycoprotein of the major histocompatibility complex class 1 (MHC-I) that presents lipid antigens. The HPV E5 protein is associated with low expression levels of CD1d on the surface of virus-infected tissue with high risk (HPV16) and also with low risk viruses (HPV6); which can alter the immune response mediated by CD1d, and thereby avoid destruction by innate immunity of the infected cells. While CD1d is hardly expressed in the cells' membrane, mRNA levels are not affected, so that the effect of E5 is at post-transcriptional level. The recognition of CD1d induces the production of inflammatory cytokines like IFN-γ. Then CD1d molecule is involved in innate immunity, and may also serve to activate adaptive immunity[47].

Cytokines

Protein molecules are secreted by various cells and act as regulators of cellular functions, and activators of the immune system[9]. Some cytokines (TGF-B, TNF, IL-1, IFN type 1 and 2) are in high concentration *in situ* and can control the growth of infected cells. In contrast, when the cytokines are in low concentration it allows viral persistence, disease progression and malignant transformation[48].

Viral transcription is an important target in the defense mechanisms of innate immunity. The IFN-1 is a family of cytokines involved in cell proliferation inhibition and interference of viral replication and transcription. Within the IFN-1 family, IFN-α, β, Ω, ε, λ and κ, are found which are the first line of antiviral defense. Within the IFN-2 family, IFN-γ is found[49,50]. IFNs are capable of activating and attracting immune cells including neutrophils, macrophages, NK and CD. However, it has been observed that E6/E7 proteins of HPV decrease the production of IFNs and molecules of the IFN signaling pathway. In this pathway participates the ISF-3 transcription complex, a mediator of IFN signaling. This transcription complex is formed by the association of STAT-1, STAT-2 and p48 proteins. In presence of E6 protein, there is a decrease of IFN-α, IFN-β and STAT-1 mRNA expression[33]. Attached to this, it has been observed that IFN-α not effectively inhibits E6/E7 transcription in human cervical cell lines. It has been shown that IFN-γ is present in the tissue of those with HPV infection regression.

Moreover, it has been found that patients with negative response to IFN-α treatment have high E7 levels[1].

There are conflicting studies on the therapeutic effectiveness of IFNs. Scott (2001) reported that IFN-γ but not β can inhibit the E6 and E7 transcription of HPV 16, 18 and 33 in immortalized KCs. In HPV transformed keratinocyte line (HPK-IA), IFN-β reduces the transcription of HPV 16 E6 and E7 genes, but has no effect on the IFN-γ or α[48].

The mechanism of inhibition by E7 is unclear. On the one hand it is known that the HPV E7 protein inhibits the IFN-α mediated signal transduction. Moreover *in vivo* studies demonstrate that HPV18 E7 expression reduces IFN-β by inhibiting the function of IRF-1[30]. By contrast, another study found that E7, does not directly affect the IFN pathway, and requires the coexistence with E6, it means E6/E7 complex is even more effective than only E6[33]. It has been observed that E6 might decrease the IL-8 transcription[1]. Another important cytokine is IL-2, a central mediator in both the innate and adaptive immunity because it induces the IFN-γ secretion produced by NK, NKT, T helper and cytotoxic T cells[47]. For other interferons, it has been reported that the constitutive transcription of IFN-k, is inhibited in HPV-positive keratinocytes and it has also been demonstrated that the E6/E7 genes of HPV16, 18, or 31 are sufficient to inhibit IFN-k transcription[51].

There are several reports on the expression of cytokines. Jayshree in 2009, showed a cytokine profile in SIL characterized by an increase in the expression of IL-2R, IL-4, TGF-β and IL-10 and a decrease in the expression of IL-2 and IFN-γ. In a comparative study of patients with SIL, CC, and normal cervix there were no differences in IL-1α, IL-6 and TNF-α mRNA expression levels, while patients with SIL and CC, had a diminished expression of IFN-γ[52]. In other study using the immunofluorescence method, it was observed that the expression of TNF-α, IFN-γ and IL-2 α was raised in patients with SIL[53].

