

How to make dendritic cells work in cancer

Michael R. Shurin, Gurkamal S. Chatta. University of Pittsburgh Medical Center and University of Pittsburgh Cancer Institute, Pittsburgh, PA 15213

Dendritic cells (DC) perform a pivotal role in the initiation and regulation of both innate and adaptive immunity, including antitumor responses. It is, therefore, not surprising that many tumors have developed mechanisms that unfavorably impact DC function. Recent studies on the generation, maturation, longevity, and function of DC in cancer suggest that (i) tumor-induced apoptosis of DC and (ii) inhibition of DC capacity to present tumor antigen(s) (TA) are the two principle mechanisms employed by different tumor types to suppress the DC system and, thus, increase the likelihood of evading immune recognition (44, 52, 54). Given the current interest in applying DC-based immunotherapy to cancer treatment (2, 3), an understanding of the molecular defects responsible for DC dysfunction in cancer is essential for designing and testing the next generation of DC vaccines.

DC loaded *ex vivo* with TA for immunizing cancer patients, to induce or boost a therapeutically meaningful antitumor immune response is currently the most common approach utilizing DC (34). Examples of loading DC include: DC pulsed with defined peptides, proteins, tumor cell lysates or exosomes; DC pulsed with apoptotic or necrotic tumor cells/bodies; DC genetically modified to express tumor antigens; and DC fused with tumor cells. However, studies in a variety of solid tumors with the above strategies have failed to stimulate curative responses (24, 31, 45). In addition, there are several potential problems with the above strategies, namely, (i) the homing efficiency of cytotoxic T lymphocytes (CTL) induced by antigen-loaded DC to the tumor sites may be relatively low; (ii) the TA are not been recognized in many malignancies; and (iii) it is difficult to induce antigen-specific CTL suitable for the full repertoire of TA expressed by tumors. Furthermore, despite eliciting vigorous immune responses, to date, most DC vaccines have had limited clinical impact justifying the rationale for developing alternative DC-based immunotherapeutic strategies.

One of the methods to overcome the problem of identifying and introducing appropriate TA in DC is the intratumoral injection of *ex vivo* generated DC (9). Several lines of evidence suggest that intratumoral DC play an important role in antitumor immune responses and there is a strong rationale for exploitation of intralesional administration of DC vaccines in different tumors, such as melanoma, squamous cell carcinoma of the head and neck (SCCHN), and probably other solid tumors including prostate, breast, and lung cancer.

First, increased numbers of tumor-infiltrating DC are associated with better outcome in cancer patients with a variety of tumors (4, 44). For instance, evaluating the prognostic significance of DC in 132 specimens from patients with primary SCCHN, Reichert et al. reported that a low number of tumor-infiltrating DC were more predictive of poor survival than lymph node (LN) involvement (38). Analyzing the distribution of DC in the primary tumor, adjacent tissue, and regional LN, Kikuchi et al. revealed that the number of DC in the tumor predicts overall survival, disease free survival, and time to disease recurrence in patients with SCCHN (22).

Second, we have recently reported that the lost expression of chemokine CXCL14 in human SCCHN may be associated with decreased migration of DC to the tumor site and, thus, suboptimal induction of antitumor immune responses (42). Using two murine SCCHN models, we have demonstrated that migration of DC into CXCL14-transfected tumors is associated with inhibition of tumor growth. In agreement, overproduction of CCL20 (MIP-3 α) by tumor cells causes the local accumulation of DC and activation of tumor specific CTL in four murine tumor models (16). Together with clinical evidence demonstrating that infiltration of tumor mass by DC is associated with better patient survival, these results suggest that administration of DC vaccines into the tumor site might provoke proficient antitumor immune responses.

