Immune response in cervical intraepithelial neoplasms

Priscila Thais Silva Mantoani[1], Daniela Rodrigues Siqueira[1], Millena Prata Jammal[1], Eddie Fernando Candido Murta[1], Rosekeila Simões Nomelini[1,*]

[1] *Department of Gynecology and Obstetrics, Research Institute of Oncology (IPON), Federal University of Triângulo Mineiro, 38025-440 Uberaba, Brazil*

*Correspondence: rosekeila@terra.com.br; rosekeila.nomelini@pesquisador.cnpq.br (Rosekeila Simões Nomelini)

Objective: The current study aims perform a comprehensive overview of the topic immune response in cervical intraepithelial neoplasms, summarizing the findings of literature. *Data sources*: PubMed database. *Methods of study selection*: A search for the following descriptors was performed in the PubMed database: descriptor "immune response in cervical cancer and Human Papilloma Virus (HPV)"; "immunotherapy in premalignant cervical lesions". *Tabulation, integration and results*: The articles identified were published between 1967 and 2021. We selected 85 articles for review on the subject (reference 16 onwards). This literature review shows the important role that the immune system plays in the development and progression of cervical cancer. Immune response in pre-neoplastic cervical lesions includes host defense mechanisms against the HPV, adaptive immunity and the function of cytokines. Predictive factors of viral persistence and progression of premalignant lesions may also be associated with immune response. *Conclusion*: One of the determining factors for the persistence or elimination of HPV infections and their evolution to pre-neoplastic lesions is the cellular immune response, as the progression or regression of the tumor depends on the type and amount of cytokines secreted by the body. The investigation of these immune reactions may provide new therapeutic targets for cervical intraepithelial neoplasms.

Keywords

Cervical intraepithelial neoplasms; Immune response; Cervical cancer; Human Papilloma Virus; Immunotherapy

1. Introduction

Initially, cervical lesions that predispose to cervical cancer were classified as mild, moderate and severe dysplasias. With the Bethesda classification system, cytological changes were differentiated from histopathological changes, being called, respectively, intraepithelial lesion and cervical intraepithelial neoplasia. The low-grade squamous intraepithelial lesion (LSIL) is the cytological expression of cervical intraepithelial neoplasia (CIN) 1, representing a transient action of Human Papilloma Virus (HPV) virus in an episomal manner in the lower third of the cervical epithelium close to the basement membrane. Consequently, the high-grade squamous intraepithelial lesion (HSIL) would be the cytological correspondent of CIN 2 and 3, that represents the true precursor lesion of cervical cancer in which HPV causes more intense cytoarchitectural changes in at least two thirds of the thickness epithelial of the uterine cervix [1].

Cervical cancer is characterized by uncontrolled cell replication of the epithelium, which can extend to the stroma and become invasive to adjacent structures and organs. The epidermoid histological type occurs in 90% of cervical neoplasms, affecting the squamous epithelium, while adenocarcinoma affects approximately 10% of women, with cellular changes in the glandular epithelium. Its main cause is persistent infection with oncogenic types of HPV, with approximately 570,000 new cases expected per year worldwide, 85% of which are diagnosed in developing countries and 15% in developed countries [2].

HPV is a virus with a diameter of 52 to 55 nm. It has an isoecahedral capsule with 72 capsomeres whose double helix genome of DNA (deoxyribonucleic acid) contains approximately 8000 base pairs. These pairs are encoded in three main portions: early (E1 to E8) which encodes non-structural proteins that regulate cell activity, late (L1 and L2) responsible for coding capsid and regulatory proteins (long control region—LCR) capable of regulating gene expression and viral replication [3].

They are cutaneous-mucous tropism viruses with more than 200 subtypes classified as low and high risk according to the progression of the lesions to malignancy [4]. The oncogenic potential is directly related to the ability to integrate the viral genome into the host cell and the production of E6 and E7 oncoproteins that interfere with cell autoregulation by inhibiting p53 and pRb promoting their degradation or inactivation, preventing the apoptosis of cells from inadequate division cellular [5, 6].

The types of high-risk HPV that infect the ano-genital tract include 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 leading to a greater propensity infected cells evolve into invasion [7]. The types of HPV of low oncogenic risk are found in the episomal form in the host cell, that is, they are not linked to its DNA and, thus, its cell clearance mechanism is facilitated.

HPV is transmitted through sexual contact and has a high prevalence worldwide, being the main sexually transmitted infection. It is known that up to 80% of women in the world have contact with the virus at some point in their lives [8, 9].

Factors related to the virus, such as its genotype and the presence of more than one oncogenic type, and to the host,

have been related to the persistence of the infection, which represents the central factor of progression. Factors related to the host are immunosuppression, smoking, other sexually transmitted infections, nutritional deficiencies and use of hormonal contraceptives [10]. Non-sexual transmission of HPV is still controversial, less important and therefore undervalued in the study of this viral infection. Another form of HPV transmission is vertical transmission, through the umbilical cord and placenta [11], leading to perinatal infection [12] and newborn laryngeal papilloma. In addition, there is transmission by fomites and self-inoculation [13], proving viral infectivity at room temperature, being considered important non-sexual forms of HPV transmission [14].

According to the International Agency for Research on Cancer [2], the number of new cases of cervical cancer worldwide reaches 569,847. Regarding deaths in the same period, 311,365 lives were lost globally, a total of 3.3% of all cancer deaths in women [15].

In Brazil, despite significant regional differences, cervical cancer is the third in incidence in women, surpassed only by malignant breast and colorectal neoplasms. There are an estimated 16,710 new cases, with an estimated risk of 16.35 cases per 100,000 women for the 2020–2022 period. It is the fourth leading cause of female death from cancer, with 6526 deaths expected in 2020 according to data from the National Cancer Institute. It is a rare malignant tumor before 30 years and has a peak incidence between 45 and 50 years, with progressive mortality rates from the fourth decade of life [2].

HPV infection induces innate and acquired immune responses in the cervical stroma. The current study aims perform a comprehensive overview of the topic immune response in cervical intraepithelial neoplasms summarizing the findings of literature.

2. Methods

A search for the following descriptors was performed in the PubMed database: "immune response in cervical cancer and Human Papilloma Virus"; "immunotherapy in premalignant cervical lesions". The articles identified were published between 1967 and 2021. We selected 85 articles for review on the subject (reference 16 onwards) by one of the authors and after, the other authors helped to write the topics covered.

