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Monocytes to functional dendritic cells is often a bridge too far for cancer therapy

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Interferons are a class of pro-inflammatory cytokines with important immunological functions, especially in regards to host defense against viral infections. Interferons are also unique cytokines in their ability to both be produced by and activate numerous cell types, of both hematopoietic and stromal origin. For example, interferons stimulate the activation of immune cells through the upregulation of major histocompatibility complex (MHC) molecules, promoting antigen presentation and lymphocyte activation(1). In addition, interferon has potent anti-proliferative/pro-apoptotic effects, and can signal many cell types to degrade mRNA and limit protein synthesis, in an attempt to limit viral replication in infected (or potentially infected) cells(2).

Interferon's ability to both activate the immune system and limit protein synthesis in a wide range of target cells makes it an attractive immunotherapy option for cancer, where concurrent activation of the immune system and blockade of tumor proliferation would appear beneficial. Recombinant interferon treatment has been used for the past decade as an immunotherapy option, with efficacy against hematologic tumors (such as leukemia and lymphoma(3)) and malignancies of the skin (such as multiple myeloma and AIDS-related Kaposi's sarcoma(4, 5)). Unfortunately, not all malignancies show responsiveness to interferon treatment; for example, treatment of cervical cancer patients with recombinant interferon $\alpha 2\beta$ (IFN $\alpha 2\beta$) often lacks efficacy, especially when used in patients suffering from advanced malignancies(6). A better understanding of the mechanisms underlying the refractory nature of non-hematologic/non-cutaneous tumors to interferon treatment is essential for improving the therapeutic efficiency of interferon, as well as providing a better understanding of the mechanisms of interferon activity.

In this issue of *Translational Research*, Roy and colleagues look to study the effects of IFN $\alpha 2\beta$ treatment on cervical cancer patients through studying immune responses in advanced cervical cancer patients (CaCx-IIIIB), with an emphasis on the modulation of monocyte function as compared to healthy controls(7). The authors compare the phenotype and effector function of peripheral blood monocytes (PBMCs) from CaCx-IIIIB patients with healthy controls, to determine if monocytes from CaCx-IIIIB suffer from activation defects prior to activation with IFN $\alpha 2\beta$. Secondly, the authors used the PBMCs from both CaCx-IIIIB patients and healthy controls to generate immature dendritic cells (DCs) *in vitro*, and then study their maturation using IFN $\alpha 2\beta$. The authors conclude that PBMCs from CaCx-IIIIB patients exhibit decreased activation potential as compared to healthy control PBMCs, and that activation with IFN $\alpha 2\beta$ fails to overcome this activation defect. In addition, DCs derived from CaCx-IIIIB patients exhibit significantly decreased antigen-presenting function,

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as well as a deficiency in their ability to promote cytotoxic T_H1-type responses in cognate T cells. The authors conclude that both the decreased activation potential of PBMCs and the refractory IFN α 2 β responsiveness of PBMC-derived DCs from CaCx-IIIb patients may in part explain IFN α 2 β 's ineffectiveness as a cervical cancer immunotherapy option.

Dendritic cells play a critical role in promoting antitumor immunity both through the activation of cytotoxic CD8⁺ T cells and cytokine-producing CD4⁺ T cells(8). To accomplish this, host dendritic cells must upregulate antigen presentation machinery (such as MHC class I and II) and costimulatory factors (such as CD80/86) to activate the anti-tumor effector functions of antigen-specific T cells(9). Type I interferons such as IFN α 2 β have the ability to mature human monocytes both *in vitro* and *in vivo* (studies summarized by Santini *et al*(10)), and as such they make a promising candidate for cancer immunotherapy. However, the results of the study by Roy *et al* suggest that monocytes from patients suffering from advanced malignancies may be unresponsive to immunomodulation with IFN α 2 β . This also results in a decreased ability of IFN α 2 β -stimulated DCs from CaCx-IIIb patients to promote cytotoxic T cell activation and proliferation in response to tumor-derived antigens (i.e. HeLa cell extract). This may explain the reduced efficacy of IFN α 2 β treatment in the treatment of cervical cancer patients, especially in those patients suffering from advanced stages of malignancy. In addition, the identification of an interferon-unresponsive phenotype in CaCx-IIIb patients may be used as a biomarker for the appropriate use of IFN α 2 β treatment; for example, simple assays of PBMCs from cervical cancer patients may be used to determine if a patient is a prime candidate for IFN α 2 β treatment.

