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The Use of Interferons in the Treatment of Pre-Invasive Cervical Neoplasias

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Abstract

Machado FA, Michelin MA, Murta EFC. The Use of Interferons in the Treatment of Pre-Invasive Cervical Neoplasias. ARBS Annu Rev Biomed Sci 2011;13:17-22. The evolution of cervical intraepithelial neoplasias (CINs) can lead to the development of cervical carcinomas and is closely tied to infection with human papillomavirus (HPV). Interferon (IFN)'s antitumor effects have a direct bearing on the proliferation or antigen composition of tumor cells, and act on specific tumor cells through immunomodulation. Moreover, some studies have indicated that type 1 IFN can have an antitumor effect by increasing levels of cytotoxic T cells, natural killer (NK) cells and dendritic cells (DCs). Immunotherapy with IFN has recently been used on cervical intraepithelial lesions and invasive cervical cancer with promising results. The objective of this mini-review is to discuss IFNs' function, importance, and mechanism of action in the treatment of pre-malignant cervical neoplasias.

Keywords: intraepithelial neoplasias; human papillomavirus (HPV); immunotherapy; cytokines and interferon (IFN).

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1. Introduction

Cervical intraepithelial neoplasias (CINs) are subdivided into grades I, II, and III, corresponding to mild, moderate, and serious dysplasia (Richart, 1968). CIN I is a dysplasia confined to the lower third of the epithelium; CIN II is a dysplasia that affects the interior two thirds of the epithelium; and CIN III is a squamous intraepithelial lesion in which changes to maturation and nuclear anomalies affect more than two thirds of the epithelial layer. The Bethesda system of cytology classification recommends that human papillomavirus (HPV)-related lesions and CIN I be included in the same category, termed low-grade squamous intraepithelial lesions (LSILs), while moderate and acute dysplasias, namely CIN II and CIN III,

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be referred to as high-grade squamous intraepithelial lesions (HSILs) (Kurman & Solomon, 1994). Thus, the terms LSIL and HSIL are used to refer to the grade of intraepithelial lesions otherwise classified as CINs (Washington, 2004).

The evolution of CINs can lead to the development of cervical carcinomas and is closely tied to HPV infection. Because HPV infection is common among women, it is the most important risk factor in the etiopathogenesis of cervical cancer and its precursor lesions, CINs. The HPV's genetic material consists of approximately 8 kb of double-stranded DNA, with three principal regions. The first region contains long control region (LCR) genes that regulate the expression of viral genes; the second region contains the E genes (E1–E7) that control the life cycle of the virus; and the third region contains genes (L1 and L2) that codify the HPV's capsid proteins. The HPV genes that have been most studied are E6 and E7 in HPV subtype 16, the most common high-risk subtype of HPV (De Villiers *et al.*, 2004). This double-chain virus is responsible for genital condylomas and proliferative epithelial lesions. The majority of the HPV subtypes cause benign epithelial lesions (condylomas). However, a few specific types (especially 16 and 18) have a tendency to provoke CINs and play an essential role in the genesis of female genital cancer. The incidence of HPV infection is higher in individuals who are immunodepressed because of having received transplants, corticosteriod use, radiation treatment, chemotherapy, or an HIV-positive status (Belsito *et al.*, 1982; Chopra & Tyring, 1997; Southern & Herrington, 1998).

Advances in our understanding of the immune system and in defining tumor cell antigens have led to many new strategies. Thus, immunotherapy has the potential to become an extraordinarily specific treatment modality for tumors. Immunotherapy with interferon (IFN) has recently been used on cervical intraepithelial lesions and invasive cervical cancer with promising results (Murta & Tavares-Murta, 2004; Ferrantini *et al.*, 2008).

