Immune response and immunotherapy in intraepithelial and invasive lesions of the uterine cervix

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Summary

Infection with the human papillomavirus virus (HPV) induces innate and acquired immune responses in the cervical stroma, which are a delicate, balanced and generally unpredictable immunological defense. Because of the immunological breaks that the HPV virus causes, eradication of infected cells does not occur, potentially leading to development of intraepithelial and invasive lesions. Advances in our understanding of the immune system and in the definition of antigens in tumor cells has led to many new treatment strategies. As a result, immunotherapy has the potential to be the most specific treatment for tumors, and one that requires elaboration. Recently, immunotherapy with interferon and dendritc cells has been used on intrapepithelial and invasive cervical lesions with promising results.

Key words: Cervical intraepithelial neoplasia; Human papillomavirus; Immunotherapy; Interferon and dendritc cells.

Introduction

With approximately 500,000 new cases in the world each year, cervical cancer is the second most common cancer among women and is responsible for the death of approximately 230,000 women each year. Its incidence is two times higher in less developed countries than in developed ones [1]. In some developing countries it is the most common form of malignant neoplasia in woman and may comprise up to 25% of all cancers among women [2].

In cervical intraepithelial neoplasias (CINs), the arrangement of the squamous cells of the ectocervix remains disorganized and the cells stay atypical. When disorganization occurs only in the deepest third, there is light dysplasia, or CIN grade 1. When the disorganization involves the two deepest thirds of the epithelium, preserving only the most superficial layers, there is moderate dysplasia, or CIN grade 2. If the disorganization is observed at all levels, involving more than two thirds of the epithelium, there is CIN grade 3 or carcinoma *in situ*. The high-grade cervical intraepithelial squamous lesion classification includes CIN grades 2 and 3 and carcinoma *in situ* [3]. In accordance with the Bethesda classification, low-grade squamous intraepithelial lesions (LSILs) correspond to light dysplasia/CIN 1, and cell changes associated with human papilloma virus (HPV) and high-grade squamous intraepithelial lesions (HSILs) correspond to moderate dysplasia/CIN 2, severe dysplasia, carcinoma *in situ*/CIN3 [4].

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Cervical intraepithelial neoplasia and the human papiloma virus

HPV is a double-stranded DNA virus, with approximately 8000 base pairs, belonging to the *Papovaviridae* family, which is transmitted through sexual relations with an infected partner or by means of fomites, which infect the skin or mucous in the form of warts [5, 6]. To date, 130 subtypes of HPV have been described [7], of which approximately 25 infect a woman's anogenital area. Based on their association with cervical cancer and precursor lesions, HPVs can be divided into high-risk HPVs (16,18,31,33,34,35,39,45,51,52,56,58,59,66,68 and 70) and low-risk HPVs (6,11,42,43 and 44) [8].

The relationship between HPV and cervical cancer is of greater significance than the relationship between smoking and lung cancer [9]. The four most common types found in cervical cancer cells are 16, 18, 31 and 45, with HPV 16 making up nearly half the cases in the US and Europe and types 18, 31 and 45 accounting for 25-30% of the cases [2]. HPV 16 and 18, the principal high-risk subtypes, have a high correlation with pre-neoplastic lesions that can evolve into cervical cancer, even higher than other types of oncogenic HPV. One study in the United States involving more than 20,000 women demonstrated that the incidence rate of HSILs was 17.2% among those infected with HPV 16 and 13.6% among those women infected with HPV 18, while the incidence of women infected with other oncogenic types of HPV was only 3% [10].

Molecular biology techniques prioritize knowledge about the epidemiological profile of HPV infection and allow the sequencing of HPV-DNA, as well as the recognition of different viral subtypes [11]. Polymerase chain reaction (PCR) is now considered the most sensitive method for detecting HPV infection, since *in situ*

hybridization is limited by the number of copies of HPV [12, 13]. Studies using molecular biology techniques have suggested that, despite having a normal cytology, 40% of sexually active young women have HPV DNA and that its prevalence diminishes with age [14, 15].

