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# The dendritic cell–tumor cross-talk in cancer

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The question as to whether the tumor grows because of or despite the host immune system is being progressively addressed with refined technology, gene targeting in mice and human translational research. The productive interplay between major actors of the antitumor immunity is actively compromised by the tumor microenvironment subverting the links between innate and cognate immunity and/or generating devastating new players. The complexity of the host–tumor equilibrium could be dissected at the reduced level of the dialogue between professional antigen presenting cells (APC), more precisely dendritic cells, and tumor cells that may profoundly dictate the outcome of the neoplasma. This review will summarize the novel mechanisms by which tumor cells regulate DC recruitment, differentiation, activation and cross-presenting functions in tumor beds and how innate players might counterbalance these interactions. Finally, we will highlight interesting strategies that harness the DC potential to fight against cancer.

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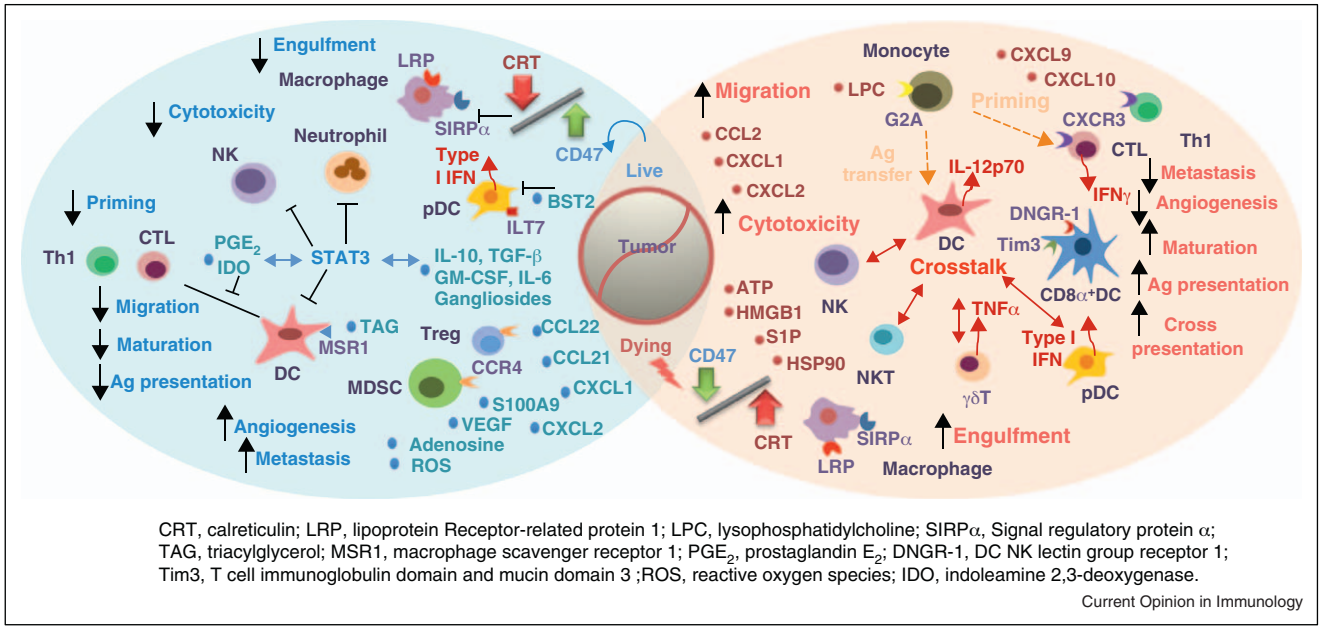
## Tumor microenvironment and deficient antigen presenting functions

Cancer-induced tolerance relies on the unresponsiveness of the host immune system to professional antigen presenting cells (APC) invading tumor beds or residing in the vicinity (tumor-draining lymph nodes) of developing tumors. Tumor progression induces defects in DC recruitment, differentiation, maturation and survival (Figure 1). The mechanisms of inadequate DC functions causing and/or resulting from tumor escape have been reviewed elsewhere [1,2]. It is noteworthy that the regulatory capacity of tumor infiltrating DC is not related to a defined DC subset but rather results from the influence of tumor microenvironment. Over the past decade, a series of immunosuppressive factors (such as GM-CSF and S100A9 associated with myeloid derived suppressor cells (MDSC), M-CSF, IL-6, VEGF, TGF-β, CXCL8, IL-10, gangliosides, altered glycosylation of tumor associated antigens, reactive oxygen species, indoleamine 2,3-deoxygenase (IDO) and extracellular adenosine) have been described to block DC recruitment and/or functions, mainly through activation of signal transduction and activator of transcription STAT3 [3].

Immunohistochemical analyses of breast cancer tissues revealed that plasmacytoid DC (pDC) invade 13% of primary tumors and predict short progression free survival [4]. In the same malignancy, Gobert *et al.* demonstrated a strong association between the DC-LAMP$^+$ DC and Tregs in a CCR4/CCL22 chemokine milieu also associated with poor prognosis if located in the periphery of tumors [5]. Interestingly, BST2 released from tumor cells can subvert pDC through ILT7 signaling and make pDC fail to respond to danger signals for type 1 IFN production. Pretreatment with IFN-α and TNF-α significantly increased BST2 secretion, suggesting that the inflammatory status of tumor microenvironment support this immunoregulatory pathway [6••].