2. The Role of Immunotherapy with IFNα-2b in Cervical Intra-Epithelial Neoplasias

IFNs include a group of cytokines that perform important antiviral, antitumor, immunomodulatory, and antiangiogenic activities in the immune system. The last of these functions is of fundamental importance for inhibiting the formation of metastases. IFNs are classified as type I (α/β) IFN, type II (γ) IFN, and type III (λ1, λ2 and λ3–respectively referred to as IL-29, IL-28A, and IL-28B) IFN. All of these types of IFNs inhibit mRNA expression through the E6 and E7 proteins in HPV-infected cells (Woodworth & Simpson, 1993; Johnson *et al.*, 1999; Khan *et al.*, 1993; Perea *et al.*, 1995; Fontaine *et al.*, 2001; Nawa *et al.*, 1990). Great technological advances have facilitated visualization of the mechanisms of action for type I IFN. IFN antitumor effects can be mediated via direct actions on tumor cell proliferation and antigen composition, or through indirect actions, as modulation of interactions of populations of effector immune cells, as cytotoxic T cells and dendritic cells, with specific tumor cells (Kufe *et al.*, 2003). IFNs can induce apoptosis, activating a cascade of caspases (Muscat *et al.*, 2006; Saidi *et al.*, 2006), including caspases 1, 2, 3, 8, and 9 in particular (Thyrell *et al.*, 2002). Moreover, some studies have suggested that type I IFN can produce an antitumor effect by increasing the presence of cytotoxic T cells, natural killer (NK) cells and dendritic cells (DCs) (Lindner, 2002).

Type I (α/β) IFNs attach themselves to a shared receptor, comprised of two subunits: IFNAR1 and IFNAR2 (Schroder *et al.*, 2004). Type I IFN receptors are associated with the enzyme tyrosine kinase (TYK), which phosphorylates other tyrosine kinases, including Janus kinase (JAK), which in turn phosphorylates STAT 1 and STAT 2 (Murray, 2007). When these STATs (signal transducers and activators of transcription) are recruited, they form a complex called IFN simulator genetic factor 3 (ISGF3), which is directed to the nucleus where it induces gene transcription of the IFN stimulation response element (ISRE) (Schindler & Plumlee, 2008) (Fig. 1).

CIN treatment involving IFN-γ has been reported to be therapeutically successful, though, in the long term, the treatment is still inferior to—that is, less effective than— surgical treatment (Iwasaka *et al.*, 1990; Sikorski & Zrubek, 2003). Various studies involving IFN-β have yielded good results in the treatment of different grades of CINs. For example, using a topical cream containing IFN-β in HPV-infected patients with CINs, Cinel *et al.* (1991) obtained a complete response in 85.36% of CIN I patients, 84.20% of CIN II patients, and 37.50% of CIN III patients (Cinel *et al.*, 1991; Penna *et al.*, 1994; Rotola *et al.*, 1995; Katesmark *et al.*, 1999; Micheletti *et al.*, 1992; Grismondi *et al.*, 1995). In studies testing IFN-α treatments, CIN remission has generally ranged from 30% to 80% of cases, though one study examining an intra-lesional IFN-α treatment found that the treated group had a similar outcome as placebo controls (Choo *et*

al., 1986; Dunham *et al.*, 1990; Stellato, 1992; Byrne *et al.*, 1986; Frost *et al.*, 1990).

The current standard treatment for HSILs is surgery (conization), which carries the risk of complications. Thus most patients that present with HSILs consequently undergo conization. However, for women of reproductive age, this surgical procedure can lead to infertility, spontaneous abortions, and premature labor. Therefore, the development of immunotherapy with IFN-α would not only advance our understanding of the immunological mechanisms of tumor regression, but would also offer a valuable clinical treatment option for patients.

Murta and Tavares-Murta (2004) demonstrated IFN therapy effectiveness when they obtained complete resolution in a patient with invasive vaginal carcinoma using intra-lesional IFNα-2b. Colposcopy and cytology showed a total remission of the lesion. When the patient became pregnant 3 years after the treatment, she showed no signs of recurrence at her follow-up. In another case involving a patient with intraepithelial neoplasias of the vulva, vagina, and cervix, treatment with the same IFN caused partial regression of lesions in all locations and, in the case of the cervix, the disease went into complete remission (Slotman *et al.*, 1988). IFNs have also been used to treat HPV-infected patients, though some individuals responded only partially to treatment. Patients with low levels of the E7 protein have been reported to respond better to treatment than those with high levels of this protein (Arany *et al.*, 1995).