3. Results

The articles identified were published between 1967 and 2021. We selected 85 articles for review on the subject. The flow diagram shows the number of articles included in the study (Fig. 1).

3.1 Immune response in pre-neoplastic cervical lesions

3.1.1 Host defense mechanisms against the Human Papilloma Virus

The host's defense against various viral infections is based on the innate non-specific and adaptive immune response, which is specific and capable of preventing future reinfections. Physical barriers, such as intact epithelium and keratinocytes in proper differentiation, as well as protective sub-
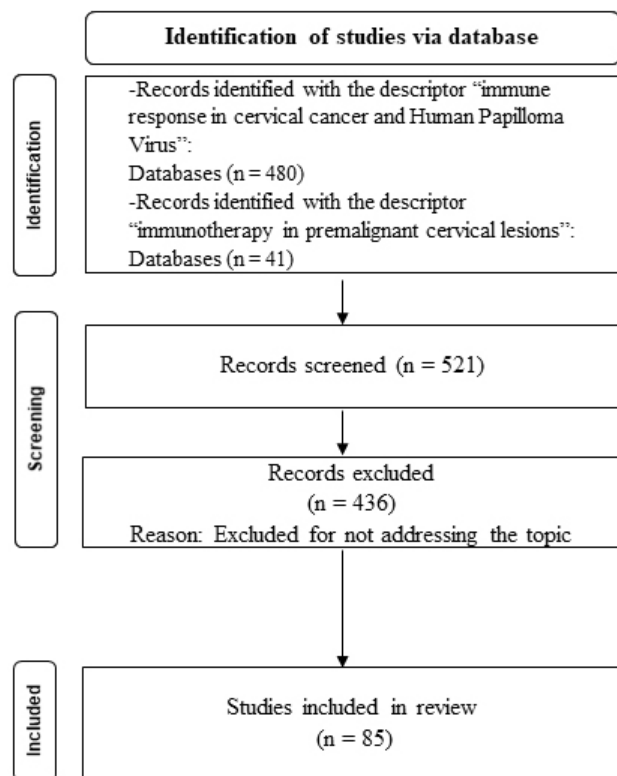

Fig. 1. Flow diagram: articles included in the study.

stances in local mucosa such as antimicrobial peptides and cytokines making local mucus more viscous [16, 17], are the first barrier of host against HPV infection.

The innate immune response triggered by HPV infection recruits local defense cells such as natural killer cells, dendritic cells, Langerhans cells, and natural killer T cells for the HPV-infected microenvironment [18].

In the adaptive immune response, TCD4 lymphocytes generate an immune response in two Th1 and Th2 pathways depending on the type of cytokine produced. The Th1 pathway produces cytokines responsible for the activation of the cellular immune response and the Th2 pathway for the humoral immune response [19].

In general, the majority of the population has acquired HPV infections. However, only 10 to 15% of these individuals develop persistent infections throughout their lives, and of this group, few have the potential to progress to neoplasia, which suggests adequate immune defense for initial infections [20]. In a small portion of the infected population, the virus develops mechanisms to evade the host's defenses.

Because it is an intraepithelial virus that does not generate systemic infections and does not have a viremia phase in its cycle, a small number of its copies are exposed to the host's immune defense and remain invisible for long periods [21].

The infection and evolution potential of the HPV virus is totally dependent on the differentiation of keratinocytes from basal to superficial cells, thus small abrasions on the skin and mucosa damaging physical barriers facilitate the viral process.

However, being in keratinocytes, HPV is able to evade the innate immune response, with no mechanisms of apoptosis or cytolysis in these cells that complete their differentiation from the basal layer to the superficial layer away from the local immune defense, without an inflammatory process [21].

High-risk HPV is able to promote a reduction in the immune response dependent on IFN-α and IFN-β, which are fundamental in antiviral immune defense by activating the innate and adaptive immune response through viral transcription [22].

Through pinocytosis, HPV goes beyond the extracellular matrix reaching the interior of the host cell, passing through endosomes/lysosomes reaching the Golgi complex towards the cell nucleus [23, 24]. In the nucleus of the host cell, HPV finds a favorable environment for replication and proliferation, a fundamental point in the infectious process of this virus.

By escaping autophagy [25], promoting the loss of the nuclear envelope in the host cells [26] and inhibiting the action of α-defensins [27], HPV achieves success in the infection process.

3.1.2 Innate immunity

HPV is a DNA virus that migrates in the host cell towards the nucleus, where it finds a suitable environment for its replication using biological instruments already in activity. During this process of movement, the viral DNA would be easily recognized by the antiviral defense system that understands any genetic material outside the cell nucleus as foreign, with the immediate elimination of viral genetic material foreign to that of the host cell [28]. In the host cell, HPV has its DNA recognized and eliminated by substances such as interferon-γ (IFN-γ) 16 (IFI16), and cyclic guanosine-adenosine synthase (cGAS) [29]. In addition, inflamosome AIM2 is released by the host DNA itself, which fights viral DNA with caspase-1 and interleukin-1β (IL-1β), activated especially in keratinocytes infected by HPV 16 [30].

IFI16 restricts the replication of the HPV genome and the transcription of the gene, increasing the association of heterochromatin with early and late promoters [31].

The greater expression of toll-like receptors (TLR) (TLR2, TLR3, TLR7, TLR8 and TLR9) is common in women who manage to eliminate the initial HPV infection [32]. The increased production of cytokines IFN-α and IFN-β is the result of the recognition of the host cell of viral pathogens, including HPV [33], and treatment with IFN-β prevents the entry of the virus and promotes the elimination of latent episomal form in infected cells [34].

Dendritic cells (DC), Langerhans cells (LC), natural killer cells (NK) and natural killer T cells (NKT), essential in the innate immune response, are activated in response to HPV infection [18], with the release of several cytokines IFN-α, IL-6, tumor necrosis factor α (TNF-α) and IL-8 [35]. In addition, the increase in HPV infection and the incidence of cancer associated with HPV have been observed in individuals with various functional deficiencies of NK cells [36].

3.1.3 Adaptive immunity

As it does not cause viremia or cilolysis, HPV suffers a reduced action from the systemic immune response, despite the proven action of host T cells [37], which stands out in the host clearance of this viral infection [38–41].

In general, the antibody titers produced from the infection are low to be effectively protective, which suggests that HPV is able to efficiently prevent the host's response during the natural course of the infection [33].

