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Plasmacytoid Dendritic Cells in Melanoma: Can We Revert Bad into Good?

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Tumor-infiltrating plasmacytoid dendritic cells (pDCs) promote an immunosuppressive milieu that drives tumor growth in melanoma. This phenomenon typically results from the lack of appropriate pDC activation signals in the tumor microenvironment, but it is also actively controlled by tumor cells, which have evolved strategies to inhibit type I IFN production by pDCs. In this issue, Camisaschi *et al.* identify a new mechanism in which tumors avoid type I IFN production by triggering LAG-3-dependent activation of pDCs. Combination therapies that restore pDC functionality and trigger innate activation to produce type I IFN should be envisaged to induce effective antitumor immunity.

Journal of Investigative Dermatology (2014) **134,** 1797–1800. doi:10.1038/jid.2014.155

Melanoma is considered a prototypical immunogenic tumor expressing several melanoma-associated antigens that can be recognized by T cells. Despite the expression of these antigens, melanoma patients usually fail to mount spontaneous immune responses that are capable of rejecting tumors. One of the reasons for this is lack of an adequate innate immune response required to initiate the immune apparatus. Another reason is the dominant immunosuppressive microenvironment orchestrated by the tumor itself through production of immunosuppressive cytokines (vascular endothelial growth factor, IL-10, and transforming growth factor-β) and the recruitment of T regulatory cells or myeloid-derived suppressor cells. Recently, plasmacytoid dendritic cells (pDCs) were found to contribute to the establishment of an immunosuppressive milieu in many cancers. In contrast, pDCs and their activation to produce type I IFNs were found to induce protective antitumor immunity. Understanding the delicate balance between these apparently divergent functions of pDCs may provide new therapeutic avenues for treating melanomas and possibly other cancers.

pDC and type I IFN-driven immunity

pDCs are known for their role in antiviral immunity, owing to their ability to produce massive amounts of type I IFNs in response to viral nucleic acid upon recognition by Toll-like receptors (TLRs) 7 and 9 (Gilliet *et al.*, 2008). Through the production of type I IFNs, pDCs initiate antiviral immunity by inducing maturation of mDCs, activation of natural killer cells, antibody production by plasma cells, proliferation and cross-priming of Th1 cells, and inhibition of T regulatory cell function (Theofilopoulos *et al.*, 2005). pDC-derived type I IFNs are also involved in the pathogenesis of autoimmune disorders, as their aberrant recognition of self-nucleic acids triggers chronic type I IFN production and sustained immune activation (Gilliet *et al.*, 2008).

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pDCs in melanoma

The presence of pDCs has been described in the tumor microenvironment of many cancers including ovarian, breast, head and neck, and thyroid cancers, and in multiple myeloma, and it has been linked to tumor progression and poor patient survival. pDCs have also been detected in both primary melanoma and in melanoma metastases, whereas they are not present in normal skin or melanocytic nevi (Vermi *et al.*, 2003; Gerlini *et al.*, 2007). In melanoma, pDCs are found mainly in clusters around blood vessels and in close contact with tumor cells, where they are recruited by SDF1 and CCL20 produced by tumors or peritumoral cells (Charles *et al.*, 2010). These pDCs normally do not produce type I IFN, and their presence is associated with the growth of melanoma (Vermi *et al.*, 2003; Gerlini *et al.*, 2007).

Mechanisms of pDC-driven immunosuppression

In recent years, there has been increasing effort devoted to understanding how pDCs drive immunosuppression in melanoma and other types of cancer. Although several mechanisms were identified, they all share the lack of an efficient type I IFN production by pDCs as a common feature.

Lack of pDC activation. Tumor pDCs are often present in a nonactivated state, because of the lack of TLR7 and TLR9 signals in the tumor microenvironment. These nonactivated pDCs express high levels of the ICOS ligand, along with low levels of CD80 and CD86 (Ito *et al.*, 2007), a unique constellation of costimulatory molecules that selectively trigger the expansion of a subset of ICOS + FoxP3 + T regulatory cells (Gilliet and Liu, 2002; Ito *et al.*, 2008). Within tumors, pDCs were found in close proximity to ICOS + FOXP3 + regulatory T cells, and their number correlates directly with that of this regulatory T-cell subset (Conrad *et al.*, 2012), suggesting that tumor pDCs promote immunosuppression by activating and expanding ICOS + FOXP3 + T regulatory cells through ICOS costimulation. Independent studies have indeed demonstrated that both pDCs and ICOS + T regulatory cells constitute strong predictors of disease progression and poor

Clinical Implications

- Tumor-infiltrating plasmacytoid dendritic cells (pDCs) promote an immunosuppressive milieu that drives tumor growth.
- Tumor cells actively sustain the immunosuppressive functions of pDCs by inhibiting their type I IFN production.
- Therapeutic activation of tumor pDCs using Toll-like receptor-7 (TLR7) and TLR9 agonists can induce type I IFN production and antitumor immunity.
- Combined strategies to restore pDC functionality and stimulate TLR-mediated activation of pDCs may be effective in increasing systemic antitumor immunity.

clinical outcome in patients affected by ovarian, breast, and thyroid cancer (Conrad *et al.*, 2012; Faget *et al.*, 2012). High numbers of ICOS+ T regulatory cells have also been identified in human melanoma (Martin-Orozco *et al.*, 2010), although a link to pDC infiltration has not been established. Another mechanism for T regulatory cell expansion by pDCs on PD-L1-PD-1 costimulation has been described in a model of murine melanoma (Sharma *et al.*, 2007).