Several studies performed by the IPON-UFTM research group in recent years have shown IFNs to be very effective cytokines for treating CIN II and CIN III (HSILs). Using RT-PCR, Tirone *et al.* (2009) evaluated the mRNA expression of IFN-α receptors (IFNAR1 and IFNAR2) and of an IFN stimulation responsive element (ISRE) located in the region of the genes that are induced by IFN-α. Examining mRNA expression of the 2´5´ Oligoadenylate synthetase (2´,5´OAS) enzyme in biopsies from patients with CIN I, II, and III, they further found that the CIN samples had HPV DNA in 50% (24/28) of the patients, and low mRNA expression of the IFN receptor subunits 1 and 2. Expression of IFNAR1 mRNA was found to be more frequent among controls (58.3%) than among CIN patients (14.2%; p = 0.0018 vs. controls). Expression of IFNAR2 mRNA was also more prevalent in controls (64.7%) than in patients with CIN lesions (7.1%, p=0.0018). Simultaneous expression of the two receptors was observed only in the control group, at 47.0% (p = 0.0018 vs. CIN patients). Simultaneous expression of the two receptor subunits was found only in the control group, and the likelihood of mRNA expression of IFN-α and 2´5´OAS did not differ between the control and CIN groups.

IFN-α's target receptor proteins, IFNAR 1 and 2, are expressed in various cell types, including tumor and immune system cells that are fundamental to clinical responses. Mardegan (2011) reported that 62.5% of patients treated with intralesional IFNα-2b responded to the treatment, meaning that they showed either complete disappearance of the high-grade lesion or a low-grade lesion grade, as confirmed by histological study. Flow cytometry experiments showed that IL-6 and TNF-α concentrations in vaginal secretions were much higher during treatment in the group of patients who experienced therapeutic failure than in the group that responded to therapy, particularly at the time of the sixth application. In addition, responsive patients' HPV viral loads showed a significant drop after the application, versus before, as demonstrated by the hybrid capture technique. Using the same type of IFN, but on a larger number of patients, Ramos *et al.* (2010) obtained satisfactory clinical responses in 60% of patients with high-grade CINs; the Th1 (IFN-γ, TNF-α, IL-2) immune response was related to a reduction in CIN grade following treatment with IFNα-2b in patients who responded well. Patients who responded to the treatment also showed a significant reduction in their high-risk HPV viral loads, as evaluated using the hybrid capture technique. In PCR and subsequent electrophoresis experiments, it was also observed that patients with therapeutic failure had concomitant expression of IL-12/TGF-β2 and IL-4/TGF-β3, suggesting that the Treg immune response could have modulated the Th1 and Th2 immune response during IFN treatment, perhaps leading to the failure of the therapy. Expression of TGF-β, after treatment with IFN α-2b, in patients with therapeutic failure suggests that this cytokine may play an immunomodulating role in which it inhibits the Th1 protective response.

In a study in which cytokine levels in the blood of patients with high-grade CINs treated with intra-lesional IFNα-2b were quantified, Misson et al. (2011) observed that 50% of the patients achieved a therapeutic response; among those who responded, only 16.6% were smokers, while 66.6% of those whose therapy failed were smokers. Thus the use of tobacco was associated with treatment failure. Meanwhile, the group of patients with successful therapy showed a significant increase in Th1 profile cytokines, which are associated with stimulating reductions in Treg profile cytokines. An analysis of the cytokines, via ELISA, showed that the average concentration of IL-12 in the blood of patients with successful therapy was very significantly elevated on day 12 of IFNα-2b administration compared to patients whose therapy failed.

IL-10 and TGF-β dropped significantly following day 12 in patients with successful therapy, suggesting that the increase in the Th1 profile cytokines stimulated a drop in Th3 profile cytokines. In total, 18 applications of intralesional IFNα-2b were delivered on alternate days.

The above studies reveal that the immune response is delicate and complex, and there is still much to be elucidated with respect to the interactions between cells, cytokines, and cytokine receptors in order to attain successful therapy protocols. However, advances, such as those related to our understanding of the immune system and defining specific immunotherapies, have prompted new strategies based on the mechanisms involved in immunity as mediated by cytokines such as IFN.