3.1.4 Escape mechanism of the host's immune system

HPV has mechanisms that allow it to escape from the host's immune system. During viral replication, the high expression of these proteins is limited to keratinocytes and as the viral infection is unable to lyse keratinocytes, antigen presenting cells (APC) and Langerhans cells (LC) have minimal chance of initiating an immune response such as phagocyte virions and subsequently present viral antigens [42]. This, the cervical epithelium ends up being a protective niche for HPV [43]. Another escape mechanism is the resistance to apoptosis via the FAS receptor that cells infected with HPV acquire [44].

HPV is also able to modulate the expression of the host gene by deregulating the methylation of the host's DNA, modifying histones and transcription factors [16]. In addition, viral infection disrupts the functions of numerous proteins, for example, HPV E6 and E7 oncoproteins have no enzyme activity, desired locations and hijacking their activities to increase replication, virus persistence and inadvertently induce a greater degree of malignancy [45, 46]. These oncoproteins, E6 and E7, often inhibit the host protein functions by direct binding [16], repress the TNF-α-inducible NF-kB activity in cervical cancer cells, promoting the appearance of cervical cancer. This loss of NF-kB allows the virus to restrict the host's immune response and stimulate continued HPV infection, promoting cancer growth and progression [47].

For effective control of HPV infection, both the innate and adaptive immune responses are critical. As previously described, HPV modulates a number of mechanisms to prevent host immune responses during persistent infection.

3.1.5 Cytokines in CIN and HPV

Cytokines are proteins produced by several cells of the innate and adaptive immune response with an important role in biological activities, mainly related to their capacity for pleiotropism and synergism. They act in regulating the magnitude of the immune response, inflammatory response and hematopoiesis, acting locally and systemic [48]. Thus, the individual analysis of the biological function of each of them, especially in tumor progression, is an extremely difficult task.

TCD4 lymphocytes generate an immune response in two Th1 and Th2 pathways depending on the type of cytokine produced. The Th1 pathway produces cytokines responsible for the activation of the cellular immune response and the Th2 pathway for the humoral immune response [19]. Re-

cently additional pathways of immune response have been identified Th17, Th9 and Th22 [49–51].

The antitumor immune response requires an adequate balance in the production of cytokines and, consequently, tumor progression or regression depends on the type and amount of cytokines secreted in the body [52].

Tumor progression is dependent on suppression of cellular immunity and the development of an immunosuppressive pathway for T reg lymphocytes. Cytokines of T reg lymphocytes are considered to suppress immune response and T reg lymphocytes are potent inhibitors of the antitumor immune response associated with a worse prognosis in many types of cancer [53, 54].

The amount of T reg lymphocytes does not seem to be important in the progression of the lesion, but rather the amount of cytokines produced. In this way, patients with a small amount of these cells but with a large release of cytokines have a greater chance of progressing the tumor lesion. The alteration of the cytokine balance in the tumor microenvironment seems to be the fundamental point of cervical tumor progression when generating conditions for these cells to evade the surveillance of the immune system [52].

Studies suggest that in the evolution of high-grade cervical lesions and cervical cancer, there is a reduction in the amount of Th1 cells and an increase in Th2 cells [55, 56].

The progression of cervical intraepithelial neoplasia leads to a change in the pattern of cytokine secretion. There is a reduction of IL-2, IL-12 and TNF, and an increase of TGF-β. Furthermore, regulatory T lymphocytes actively participate in the induction of cell damage [52].

The reduction in the synthesis of IFN-c and IL-2 has been associated with an evolution of the histological grade of CIN and progression to cancer [57, 58]. Likewise, the development of an injury has been associated with cytokine secretion from the Th2 pathway with a reduction in IL-2 and IL-10 [59]. Immunosuppressive cytokines such as IL-10, IL-4 and TGF-B increase with tumor progression [60].

3.1.6 Predictors of viral persistence and progression of premalignant lesions

HPV infection in the metaplastic transformation zone with viral persistence, the progression of intraepithelial lesions and the invasion of altered cells beyond the basement membrane of the epithelium are decisive factors in the development of cervical cancer [20].

The cellular immune response has been shown to be one of the most important determining factors in the persistence or clearing of HPV infections and their evolution to pre-neoplastic lesions [61, 62]. Although the role of T cells in this process is not yet fully understood, it is known that in many epidemiological studies the expression of the leukocyte antigen-DRB1*1301 can be considered a protective factor [63]. In addition, the innate immune response present in the cervical mucosa plays an important role in viral clearance [61].

The integration of viral DNA with that of the host cell with persistent infection and viral load are decisive points in the evolution to high-grade neoplasms and cervical cancer [10, 64]. Although some studies question whether viral load should really be considered clinically important in this evolution, as it may not be directly implicated in the higher risk of disease progression, except for HPV 16 [65, 66].

The evolution from cervical preneoplastic lesions to invasive cancer can occur after decades in a small number of cases. In less than 10% of HPV infections, there is viral persistence and cervical intraepithelial neoplasms, which occur between 5 and 10 years of evolution. Most HPV infections with or without cytological abnormalities undergo a process of suppression or clearing by cell immunity between 1 and 2 years [61].

The longer the period of persistence of a particular viral type in the cervical epithelium, the greater the chances of an increase in the interval for clearing the virus and the possibility of establishing pre-neoplastic lesions [67].

Rarely after the viral bleaching process, HPV can reappear, occurring mainly due to a viral replication from a previous latent infection as occurs in cases of patients immunosuppressed by the HIV virus [68].

The viral types of HPV that promote persistent infections are more common, such as HPV 16. The risk conditions that lead to viral persistence are not fully understood, however the HPV subtype involved seems to be the main determining factor that leads to a persistent viral infection. progressing to pre-neoplastic lesions [69, 70].

Although the dimension in which it occurs is not fully elucidated, it is known that women with viral infections due to multiple HPV subtypes are at higher risk of developing preneoplastic lesions when compared to women with only one viral subtype [71].

Some studies suggest an association between condom use and decreased viral persistence and progression of cervical lesions [72, 73].