Third, the clinical benefits from intratumoral administration of DC, including tumor rejection, prolonged survival, and induction of immune memory, have been reported in mice and rats (1, 6, 11, 27, 37, 47). A pilot study in patients with melanoma and breast carcinoma demonstrated marked antitumor potential of intratumoral delivery of DC without addition of tumor antigens (50). Other clinical trials, evaluating intralesional administration of IL-12-transfected DC in patients with hepatocellular carcinomas and metastatic pancreatic and colorectal malignancies, concluded that intratumoral injection of DC is feasible and well tolerated (15, 26). Similarly, injection of autologous immature DC into tumor in conjunction with radiotherapy in patients with advanced hepatoma, revealed its safety and also the induction of tumor-specific and innate immunity (7). Evidence has been obtained showing that intratumoral DC can capture and process tumor antigens to be presented to T lymphocytes (28). Interestingly, DC were recently reported to be cytotoxic for several tumor cell lines suggesting that this may have important consequences for their ability to stimulate tumor-specific CTL (46, 51, 53).

Thus, intratumoral approaches are appealing because they may direct antigen-specific responses at the tumor site, exploit the presence of multiple undefined tumor antigens present in the endogenous tumor, and would be expected to reduce systemic toxicity (9). However, clinical efficacy of intratumoral delivery of DC might be limited by suppression of DC longevity and function in the tumor environment. We and others have reported that induction of DC apoptosis and suppression of DC function are the two main mechanisms mediating suppression of the DC system in the local tumor microenvironment (12, 17, 19, 35, 44). Of special interest is the fact that tumor-induced apoptosis of DC or inhibition of DC function maybe tumor-specific: melanoma, lung carcinoid, and several prostate adenocarcinoma cell lines were strong inhibitors of DC survival (13, 21, 36), while neuroblastoma and SCCHN cell lines did not induce DC apoptosis but suppressed antigen presentation by DC (43, 48). Therefore, in the intratumoral DC delivery strategy, for the purpose of enhancing antigen-specific antitumor immunity in the absence of define tumor antigens, it is important to increase antigen processing in DC and prolong survival of DC at local tumor sites.

Several different approaches can be used to increase the antitumor efficacy of intratumorally delivered DC vaccines. Thus transfection of DC with the anti-apoptotic gene Bcl-xl significantly increases their resistance to prostate cancer-induced apoptosis and is associated with significant antitumor potential upon intratumoral delivery *in vivo* in animal models (37). Interestingly, pre-treatment of DC with certain cytokines, including TNF- α , CD40L, and IL-12, also protects DC from tumor-induced apoptosis (13, 35, 36, 49). This effect is mediated by up-regulation of expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL and down-regulation of the pro-apoptotic protein Bax. Many agents, including TNF- α , IFN- γ , CD40L, TRAIL, LIGHT, CpG, TLR ligands, IL-12, IL-15, and IL-1, have been shown to stimulate DC maturation and function and might protect DC longevity and function in the tumor microenvironment. The success of intratumoral DC vaccines is dependent on several essential factors, namely: (i) a well-preserved endocytosis in DC for picking up tumor antigens, and (ii) normal or enhanced ability to process these antigens after being exposed to tumor-derived immunosuppressive factors. (8, 41). Our data comparing the effect of different factors on DC maturation and function, demonstrate that IL-15, protects DC from tumor-induced inhibition of antigen-processing machinery (APM) component expression without affecting the endocytic capacity of DC. IL-15 also preserves the potential of DC to present antigenic peptides to specific autologous T cells after being exposed to tumor-derived factors. Importantly, IL-15 not only protects APM in DC but also stimulates recovery of APM in DC from tumor-induced suppression (49). Thus, IL-15 not only increases survival of DC in the tumor microenvironment, but can also protects or recovers tumor-induced suppression of DC function, Hence, IL-15 is an important candidate factor for stimulating DC prior to their intratumoral administration.