Tumor-mediated suppression of IFN production by pDCs. Tumor cells produce immunosuppressive cytokines PGE2, IL-10, and transforming growth factor-β, which directly suppress type I IFN production by inhibiting TLR and IRF7 expression in pDCs (Bekeredjian-Ding *et al.*, 2009). Furthermore, melanoma cells express Wnt5a, which inhibits TLR-mediated pDC activation and type I IFN production. Type I IFN production is also inhibited by ILT7, a cell-surface receptor specifically expressed by pDCs. ILT7 interacts with the transmembrane protein BST2 induced in cells upon type I IFN exposure and constitutively expressed by many cancer cells such as melanoma, lung cancer, renal cell carcinoma, and breast cancer. The interaction between BST2 and ILT7 leads to inhibition of TLR-induced type I IFN induction, whereas upregulation of CD80 and CD86 costimulatory molecule expression is unaffected. Under physiological conditions, this may be an important negative feedback mechanism for preventing prolonged IFN production after viral infection and a sustained inflammatory response. In contrast, constitutive expression of BST2 by cancer cells may inhibit TLR-mediated IFN production by pDCs and promote tumor immunosuppression.

Alternate maturation of pDCs by tumor cells. In this issue, Camisaschi *et al.* (2014) have identified the role of a subset of pDCs expressing the lymphocyte activation gene-3 (LAG-3), a CD4-related costimulatory receptor that binds major histocompatibility complex class II molecules in cancer immunosuppression. LAG-3+ pDCs were found to infiltrate the tumor microenvironment of melanoma and to interact with HLA-DR+ melanoma cells *in vivo*. *In vitro*, the authors show that HLA-DR+ melanoma cells stimulate LAG-3+ pDCs to mature and produce IL-6 without inducing type I IFNs. Accordingly, LAG-3-expressing pDCs displayed a partially activated phenotype and produced IL-6 *in vivo*. pDC-derived IL-6 induced CCL2 production by monocytes, a key chemokine in the recruitment of myeloid-derived suppressor cells into the tumor site. Thus, the recruitment of LAG-3+ pDCs into the tumors and their activation in the absence of type I IFN production may drive myeloid-derived suppressor cell-mediated immunosuppression. An alternate activation pathway induced in pDCs by tumor cells has been observed in multiple myeloma (Chauhan *et al.*, 2009). Myeloma cells were found to secrete low levels of IL-3, an inducer of pDC activation and maturation. IL-3-activated pDCs do not produce type I IFNs but mature rapidly into DCs that drive the generation of CD4+ and CD8+ T regulatory cells producing IL-10 (Gilliet and Liu, 2002; Ito *et al.*, 2007). Thus, multiple myeloma cells drive an alternate pathway of pDC activation leading to T regulatory cell–mediated immunosuppression.

Therapeutic activation of tumor pDCs to produce type I IFN

Spontaneously regressing melanomas are characterized by the presence of activated pDCs that produce type I IFN, suggesting a role of these cytokines in triggering antitumor immune responses (Wenzel *et al.*, 2005). Furthermore, treatment of skin tumors with TLR7 and TLR9 agonists appears to activate pDCs to produce type I IFN and to induce local tumor regressions. Topical application of the TLR7 agonist imiquimod is currently used for the treatment of superficial basal cell carcinomas, actinic keratosis, and lentigo maligna and was shown to induce type I IFN production by tumor pDCs as a central event in the local antitumor effect (Urosevic *et al.*, 2005). Some regression of primary human melanoma and superficial in-transit melanoma metastasis has also been reported, although topical imiquimod is usually ineffective for this indication as monotherapy. The local tumor regression induced by topical imiquimod is dependent on both pDCs and type I IFN, as it was largely abrogated in pDC-depleted or IFNAR−/− mice in a melanoma tumor model (Drobits *et al.*, 2012). The antitumor effect was local, entirely independent of T cells, but mediated by cytotoxic molecules TRAIL and granzyme induced in pDCs by autocrine type I IFN signaling (Stary *et al.*, 2007). Another study, however, using a melanoma mouse model, led to the elicitation of systemic T cell–mediated antitumor immunity through intratumoral injection of the TLR9 agonist CpG-ODN. This study demonstrated that the induction of systemic T cell–mediated antitumor immunity was driven in part by activation of intratumor pDCs (Lou *et al.*, 2011). The potential of activated pDCs to stimulate systemic T cell–mediated immunity at the tumor site was confirmed by an elegant study showing that intratumoral injection of blood-derived CpG-activated pDCs (therefore not conditioned by the tumor) would elicit systemic T cell–mediated tumor regression (injected versus non-injected tumors) by promoting activation of natural killer cells and cDCs that prime tumor-specific T cells (Liu *et al.*, 2008). Although type I IFN had a central role in this process, a contribution of other factors such as