Immunosuppression favors the persistence and progression of cervical lesions. Patients with autoimmune diseases, particularly systemic lupus erythematosus, may be prone to HPV infection and cervical dysplasia. However, the risk factors for the development of persistent HPV-related infection should be further assessed and the issue seems to be unclear [74]. Transplant patients have a chronic condition of immunological depression that predisposes them to the appearance of tumors, in particular those that are induced by cervical, vaginal, vulvar and perianal viruses [75, 76]. Studies have shown an increased susceptibility of transplant patients to human papillomavirus cervical infection [77, 78].

3.2 Immunotherapy in premalignant cervical lesions

The search for conservative treatments in pre-neoplastic and cervical neoplastic diseases, aiming at preserving the reproductive future of some women and avoiding the persistence of HPV, motivates current studies that establish efficacy and safety in new therapies.

The immunotherapy shows great possibilities and expectations in the less aggressive and more effective treatment of CIN. Currently, a large number of new and recent researches promote constant changes and questions aiming at better and less traumatic therapeutic practices [79, 80].

Immunotherapy is a recent and promising biological treatment for several diseases, including cancer, based on the stimulation of innate and adaptive immune responses, enhancing and promoting the detection of malignant cells or cells with malignancy potential. Thus, tumor treatment is more selective and with less damage to normal cells. They can be made in the form of monoclonal antibodies (artificial versions of proteins of the immune system), immune control inhibitors (drugs that increase the action of the immune system) and vaccines (produced from the body's own tumor cells). The focus of the target immunotherapy is to eliminate or decrease cell infection through the action of cytotoxic T cells against infected cells by increasing the expression of MHC I [81].

Two distinct phases of the oncogenic infection process, the initial and the one already established over the years, are targets of immune therapy based on vaccination. With a focus on preventing initial HPV infection, the use of prophylactic vaccines is already well established in clinical practice. These vaccines induce the formation of antibodies against the viral L1 portion, preventing HPV from promoting its adhesion and entry into the host cell. Prophylactic vaccines have no global coverage and have no therapeutic effect [82, 83]. On the other hand, prophylactic vaccines for HPV infection of high oncogenic risk for cevical cancer are accepted worldwide with their established and widespread use in prevention programs, other less invasive and safe therapeutic alternatives for the treatment of pre-neoplastic cervical lesions are studied, with emphasis therapeutic vaccines.

Therapeutic vaccines have their models based on the episomal form of viral replication or on the viral sequence already integrated into the cell, with a primary focus on viral proteins E6 and E7 from infected cells of the basal layer of epithelium and cervical cancer cells that do not express viral proteins strongly L1/L2. HPV oncogenes are the main target of immunotherapy in cervical cancer. Therapeutic HVP DNA vaccines have been shown to be effective, safe and have great immunogenic potential. For this reason, they have been studied with great success, becoming more and more a modern and real possibility of pharmacotherapy against cervical diseases. The combination of anti-HPV DNA vaccines with radiotherapy, chemotherapy, immunomodulators or immune adjuvants shows a great perspective in the treatment of cervical malignant tumors [84].

Therapeutic vaccine technology is based on peptide/protein vaccines, nucleic acid vaccines (DNA for NIC), viral or bacterial vector vaccines. These cells present in vaccines are derived from dendritic cells (applied in the treatment of CINs) or tumor cells [79, 80].

Therapies with blockade of the Hippo-YAP pathway would be promising in the treatment of malignant cervical tumors. The Hippo-YAP regulatory pathway for growth, homeostasis and tumorigenesis is already well established, aiming at cell growth through the adequate control of nuclear proteins YAP1/TAZ. Its localization or regulation abnormalities lead to the formation, progression and metastasis of numerous malignant tumors, especially squamous cell carcinoma. In the laboratory, cofactors increase YAP1/TAZ in these tumors, promoting the rapid appearance of carcinoma in situ and its evolution. In cervical squamous cell carcinoma, in addition to inhibition of P53 by the E6 and E7 portions of HPV, E6 also promotes activation and accumulation of the YAP1 pathway. As a result, there is uncontrolled abnormal cell proliferation, lymph node metastasis and recurrence. Thus, anticancer treatment models such as therapeutic vaccines with blockade of the Hippo-YAP pathway would be revolutionary [85].

Studies have shown that patients with advanced or recurrent cervical cancer treated with autologous dendritic cell-derived monocytes associated with recombinant HPV-16/18 E6 or E7 vaccines have an adequate T cell response [86, 87].

A large number of studies involving gene optimization, strong viral epitopes, advanced methods of local immune reach and great molecular adjuvant efficiency in addition to immunomodulators are promising in clinical research of therapeutic vaccines. Therapeutic vaccines are not only safe and generate an immune response, but also have great clinical efficacy. In addition, they may be more advantageous in preneoplasic cervical lesions when they demonstrate the same efficacy and safety as surgical treatments, being less invasive. And they can also be a therapeutic option in conjunction with other established clinical therapies [84].

Antigen-presenting cells such as dendritic cells have the role of increasing the activity of T cells by presenting antigens from infected or tumor cells. Therapeutic vaccines trigger the activation and maturation of dendritic cells promoting the formation of cytotoxic CD8 + anti-tumor T cells. E6 and E7 viral proteins are considered ideal target molecules to trigger a strong immune response against cells infected by HPV [88].

The study by Trimble *et al.* [89] in 2015 was the first therapeutic study of DNA vaccines not associated with surgical treatment with proven efficacy. They demonstrated a regression of 48.2% of treated CIN II/III cases [89]. Currently, there are eighteen therapeutic vaccines in phase 2 and 3 of studies with different stages of evaluation showing good and promising clinical results, most of which are well tolerated with mild adverse effects at the application site and nonspecific flu-like symptoms. The use of therapeutic vaccines showed efficacy in regression from CIN 2/3 to CIN 1 or absence of CIN between 17 and 59% over at least 12 weeks of follow-up [90].

Although specific therapeutic vaccines for tumor antigens (NY-ESO-1 or HER2) and therapeutic vaccines against viral antigens of HPV 16 and 18 have shown great efficacy, these studies do not include infections by other types of HPV [91].

Imiquimod is an immunomodulatory drug capable of ini-

tiating the local immune response, through the expression of dendritic dermal cells of cytokines such as TNF-alpha, interferon (IFN)-alpha and interleukins 1, 6 and 8. There is an increase in the production of INF by dendritic plasma cells attracted from the bloodstream. This increase in cytokine production stimulates natural killer cells to eliminate cells infected with HPV or already undergoing carcinogenic transformation. Imiquimod has an action on the innate and adaptive immune response, this through the migration of mature Langehans cells to lymph nodes, presenting viral antigens and initiating the Th1 response. Specific tumor T cells produced infiltrate the affected skin. B cells produce antibodies that enhance the presentation of antigens. Thus, imiquimod has a synergistic antiviral and antitumor effect [92, 93].