IL-15 has a broad spectrum of biological activities on various types of cells, including T, B, NK, and mast cells, granulocytes, monocytes/macrophages, and DC. IL-15 activates DC both *in vivo* and *in vitro*. We have recently reported that stimulation of DC with IL-15 prior to their co-incubation with

tumor cells resulted in a significant increase in DC survival, mediated by increased expression of the anti-apoptotic proteins Bcl-x_L and Bcl-2 in DC (35, 49). Other groups have also identified IL-15 as a stimulatory cytokine for DC with the potential for autocrine activity and linked its effects to the expression of interferon (25). We and others have reported that human DC cultured with IL-15 are potent antigen-presenting cells (APC) with the ability to induce both primary (mixed leukocyte reaction, MLR) and secondary (recall responses to flu-matrix peptide) immune responses (5, 29, 40). Interestingly, monocyte-derived DC from patients with multiple myeloma have a significantly higher ability to present antigens to T cells, if IL-15 was added to cultured DC (S. Bykovskaia, personal communication). Furthermore, upon IL-15 stimulation, DC express MHC class I-related chain A and B (MICA/B), ligands for NKG2D, and can activate resting NK cells, which is solely dependent on the MICA/B-NKG2D interaction (20). IL-15 is required for IL-2 production by DC and DC-derived IL-2 increases DC-mediated NK cell activation and T-cell priming both *in vitro* and *in vivo* (14, 18). IL-15 activated DC result in the up-regulation of costimulatory molecules, an increase in production of IFN- γ , IFN- α , IFN- β and IL-12, and an enhanced ability of DC to stimulate Ag-specific CD8⁺ T cells (20, 25, 32). IL-15 also blocks IL-10 production but augments the release of TNF- α and IL-1 β by DC (39).

Recent investigations have demonstrated a unique aspect of responses to IL-15, in that cells bearing the IL-15R α chain can bind soluble IL-15 and trans-present the cytokine to other cells, allowing the latter to respond to IL-15. For instance, DC require IL-15R α to present IL-15 in trans to NK cells to support NK cell cytolytic activity and elaboration of IFN- γ (23, 30). We and others have demonstrated that stimulation of DC with IL-15 results in up-regulation of IL-15R α expression on DC (49), thus generating signaling and the trans-presentation loop on DC. Importantly, recent data suggest that an autocrine IL-15/IL-15R α signaling loop in DC is essential for inducing CD8 T-cell responses and dependent Th1 response in mice *in vivo* (39). In contrast to many other cytokines, IL-15 associates with IL-15R α on the membrane of DC and is capable of stimulating directly neighboring cells in a juxtacrine manner, thus greatly extending the range of signaling options and activities of IL-15.

Hence, IL-15 could up-regulate the antitumor potential of DC through autocrine, juxtacrine, and paracrine loops, and also possibly through an intracrine loop (39). IL-15 indeed is internalized in DC by binding to the IL-15R α chain and becomes intracellularly co-localized with it, raising the possibility that IL-15 might act in DC, at least in part, through activation of an intracrine signaling loop. Another unique feature of IL-15 is its ability for recycling after intracellular uptake and subsequent reexpression on the cell surface of DC (10). The fact that IL-15 is able to promote APC survival and probably other activities even after its withdrawal from the medium (10) can be explained by an IL-15R α -mediated IL-15 recycling process that leads to the persistence of IL-15 on the membrane. The presence of IL-15 at the tumor site may also stimulate the development of antitumor immune responses (33). Thus, the traditional view of how cytokines mediate their effects has been challenged by the data that trans-presentation of membrane bound IL-15 to adjacent cells is a major dominant alternative mechanism of IL-15-mediated actions.

In summary, a cytokine or factor, which protects/recovers APM in DC in the tumor environment, is expected (i) not to inhibit endocytic activity of DC, (ii) prolong survival of DC at the tumor site, (iii) preserve migratory potential of DC in the tumor microenvironment, and (iv) participate in a unique receptor-ligand autocrine/paracrine loop, which allows DC to activate NK cells and stimulate CTL generation. All of these DC activities are required for initiating antitumor immune responses following intratumoral administration of DC. Pre-stimulation of DC with IL-15 seems to meet these requirements. Collectively, these data suggest that IL-15 has a potentiating effect on DC function and survival, which maybe useful in enhancing the therapeutic efficacy of DC-based vaccines.

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