3. Final Considerations

IFNs are a group of cytokines that have an important function in the immune system. Various studies have been performed, both in vitro and in vivo, that have demonstrated the efficacy of IFNs in combating tumors. Recent studies have shown that IFNs can be used to treat various diseases, including viral hepatitis, multiple sclerosis, and cancer. Since the early 1980s, studies examining IFN treatments of gynecological cancers have revealed varying responses (Borden *et al.*, 2007; Nomelini *et al.*, 2007). In recent decades, the treatment of CINs with IFNs has been extensively discussed, studied, and analyzed. Given that surgical treatment can cause changes in the cervix that lead to complications during pregnancy, the principal aim of developing intra-lesional IFN treatments for CINs is to make such treatments a viable option for fertile patients such that the anatomy of the cervix remains unaltered by CIN treatment given. Today, many investigators are focusing their research interests on IFNs' antiproliferative and immunoregulatory actions.

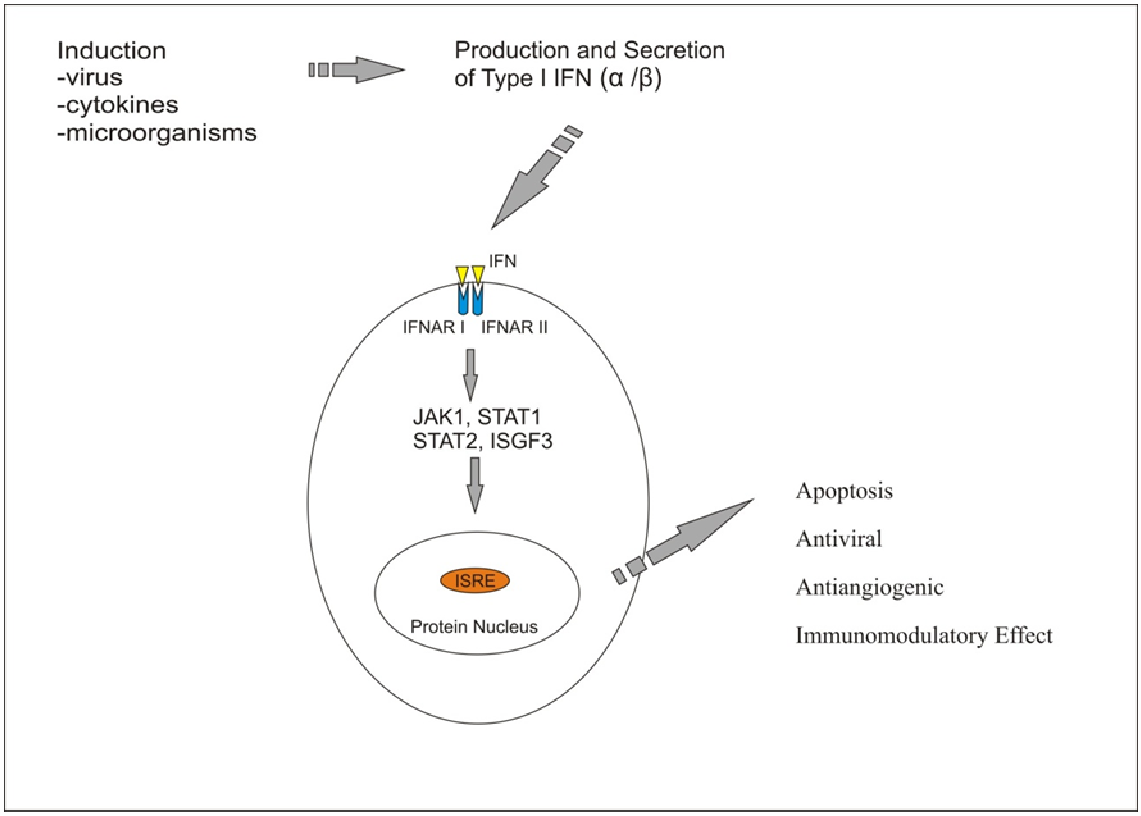

Figure 1: Induction, production, and actions of Type I IFN (Based on Nomelini *et al.*, 2007) .

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