The activation of innate immune response leads to adverse local changes such as hyperemia, burning and edema. Simple side effects are common such as vaginal desquamation with mild leukorrhea, vulvar itching, adynamia, fatigue and headache. In more complex situations, mucosal erosions and fever can occur. Symptoms and signs similar to a common cold can be noticed by patients. These adverse effects are most often mild and of low intensity, with complete remission with discontinuation of use. In cases of side effects, the use of low doses does not harm the treatment in the long term, and can minimize surgical or ablative treatments that may be necessary [94].

The use of imiquimod in the treatment of vulvar intraepithelial neoplasms is already well established in the literature, with good results when compared to excisional treatment. With regard to the treatment of CIN and vaginal intraepithelial neoplasia with imiquimod, studies have limited evidence [95].

Since the early 1980s, several studies have used interferon to treat gynecological cancer [96]. Interferons (IFNs) are glycoproteins that were initially described with antiviral effects [97]. Conservative therapy with pegylated interferon may be a therapeutic option for patients of childbearing age, since it does not alter the anatomy of the cervix, which is the factor most responsible for generating complications during pregnancy [98].

A study by Ramos *et al.* [99] demonstrated that women with CIN 3 treated with IFN-α and who had tumor regression expressed more helper T-profile (Th1) type 1 cytokines (IFN-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-2), with a significant reduction in the viral load of high-risk HPV in the lesions.

Another study quantified cytokine levels after CIN treatment with Pegylated-interferon-alpha-2b, demonstrating the profile of the local and systemic immune response. The study showed that this treatment could modulate the immune response profile through the production of some cytokines [100].

The association of joint or sequential therapies of efficacy and safety already established in the literature with the application of immunotherapy is the center of modern practices of

great expectation for the future treatment of malignant and pre-neoplasic cervical lesions [84].

4. Conclusions

The cellular immune response is one of the determining factors in the persistence or elimination of HPV infections and its evolution to pre-neoplastic lesions. Although the dimension in which it occurs is not entirely clear, it is known that the progression of the cervical neoplastic lesion promotes a change in the pattern of cytokine secretion, with active participation of regulatory T lymphocytes. Thus, the antitumor immune response requires a balance in the production of cytokines because the progression or regression of the tumor depends on the type and amount of cytokines secreted. In response to HPV infection, activation of dendritic cells, Langerhans cells and natural killer cells is essential. With a focus on preventing early HPV infection, the use of prophylactic vaccines is already well established in clinical practice. Investigation of these immune reactions may provide new therapeutic targets for cervical intraepithelial neoplasms.

Author contributions

RSN and EFCM designed the research study. RSN, MPJ, EFCM, PTSM and DRS performed the research. PTSM selected the articles. PTSM, MPJ and DRS wrote the manuscript. RSN and EFCM reviewed the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Nayar R, Wilbur DC. The Bethesda System for Reporting Cervical Cytology. Definitions, Criteria, and Explanatory Notes (pp. 321). 3rd edn. In Nayar R, Wilbur D. (eds.) Cham, Switzerland: Springer International Publishing. 2015.

[2] Instituto Nacional de Câncer (INCA). Incidência do Câncer no Brasil. 2020. Available at: https://www.inca.gov.br/controle-do-cancer-do-colo-do-utero/conceito-e-magnitude (Accessed: 15 November 2020).

[3] Leto MGP, Santos Júnior GF, Porro AM, Tomimori J. Human papillomavirus infection: etiopathogenesis, molecular biology and clinical manifestations. Journal Brazilian Annals of Dermatology. 2011; 86: 306–317.

[4] Münger K, Baldwin A, Edwards KM, Hayakawa H, Nguyen CL, Owens M, et al. Mechanisms of human papillomavirus-induced oncogenesis. Journal of Virology. 2004; 78: 11451–11460.

[5] Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell. 1990; 63: 1129–1136.

[6] zur Hausen H. Papillomavirus infections—a major cause of human cancers. Biochimica et Biophysica Acta. 1996; 1288: F55–F78.

[7] Muñoz N, Méndez F, Posso H, Molano M, van den Brule AJC, Ronderos M, et al. Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. Journal of Infectious Diseases. 2004; 190: 2077–2087.

[8] Foldvari M. HPV infections: can they be eradicated using nanotechnology? Nanomedicine. 2012; 8: 131–135.

[9] Franceschi S, Herrero R, Clifford GM, Snijders PJF, Arslan A, Anh PTH, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. International Journal of Cancer. 2006; 119: 2677–2684.

[10] Rodríguez AC, Schiffman M, Herrero R, Hildesheim A, Bratti C, Sherman ME, et al. Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. Journal of the National Cancer Institute. 2010; 102: 315–324.

[11] Sarkola ME, Grénman SE, Rintala MAM, Syrjänen KJ, Syrjänen SM. Human papillomavirus in the placenta and umbilical cord blood. Acta Obstetricia et Gynecologica Scandinavica. 2008; 87: 1181–1188.

[12] Medeiros LR, Ethur ABDM, Hilgert JB, Zanini RR, Berwanger O, Bozzetti MC, et al. Vertical transmission of the human papillomavirus: a systematic quantitative review. Cadernos De SaúDe PúBlica. 2005; 21: 1006–1015.

[13] Ferenczy A, Bergeron C, Richart RM. Human papillomavirus DNA in fomites on objects used for the management of patients with genital human papillomavirus infections. Obstetrics and Gynecology. 1989; 74: 950–954.

[14] Roden RB, Kirnbauer R, Jenson AB, Lowy DR, Schiller JT. Interaction of papillomaviruses with the cell surface. Journal of Virology. 1994; 68: 7260–7266.

[15] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer. 2015; 136: E359–E386.

[16] Westrich JA, Warren CJ, Pyeon D. Evasion of host immune defenses by human papillomavirus. Virus Research. 2017; 231: 21–33.

[17] Otani S, Fujii T, Kukimoto I, Yamamoto N, Tsukamoto T, Ichikawa R, et al. Cytokine expression profiles in cervical mucus from patients with cervical cancer and its precursor lesions. Cytokine. 2019; 120: 210–219.