Hybrid capture is an extremely sensitive and easily handled but costly method [16]. Research shows that PCR detects more high-risk HPV than hybrid capture and that both PCR and hybrid capture have a high negative predictive value for high-grade lesions; although the sensitivity of PCR is greater and its cost is lower [17]. In conization material for CIN 3, the use of PCR detects HPV-DNA 16 and/or 18 in most positive cases (87%), and only 9.1% of cases present HPV 6 and 11 together. In this study, HPV 18 was the most common HPV (78.7% of cases) and was frequently associated with HPV 16 but rarely with HPV 6 and 11 [18].

Immune response in intraepithelial and invasive lesions of the cervix

Tumor immunology is a promising area of cancer research and is already used in medical therapy [19]. Tumor-specific immune responses are observed in cancer patients, given that in the tumor mass they show infiltration with cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. Altered patterns in class I and class II main histocompatibility complex (MHC) molecules are observed on the surface of tumor cells [20, 21].

The first line of the innate defense response begins in the stroma, but is also present in the epithelium or near it [22]. This non-specific resistance occurs when pathogenic agents employ mechanisms to escape the immunology system, evading an adaptive (specific) immune response. Innate immune response is measured in various ways, including interferon (IFN) induction and the activation of macrophages and NK cells, which can lead to the activation of a specific response.

Cell immunity measured by T cells plays an important role in the eradication of cells infected by HPV. Flaws in the induction or maintenance of T-cell response can lead to persistent infection and to the development of malignant neoplasias [23]. T CD4 lymphocytes help in immune response by promoting the secretion of cytokines and are mediators that activate immune response cells, as well as macrophages and B lymphocytes. The T CD8 lymphocytes promote infected or tumor cell death through the action of their toxic granules [24].

Immune responses are moderated by the liberation of different cytokines. Those which are secreted by T CD4+ lymphocytes were originally classified as T helper 1 (Th1) and T helper 2 (Th2). Recently, a new population, T helper 3 (TH3) and T regulatory (Treg), have been discovered; these can indirectly suppress immune response by reducing the expression of co-stimulators in the antigen presenting cells (APCs) or suppress it directly in T cells. The most important Th1-pattern cytokines are IL-2, IL-12, IFN-γ and TNF-α, which are responsible for activating cell immune response. Th 2-patterns, such as IL-4, IL-10 and TGF-β, are directly related to the activation of humoral immune response. The Treg type has already been characterized by the presence of IL-10 and TGF-β. This pattern seems to involve the induction of tolerance.

Regression of HPV infection has been associated with an immune response mediated by Th1-pattern cytokines and the development of CIN seems to be moderated by Th2-pattern cytokines [25]. Inflammation, which plays an important role in innate immunity, is stimulated by cytokines like IL-1 and TNF-α, which are synthesized by keratinocytes following aggression by a viral infection - HPV, for example [26]. These cytokines stimulate changes in the adherence of molecules and their capillary permeability, and also lead to the liberation of other cytokines [27]. Acute inflammation leads to the elimination of infection and tissue repair and is responsible for unleashing acquired immunity. Changes in the Th1 and Th2 cytokine profiles have been demonstrated in humans with neoplastic diseases [28]. In addition, chronic inflammation, which occurs in cases of persistent infection, has been considered to be a risk factor in the activation of carcinogenesis [29] (Table 1).

Table 1. — *Type 1 and type 2 cytokines*.

Type 1 cytokines	Type 2 cytokines
T-helper 1 response	T-helper 2 response
Immunity mediated by cells	Humoral immunity
IL-2, IL-12, IL-15	IL-4, IL-5, IL-6, IL-10, IL-13
Interferon gamma (IFN-γ)	

IL, interleukin.

Various studies have described how HPV interacts with the immune system [30] and how the virus deactivates adaptive immune response [31]. There is a decrease in the expression of class 1 MHC molecules and in T cell receptors (TCRs), impeding the presentation of antigens to T cells. In this way, the immunological recognition of HPV-infected cells on the part of the host's CD8+ lymphocytes is reduced. Moreover, the virus does not have a blood dissemination phase, or does it cause lysis of keratinocytes, and therefore does not induce an inflammatory immune response. The production and liberation of the virus also occurs in different squamous cells that are far from the immunocompetent and cytokine cells in the submucosa [30, 32, 33].