Of particular interest is the apparent refractory nature of PBMCs to IFN α 2 β stimulation, even in the context of *in vitro* maturation into DCs. These results suggest that there may be cell-intrinsic defects in the PBMCs of CaCx-IIIb patients that limit their ability to respond to exogenous IFN α 2 β . The authors present evidence for this possibility by analyzing the expression of IFN α receptors on *ex vivo* differentiated DCs from healthy and CaCx-IIIb patients, showing a decrease in receptor expression on PBMC-derived DCs from cancer patients. It is important to note that the observed differences in cell surface phenotype come after a lengthy *in vitro* cell culture protocol involving the addition of recombinant cytokines and optimized culture media; this suggests that the deficiencies observed are not reversed by removal of the PBMCs from the affected patient, or by conditioning of the PBMCs with optimized growth and differentiation stimuli. Previous studies in human patients and animal models indicate that malignancies have the ability to reprogram immune cells to promote tumor growth; one example of this phenomenon is the tumor-associated macrophage (TAM) which helps support the malignancy through the promotion of angiogenesis and the production of growth factors(11). However, this "reprogramming" effect is often observed *in vivo* in the context of the tumor; the present study indicates not only an effect on circulating immune cells far from the site of tumorigenesis, but also that this phenotype may be hard-wired in the progenitor cell and any daughter cells which may arise.

Recent studies into the effect of inflammation on subsequent immune responses have identified epigenetic regulation of gene expression as a possible mechanism for the maintenance of immunosuppressive phenotypes in cells. For example, increases in repressive histone modifications (resulting in gene silencing) have been observed in immune cells following significant inflammatory events, such as severe sepsis. In particular, peripheral dendritic cells from post-septic mice exhibit decreased activation potential, evidenced by decreased IL-12 production, which then correlates with repressive histone modification events at the *IL12* promoter in these cells(12). Additionally, CD4⁺ T cell subsets from post-septic animals exhibit increases in permissive histone modification events at the promoter regions of *Foxp3*, correlating in increased regulatory T cells in these

animals, increased suppressive activity of post-septic regulatory T cells, and an increased susceptibility of post-septic animals to solid tumor challenge(13). Similar evidence of epigenetic regulation has been shown in TAMs as well, as decreases in permissive histone modifications correlates with decreased expression of antigen presentation machinery, specifically MHC II(14). It is intriguing to hypothesize that a similar phenomenon may be occurring in the PBMCs of CaCx-IIIB patients, whereby an as-yet unknown mechanism is driving the epigenetic repression of IFN α 2 β receptors, resulting in unresponsiveness to type 1 interferon treatment. In such a scenario, coupling IFN α 2 β treatment with other treatments aimed at modifying or reversing epigenetic marks may help rescue IFN α 2 β 's efficacy in unresponsive cancer patients.

Clearly, solid tumors and advanced malignancies have a powerful ability to modulate the immune system, resulting in promotion of tumor growth at the expense of protective anti-tumor immunity. It therefore becomes critical to properly identify which patients will respond positively to immunotherapy. Also, treatments aimed at promoting tumor immunity must be able to overcome the immunosuppression mediated by advanced malignancies. In the present study, Roy *et al* have identified an important phenotype of cervical cancer PBMCs, providing an explanation for the refractory nature of IFN α 2 β treatment in these patients, and providing a useful phenotype for identifying candidates for IFN α 2 β in a clinical setting.

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