[18] Amador-Molina A, Hernández-Valencia JF, Lamoyi E, Contreras-Paredes A, Lizano M. Role of innate immunity against human papillomavirus (HPV) infections and effect of adjuvants in promoting specific immune response. Viruses. 2013; 5: 2624–2642.

[19] Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. i. Definition according to profiles of lymphokine activities and secreted proteins. Journal of Immunology. 1986; 136: 2348–2357.

[20] Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet. 2007; 370: 890–907.

[21] Stanley M. Pathology and epidemiology of HPV infection in females. Gynecologic Oncology. 2010; 117: S5–S10.

[22] Diamond MS, Farzan M. The broad-spectrum antiviral functions of IFIT and IFITM proteins. Nature Reviews. Immunology. 2013; 13: 46–57.

[23] Schelhaas M, Shah B, Holzer M, Blattmann P, Kühling L, Day PM, et al. Entry of human papillomavirus type 16 by actin-dependent, clathrin- and lipid raft-independent endocytosis. PLoS Pathogens. 2012; 8: e1002657.

[24] DiGiuseppe S, Bienkowska-Haba M, Sapp M. Human Papillomavirus Entry: Hiding in a Bubble. Journal of Virology. 2016; 90: 8032–8035.

[25] Griffin LM, Cicchini L, Pyeon D. Human papillomavirus infection is inhibited by host autophagy in primary human keratinocytes. Virology. 2013; 437: 12–19.

[26] Pyeon D, Pearce SM, Lank SM, Ahlquist P, Lambert PF. Establishment of human papillomavirus infection requires cell cycle progression. PLOS Pathogens. 2009; 5: e1000318.

[27] Wiens ME, Smith JG. Alpha-defensin HD5 inhibits furin cleavage of human papillomavirus 16 L2 to block infection. Journal of Virology. 2015; 89: 2866–2874.

[28] Chan YK, Gack MU. Viral evasion of intracellular DNA and RNA sensing. Nature Reviews. Microbiology. 2016; 14: 360–373.

[29] Li X, Shu C, Yi G, Chaton CT, Shelton CL, Diao J, et al. Cyclic GMP-AMP synthase is activated by double-stranded DNA-induced oligomerization. Immunity. 2013; 39: 1019–1031.

[30] Reinholz M, Kawakami Y, Salzer S, Kreuter A, Dombrowski Y, Koglin S, et al. HPV16 activates the AIM2 inflammasome in keratinocytes. Archives of Dermatological Research. 2013; 305: 723–732.

[31] Lo Cigno I, De Andrea M, Borgogna C, Albertini S, Landini MM, Peretti A, et al. The Nuclear DNA Sensor IFI16 Acts as a Restriction Factor for Human Papillomavirus Replication through Epigenetic Modifications of the Viral Promoters. Journal of Virology. 2015; 89: 7506–7520.

[32] Daud II, Scott ME, Ma Y, Shiboski S, Farhat S, Moscicki A. Association between toll-like receptor expression and human papillomavirus type 16 persistence. International Journal of Cancer. 2011; 128: 879–886.

[33] Viscidi RP, Schiffman M, Hildesheim A, Herrero R, Castle PE, Bratti MC, et al. Seroreactivity to Human Papillomavirus (HPV) Types 16, 18, or 31 and Risk of Subsequent HPV Infection. Cancer Epidemiology Biomarkers & Prevention. 2004; 13: 324–327.

[34] Warren CJ, Van Doorslaer K, Pandey A, Espinosa JM, Pyeon D. Role of the host restriction factor APOBEC3 on papillomavirus evolution. Virus Evolution. 2015; 1: vev015.

[35] Bontkes HJ, Ruizendaal JJ, Kramer D, Meijer CJLM, Hooijberg E. Plasmacytoid dendritic cells are present in cervical carcinoma and become activated by human papillomavirus type 16 virus-like particles. Gynecologic Oncology. 2005; 96: 897–901.

[36] Orange JS. Natural killer cell deficiency. Journal of Allergy and Clinical Immunology. 2013; 132: 515–525.

[37] Woo YL, Sterling J, Damay I, Coleman N, Crawford R, van der Burg SH, et al. Characterising the local immune responses in cervical intraepithelial neoplasia: a cross-sectional and longitudinal analysis. BJOG: an International Journal of Obstetrics and Gynaecology. 2008; 115: 1616–1612.

[38] Da Silva DM, Woodham AW, Skeate JG, Rijkee LK, Taylor JR, Brand HE, et al. Langerhans cells from women with cervical precancerous lesions become functionally responsive against human papillomavirus after activation with stabilized Poly-i:C. Clinical Immunology. 2015; 161: 197–208.

[39] Handisurya A, Day PM, Thompson CD, Bonelli M, Lowy DR, Schiller JT. Strain-specific properties and T cells regulate the susceptibility to papilloma induction by Mus musculus papillomavirus 1. PLoS Pathogens. 2014; 10: e1004314.

[40] Uberoi A, Yoshida S, Frazer IH, Pitot HC, Lambert PF. Role of Ultraviolet Radiation in Papillomavirus-Induced Disease. PLoS Pathogens. 2016; 12: e1005664.

[41] Stanley MA. Epithelial cell responses to infection with human papillomavirus: results from a population-based study in Costa Rica. Clinical Microbiology Reviews. 2012; 25: 215–222.

[42] Evans M, Borysiewicz LK, Evans AS, Rowe M, Jones M, Gileadi U, et al. Antigen processing defects in cervical carcinomas limit the presentation of a CTL epitope from human papillomavirus 16 E6. Journal of Immunology. 2001; 167: 5420–5428.

[43] Patel S, Chiplunkar S. Host immune responses to cervical cancer. Current Opinion in Obstetrics & Gynecology. 2009; 21: 54–59.

[44] Zoodsma M, Nolte IM, Schipper M, Oosterom E, van der Steege G, de Vries EGE, et al. Interleukin-10 and Fas polymorphisms and susceptibility for (pre)neoplastic cervical disease. International Journal of Gynecological Cancer. 2005; 15: 282–290.

[45] Roman A, Munger K. The papillomavirus E7 proteins. Virology. 2013; 445: 138–168.

[46] Vande Pol SB, Klingelhutz AJ. Papillomavirus E6 oncoproteins. Virology. 2013; 445: 115–137.