Systemic and local immune responses play important roles in the progression of CIN. The presence of greater infiltration of T (CD3) lymphocytes in patients with CIN 3 is linked to a greater frequency of recurrence after conization. All women with recurrent CIN 3 showed a high percentage of CD3 lymphocytes in their cell count [34].

The profile of nitrous oxide (NO) cytokines in the evaluation of local immune response in patients with bacterial vaginosis and CIN has already shown an increase in the local production of IL-8, IL-10 and NO, demonstrating an immune response against the tumor or developing tumor from these respective mediators [35]. NO has also

Table 2. — *Immunological transformations in the cervical stroma during carcinogenesis*.

Inflammation/ resistant to infection	HPV infection risk factors for carcinogenesis	CIN progression	Invasive cancer prognosis
Increase in macrophages	CD4+ reduction	Reduction in CD8+	CTL or TIL cells NK cells
Increase in NK cells	Reduction in IFN	Reduction in CD4+	Increase in CD3
Increase in IFN	Reduction in type 1 cytokines	Reduction in CTL	Increase in iNKRs
Increase in IL-1	Increase in type 2 cytokines	Reduction in IL-10	Progressive loss of type 1 cytokines
			Low IFN activity
			MHC I damaged
			IL-4r 75v HLA polymorphism

been evaluated by Fernandes and collaborators, who confirmed its liberation by tumor cells, as well as other soluble circulating mediators [36]. NO can interfere with the initial capacity for neutrophil migration, impeding immune response. Neutrophils can have anti-tumor properties, and their migration is very important for the inflammatory response.

The upper part of the female genital tract has both an innate [37] and acquired [38] immune response, which keeps it in a predominantly aseptic state. There is a delicate balance between pathogenic agents and tolerance to semen [39], pregnancy, and cervical cancer. In the early stages of cervical cancer, HPV-infected cells must bypass the immune system. As the disease progresses, there is a reduction in type Th1 cytokines, and type Th3/Treg cytokines become predominant [40]. Patients with CIN also show a reduction in the expression of receptors for IFN-α in comparison to that in normal tissue. Low levels of IFN-γ mRNA copies are seen in tumor biopsies and are related to prognosis [41].

In studies of the microenvironment of the normal cervix examining CIN 2/3 lesions in immunocompetent women and CIN 2/3 lesions in HPV-infected women, the immunocompetent women with CIN had a mixture of pro-inflammatory cytokines and regulatory cells, characterized by an increase in the production of IFN-γ by CD4+ or CD8+ T cells and NK cells, as well as an increase in the regulation of tumor growth factor beta (TGF-β) [42]. In CIN/HIV+, the numbers of cells and regulatory cytokines were down-regulated. Cervical cancer cells can promote the expression of inhibitory natural killer cell receptors (iNKRs) via IL-15 and, possibly via a mechanism mediated by TGF-β and by the antitumor cytotoxic cancellation of TILs (tumor infiltration lymphocytes) [42]. Advances in our understanding of the immune system and in the definition of antigens in tumor cells have encouraged a number of new strategies. Therefore, immunotherapy has the potential to be the most specific treatment that can be developed for tumors.

Practical application of the study of immune response: the role of immunotherapies

The objectives of immunotherapy are activating the immune response, bolstering its natural efficiency, detecting the multiplication of malignant cells, and selectively eradicating tumors without causing lesions in the patient. A vaccine for certain types of HPV that could reduce the frequency of cervical cancer is already on the market. However, this vaccine should not be associated with the prevention of this cancer, but with protection against some types of low- and high-risk HPV [43]. Recently, immunotherapy with interferon (IFN) and dendrite cells has been used on cervical intraepithelial and invasive lesions with promising results [44, 45].

IFN, which has been under study since 1957, was discovered during research on the phenomenon of viral interference. But its mechanism of action is not completely understood. It is known that it encompasses a group of cytokines with important immune system functions, such as inhibition of viral multiplication, immune modulation to stimulate NK cells and monocytes, and an antiproliferative effect, as well as an anti-angiogenic action [46]. There are more than seven types of IFN, which act on the immune system in different ways [47-49]. Recent studies have shown that IFN can be applied to many different kinds of pathologies, including viral hepatitis, multiple sclerosis and cancer. Since the early 1980s, various studies have used IFN in the treatment of gynecological cancer with varying results [50, 51]. Murta and Tavares achieved good results in demonstrating its efficiency, obtaining a complete response in the treatment of a patient with invasive vaginal carcinoma using intralesional IFNα-2b with complete remission of the lesion [44]. The actions of IFN, both anti-proliferative and immunoregulatory, are today an area of interest to many researchers.