Contradictorily, Scott (2001) found that in women with HPV infection the IFN-γ transcriptional level was increased, whereas IL-4 expression was decreased[54]. Constitutively, the KCs secrete low levels of cytokines but they increase the secretion in response to various stimuli. For example, in HPV transformed KCs, TGF-β and TNF are increased, which may inhibit the growth of HPV immortalized epithelial cells[48]. Other diminished genes are PKR and MxA, 2 −5 oligoadenylate synthetase 2[55, 56, 57].

Evasion

The viruses use mechanisms that deregulate the pathway of antiviral innate immunity, providing, in this way, their immune evasion and the persistence of the infection in the host[10]. The KCs are programmed to natural cell death in a term of approximately 70 days, explaining the fact that the infected cells generate no danger signals, due to lack of exposure to the host immune system. Moreover, in the initial phase of HPV infection there is no inflammation, since the HPV cycle is lytic with no production of pro-inflammatory signals that could activate the DCs. Similarly, the HPV DNA is amplified in small quantities (30-50 copies of viral DNA per infected cell), so that it cannot be detected by the host immune system and the answer to the virus does not occur or occurs very late[1,13].

During the CC, HPV does not activate DCs by selective mutation of viral L1 protein, thus viral particles cannot be assembled in large quantities, and are no longer capable of activating MyD88 of the innate response. Low levels of L1 expression are produced in the upper layers of the epithelium where also the HPV16 L1 protein is unable to activate the LCs. Deletions, insertions and changes in the open reading frame of HPV16 L1 result in low L1expression in carcinomas[58].

Other form of viral evasion is molecular mimicry, defined as the process by which the structural properties of a viral molecule simulate a host molecule. Xeroderma pigmentosum group G protein (XPG) and retinoblastoma binding protein I (RBP-1) are proteins that protect against tumor formation, the former repairs DNA and the latter suppress tumors. These molecules have a common domain with E7 protein HPV16 so that it can escape immune recognition, probably by inhibiting the function of these proteins or by competition with them[1].

E6 and E7 proteins have significant activity in the innate immune evasion by HPV. These proteins interfere with the TLR expression, particularly TLR9. This receptor is inhibited in cells expressing E6 and E7 proteins of HPV16[29]. The HPV16 E7 protein inhibits the IFN- production, being a possible mechanism of HPV to avoid elimination[59] (Figure 2).

CONCLUSION

There are several mechanisms by which the innate immune system can interfere with HPV infection, from physical barriers such as skin and mucosal cells as well
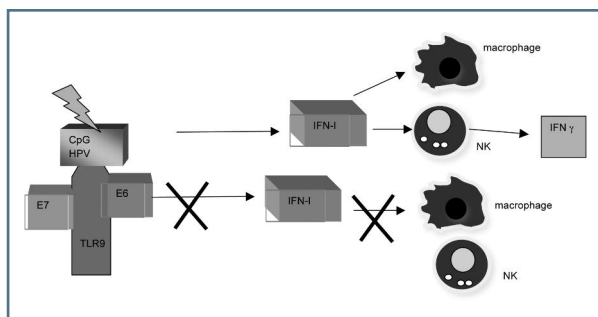
FIGURE 2. Evasion of innate immunity by E6 and E7. HPV CpG domains activate TLR, especially TLR9, which stimulates IFN type 1 synthesis, allowing the activation of macrophages and natural killer with subsequent production of cytokines such as IFN-γ. When the E6 and E7 viral proteins are produced, they are attached to TR, inhibiting IFN type 1 synthesis by preventing the activation of innate cells and cytokines

as keratinocytes to special cells of the immune system such as CD, CL and NK, hindering the development of the disease through the production of IFN or cytokines, either to eliminate the virus or otherwise activate the adaptive response. Activation of these responses has been observed in patients where there is a recovery and no persistence of the disease. Therefore innate immunity is crucial for the host response, even deleting the virus or activating the adaptive response to combat strongly against HPV.

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