[47] Vandermark ER, Deluca KA, Gardner CR, Marker DF, Schreiner CN, Strickland DA, et al. Human papillomavirus type 16 E6 and E 7 proteins alter NF-kB in cultured cervical epithelial cells and inhibition of NF-kB promotes cell growth and immortalization. Virology. 2012; 425: 53–60.

[48] Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity. 2000; 12: 121–127.

[49] Schmitt E, Klein M, Bopp T. Th9 cells, new players in adaptive immunity. Trends in Immunology. 2014; 35: 61–68.

[50] Dudakov JA, Hanash AM, van den Brink MRM. Interleukin-22: immunobiology and pathology. Annual Review of Immunology. 2015; 33: 747–785.

[51] Lee GR. The Balance of Th17 versus Treg Cells in Autoimmunity. International Journal of Molecular Sciences. 2018; 19.

[52] Peghini BC, Abdalla DR, Barcelos ACM, Teodoro LDGVL, Murta EFC, Michelin MA. Local cytokine profiles of patients with cervical intraepithelial and invasive neoplasia. Human Immunology. 2012; 73: 920–926.

[53] Hiraoka N, Onozato K, Kosuge T, Hirohashi S. Prevalence of FOXP3+ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. Clinical Cancer Research. 2006; 12: 5423–5434.

[54] Fu J, Xu D, Liu Z, Shi M, Zhao P, Fu B, et al. Increased Regulatory T Cells Correlate with CD8 T-Cell Impairment and Poor Survival in Hepatocellular Carcinoma Patients. Gastroenterology. 2007; 132: 2328–2339.

[55] Gauza JE, Pimenta ATM, Moreira FV, Melli PPS, Quintana SM. IL-10 como preditor da progressão das lesões intraepiteliais do colo uterino: revisão da literatura. Arquivos Catarinenses de Medicina. 2013; 42: 92–97.

[56] Iwata T, Fujii T, Morii K, Saito M, Sugiyama J, Nishio H, et al. Cytokine profile in cervical mucosa of Japanese patients with cervical intraepithelial neoplasia. International Journal of Clinical Oncology. 2015; 20: 126–133.

[57] Tsukui T, Hildesheim A, Schiffman MH, Lucci J, Contois D, Lawler P, et al. Interleukin 2 production in vitro by peripheral lymphocytes in response to human papillomavirus-derived peptides: correlation with cervical pathology. Cancer Research. 1996; 56: 3967–3974.

[58] Tartour E, Gey A, Sastre-Garau X, Lombard Surin I, Mosseri V, Fridman WH. Prognostic value of intratumoral interferon gamma messenger RNA expression in invasive cervical carcinomas. Journal of the National Cancer Institute. 1998; 90: 287–294.

[59] El-Sherif AM, Seth R, Tighe PJ, Jenkins D. Quantitative analysis of IL-10 and IFN-gamma mRNA levels in normal cervix and human papillomavirus type 16 associated cervical precancer. Journal of Pathology. 2001; 195: 179–185.

[60] Clerici M, Merola M, Ferrario E, Trabattoni D, Villa ML, Stefanon B, et al. Cytokine production patterns in cervical intraepithelial neoplasia: association with human papillomavirus infection. Journal of the National Cancer Institute. 1997; 89: 245–250.

[61] Stanley M. Immune responses to human papillomavirus. Vaccine. 2006; 24: S16–S22.

[62] Wang SS, Hildesheim A. Chapter 5: Viral and host factors in human papillomavirus persistence and progression. Journal of the National Cancer Institute. Monographs. 2003; 31: 35–40.

[63] Carrington M, Wang S, Martin MP, Gao X, Schiffman M, Cheng J, et al. Hierarchy of resistance to cervical neoplasia mediated by combinations of killer immunoglobulin-like receptor and human leukocyte antigen loci. Journal of Experimental Medicine. 2005; 201: 1069–1075.

[64] Goodman MT, Shvetsov YB, McDuffie K, Wilkens LR, Zhu X, Thompson PJ, et al. Prevalence, acquisition, and clearance of cervical human papillomavirus infection among women with normal cytology: Hawaii Human Papillomavirus Cohort Study. Cancer Research. 2008; 68: 8813–8824.

[65] Ylitalo N, Sørensen P, Josefsson AM, Magnusson PK, Andersen PK, Pontén J, et al. Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma in situ: a nested case-control study. Lancet. 2000; 355: 2194–2198.

[66] Josefsson AM, Magnusson PK, Ylitalo N, Sørensen P, Qwarforth-Tubbin P, Andersen PK, et al. Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma in situ: a nested case-control study. Lancet. 2000; 355: 2189–2193.

[67] Plummer M, Schiffman M, Castle P, Maucort-Boulch D, Wheeler C. A 2-Year Prospective Study of Human Papillomavirus Persistence among Women with a Cytological Diagnosis of Atypical Squamous Cells of Undetermined Significance or Low-Grade Squamous Intraepithelial Lesion. Journal of Infectious Diseases. 2007; 195: 1582–1589.

[68] Schiffman M, Kjaer SK. Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. Journal of the National Cancer Institute. Monographs. 2003; 14–19.

[69] Schiffman M, Herrero R, Desalle R, Hildesheim A, Wacholder S, Rodriguez AC, et al. The carcinogenicity of human papillomavirus types reflects viral evolution. Virology. 2005; 337: 76–84.

[70] Kjaer S, Høgdall E, Frederiksen K, Munk C, van den Brule A, Svare E, et al. The absolute risk of cervical abnormalities in high-risk human papillomavirus-positive, cytologically normal women over a 10-year period. Cancer Research. 2006; 66: 10630–10636.

[71] Herrero R, Castle PE, Schiffman M, Bratti MC, Hildesheim A, Morales J, et al. Epidemiologic profile of type-specific human papillomavirus infection and cervical neoplasia in Guanacaste, Costa Rica. Journal of Infectious Diseases. 2005; 191: 1796–1807.

[72] Richardson H, Abrahamowicz M, Tellier P, Kelsall G, du Berger R, Ferenczy A, et al. Modifiable risk factors associated with clearance of type-specific cervical human papillomavirus infections in a cohort of university students. Cancer Epidemiology, Biomarkers & Prevention. 2005; 14: 1149–1156.

[73] Hogewoning CJA, Bleeker MCG, van den Brule AJC, Voorhorst FJ, Snijders PJF, Berkhof J, et al. Condom use promotes regression of cervical intraepithelial neoplasia and clearance of human papillomavirus: a randomized clinical trial. International Journal of Cancer. 2003; 107: 811–816.