Immune responses can often cease controlling the growth of tumors because these responses are ineffective or because the tumors have ways to escape the immune system. The principal mechanisms by which tumors escape are: reduction in production of tumor antigens; reduction in the expression of MHC molecules; and production of immunosuppressor proteins [52]. These escape mechanisms can act on dendritic cells (DCs), inhibiting their activation and their migration to secondary lymph nodes for activation of T lymphocytes.

The DCs are the main cells that present antigens. They are extremely efficient in activating CD4+ and CD8+ T lymphocytes and can maintain tolerance or unlock immune responses [53]. Because of their role in controlling immune response, interest in studying DCs has surged in recent years in the hope to fully use them as therapeutic tools. Today, the manipulation of *in vivo* or *ex vivo* DCs is thought to be one instrument that will lead to progress in the use of immunotherapy in cancers. In fact, DCs are considered an ideal tool for the development of therapeutic vaccines against cancer since numerous clinical studies have shown their efficacy against tumors [45].

Thus, bearing in mind the existence of tumor escape mechanisms that stimulate the persistence or progression of a tumor, the development of immunotherapy to treat tumors must take priority. The principal strategies for cancer immunotherapy mainly aim to prove their anti-tumor effects on patients, actively immunizing these patients against their tumors, and stimulating their own immune responses. Indeed DC therapy is one immunotherapy that seems promising in terms of stimulating anti-tumor immune responses.

With the goal of evaluating the potential of autologous DCs pulsed with the E7 antigen of HPV16 and HPV18 to serve as a therapeutic cell vaccine for patients with recurrent/metastatic cervical cancer resistant to conventional treatments, Santin and collaborators [54] obtained good results: the vaccine was tolerated well by all the patients, and it did not cause local or systemic side-effects. However, patients who developed positive delayed-type hypersensitivity (DTH) showed lower tumor progression (survival beyond 13 months), while the DTH negative patients died within five months after the start of therapy. The number of monocytes//Trx80-activated-monocytes (TAMs) and DCs was positively correlated with the expression levels of growth factor of colonies of granulocytes and macrophages (GM-CSF) and alpha tumor necrosis factor (TNF-α). This finding suggests a role for these cytokines in the differentiation of monocytes in mature DCs, which may constitute an escape mechanism for cancerous cells [55]. The DC vaccine seeks to create the conditions for these cells to develop and to allow for the execution of that which the tumor has not yet permitted-the migration to secondary lymph nodes so that antigens to the T lymphocytes can be presented, inducing their activation and, consequently, the initiation of an anti-tumor immune response.

Many technological developments in clinical immunology for research and diagnosis of HPV infections and their relationship to cervical cancer have been obtained in the last few decades. The techniques that emerged have been a direct result of immunological reactions revealed through markers (immunofluorescence, immunohistochemistry and flow cytometry). The characterization of the structure, gene expression, synthesis of selected genes and their manipulation in cells and animals is made by molecular biology. Through DC vaccine treatment, it should be possible to analyze immune response to treatment, leading to an understanding through flow cytometry of the induction mechanism, the subtype of activated T lymphocyte, the cytokines produced, and the profiles of immunological memory.

Final Considerations

In summary, infection with HPV induces innate and acquired immune responses in the patient's cervical stroma. This immunological defense is delicately balanced and not always possible to predict. Clinical practice is mainly determined by risk, evolution, and prognosis (Table 2). The study of immune responses is complex, and there is still much to be understood about cell interactions and cytokines. Immunotherapy, by stimulating the immune system through the use of biological response modifying substances, has the potential to offer the most specific treatment for tumors. Developments in the understanding of the immune system and in the definition of tumor cell antigens have led to many new treatment strategies that are based on the mechanisms involved in immunity mediated by cells or their mediators.

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