[74] David E, Belot A, Lega JC, Durieu I, Rousset-Jablonski C. Papillomavirus humain et lupus érythémateux systémique. La Revue De MéDecine Interne. 2021; 42: 498–504. (In French)

[75] Penn I. Cancer is a complication of severe immunosuppression. Surgery, Gynecology & Obstetrics. 1986; 162: 603–610.

[76] Baccarani U. De novo malignancies after kidney and liver transplantation: experience on 582 consecutive cases. Transplantation Proceedings. 2006; 38: 1135–1137.

[77] Brown MR. HPV subtypes analysis in lower genital tract neoplasm of female renal transplantation recipients. Gynecologic Oncology. 2000; 79: 220–224.

[78] Harwood C. HPV infection and non-melanoma skin cancer in immunosuppressed and immunocompetent individuals. Journal of Medical Virology. 2000; 61: 289–297.

[79] Young JL, Jazaeri AA, Darus CJ, Modesitt SC. Cyclooxygenase-2 in cervical neoplasia: a review. Gynecologic Oncology. 2008; 109: 140–145.

[80] Grabosch SM, Shariff OM, Helm CW. Non-steroidal anti-inflammatory agents to induce regression and prevent the progression of cervical intraepithelial neoplasia. Cochrane Database of Systematic Reviews. 2018; 2: CD004121.

[81] Maher J, Davies ET. Targeting cytotoxic T lymphocytes for cancer immunotherapy. British Journal of Cancer. 2004; 91: 817–821.

[82] Hildesheim A, Herrero R, Wacholder S, Rodriguez AC, Solomon D, Bratti MC, et al. Effect of Human Papillomavirus 16/18 L1 Viruslike Particle Vaccine among Young Women with Preexisting Infection: a randomized trial. Journal of the American Medical Association. 2007; 298: 743–753.

[83] FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. New England Journal of Medicine. 2007; 356: 1915–1927.

[84] Cordeiro MN, De Lima RDCP, Paolini F, Melo ARDS, Campos APF, Venuti A, et al. Current research into novel therapeutic vaccines against cervical cancer. Expert Review of Anticancer Therapy. 2018; 18: 365–376.

[85] Maehama T, Nishio M, Otani J, Mak TW, Suzuki A. The role of Hippo-YAP signaling in squamous cell carcinomas. Cancer Science. 2021; 112: 51–60.

[86] Santin AD, Bellone S, Palmieri M, Zanolini A, Ravaggi A, Siegel ER, et al. Human papillomavirus type 16 and 18 E7-pulsed dendritic cell vaccination of stage IB or IIA cervical cancer patients: a phase i escalating-dose trial. Journal of Virology. 2008; 82: 1968–1979.

[87] Rahma OE, Herrin VE, Ibrahim RA, Toubaji A, Bernstein S, Dakheel O, et al. Pre-immature dendritic cells (PIDC) pulsed with HPV16 E6 or E7 peptide are capable of eliciting specific immune response in patients with advanced cervical cancer. Journal of Translational Medicine. 2014; 12: 353.

[88] Barra F, Della Corte L, Noberasco G, Foreste V, Riemma G, Di Filippo C, et al. Advances in therapeutic vaccines for treating human papillomavirus-related cervical intraepithelial neoplasia. Journal of Obstetrics and Gynaecology Research. 2020; 46: 989–1006.

[89] Trimble CL, Morrow MP, Kraynyak KA, Shen X, Dallas M, Yan J, et al. Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial. Lancet. 2015; 386: 2078–2088.

[90] Brun J, Rajaonarison J, Nocart N, Hoarau L, Brun S, Garrigue I. Targeted immunotherapy of high-grade cervical intra-epithelial neoplasia: Expectations from clinical trials. Molecular and Clinical Oncology. 2018; 8: 227–235.

[91] Di Tucci C, Schiavi MC, Faiano P, D'Oria O, Prata G, Sciuga V, et al. Therapeutic vaccines and immune checkpoints inhibition options for gynecological cancers. Critical Reviews in Oncology/Hematology. 2018; 128: 30–42.

[92] Schiller M, Metze D, Luger TA, Grabbe S, Gunzer M. Immune response modifiers–mode of action. Experimental Dermatology. 2006; 15: 331–341.

[93] Schön M, Schön MP. The antitumoral mode of action of imiquimod and other imidazoquinolines. Current Medicinal Chemistry. 2007; 14: 681–687.

[94] Grimm C, Polterauer S, Natter C, Rahhal J, Hefler L, Tempfer CB, et al. Treatment of cervical intraepithelial neoplasia with topical imiquimod: a randomized controlled trial. Obstetrics and Gynecology. 2012; 120: 152–159.

[95] de Witte CJ, van de Sande AJM, van Beekhuizen HJ, Koeneman MM, Kruse AJ, Gerestein CG. Imiquimod in cervical, vaginal and vulvar intraepithelial neoplasia: a review. Gynecologic Oncology. 2015; 139: 377–384.

[96] Nomelini RS, De Carvalho Mardegan M, Murta EFC. Utilization of Interferon in Gynecologic and Breast Cancer. Clinical Medicine. Oncology. 2007; 1: 111–120.

[97] Friedman RM. Interferon binding: the first step in establishment of antiviral activity. Science. 1967; 156: 1760–1761.

[98] Michelin MA, Montes L, Nomelini RS, Trovó MA, Murta EFC. Helper T lymphocyte response in the peripheral blood of patients with intraepithelial neoplasia submitted to immunotherapy with pegylated interferon-α. International Journal of Molecular Sciences. 2015; 16: 5497–5509.

[99] Ramos MC, Mardegan MC, Peghini BC, Adad SJ, Michelin MA, Murta EFC. Expression of cytokines in cervical stroma in patients with high-grade cervical intraepithelial neoplasia after treatment with intralesional interferon α-2b. European Journal of Gynaecological Oncology. 2010; 31: 522–529.

[100] Mundim FV, Trovó MA, Stark LM, Jammal MP, Michelin MA, Murta EFC. Pegylated-interferon-alpha treatment modifying T cell cytokine profile in tumor microenvironment of patients with cervical intraepitelial neoplasia. European Journal of Gynaecological Oncology. 2021; 42: 96–104.