Moreover, prostaglandin $E_2$ ($PGE_2$), the major cyclooxygenase 2 metabolite released by tumor cells, can interrupt the tumor > DC > T cell cascade by inducing an IL-10-dependent reduction of DC infiltration in tumors and of DC maturation, and PGE2 compromises CCR7-dependent DC migration in LN promoting abortive CD8$^+$ T cell responses [7]. These data extended the pioneering finding that tumor growing in $PGE_2$ receptor deficient hosts exhibited markedly enhanced DC differentiation and antitumor CTL responses compared with WT littermates [8]. In humans, maturation of DC in the presence of $PGE_2$ resulted in upregulation of CD25 and IDO culminating in DC-mediated T cell inhibition, a scenario compatible with the DC phenotype found in human tumors [9]. More recently, novel mechanisms of alterations of DC cross-presenting functions were unraveled. Herber *et al.* reported accumulation of triacylglycerol through exacerbated macrophage scavenger receptor 1 (MSR1)-mediated uptake in DC from tumor bearers. Lipid-laden DC were severely altered in their ability

**Figure 1**




CRT, calreticulin; LRP, lipoprotein Receptor-related protein 1; LPC, lysophosphatidylcholine; SIRPα, Signal regulatory protein α; TAG, triacylglycerol; MSR1, macrophage scavenger receptor 1; $PGE_2$, prostaglandin $E_2$; DNGR-1, DC NK lectin group receptor 1; Tim3, T cell immunoglobulin domain and mucin domain 3 ;ROS, reactive oxygen species; IDO, indoleamine 2,3-deoxygenase.

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The yin/yang dialogue between tumor cells and immune players in cancer. Live tumor cells produce or express a variety of metabolites or proteins that subvert the capacity of bona fide antigen presenting cells to initiate tumor-specific T cell responses (at all levels: engulfment, recruitment, differentiation, migration, activation, cross-presentation) and/or contribute to activate MDSC or Treg competing against effector T cells and/or directly promoting angiogenesis or metastases (blue circle). Tumor cell death (intrinsically or extrinsically regulated) might either reenforce tumor-induced tolerance (via engulfment by inflammatory phagocytes and/or tolerogenic molecular pathways) or instead, reset immune responses by exposing appropriate 'cell death-associated molecular patterns' (rebooting APC functions and T cell polarization) and/or recruiting key innate effectors (red circle).

to cross-present soluble antigen or tumor associated antigens unless an inhibitor of acetyl-CoA carboxylase was added to DC *in vitro* and *in vivo* [10••]. Patients bearing head and neck tumors also presented with lipid accumulation in their circulating, tumoral and lymph node $Lin^{-}CD4^{+}$ DC [10••]. Human and mouse tumors releasing cholesterol metabolites could dampen the expression of CCR7 on maturing DCs by triggering liver X receptor (LXR), thereby impairing DC migration and allowing tumor escape [11••]. Interestingly, cancer stem cells may not differ from parental tumor cells in their spectrum of antigen expression [12] and the lack of immune tolerance to pluripotency antigen (such as the transcription factor octamer-binding protein 4 (OCT4)) in cancer patients suggests that tumor outgrowth may not result from a primary defect of DC recognition of tumor stem cells [13].

## Come-and-get-me signals emanating from tumor cells

During tumor progression, there is constant and in some cases prominent apoptosis (such as Burkitt lymphoma) that is part of a vicious circle. Indeed, apoptotic cells express phosphatidylserine (PS) on their surface at late stages of apoptosis, and several PS receptors or adaptors have been described [such as T cell immunoglobulin and mucin domain-containing protein 4 (Tim4), Mer tyrosine kinase (MerTK), milk fat globule-EGF factor 8 protein (MFG-E8), brain-specific angiogenesis inhibitor 1 (BAI1)] on macrophages to ensure prompt clearance of apoptotic cells and elicitation of tolerance before full blown inflammatory necrosis [14–16,17••]. During the pre-apoptosis phase, cells upregulate sphingosine kinase 1 expression, allowing the release of sphingosine-1-phosphate (S1P) that causes cytoskeletal rearrangements and chemoattraction of macrophages, even at low nanomolar ranges [18]. However, *in vivo* studies showed that tumors expanding in $S1P_2^{-/-}$ (the G protein-coupled receptor for S1P) animals exhibited a higher infiltration with $CD11b^{+}Gr1^{-}CD34^{-}$ bone marrow cells including F4/$80^{+}$ macrophages in a high VEGF, TGF-β1, basic FGF and IL-1β stromal environment with augmented tumor angiogenesis and growth [19]. S1P may even synergize with other chemokine-like factors such as lysophosphatidylcholine (LPC) [20], IL-8 or CCL2 that can be released by dying cells [18]. The G protein-coupled receptors G2A, unlike its relative GPR4, is involved in the chemotaxis of monocytic cells and could be a phagocyte receptor for find me signals such as LPC secreted by dying cells [21].

However, contrasting with the theory whereby inflammatory phagocytes mediate a status of clearance and tolerance of tumor cell debris, a different view positioning

macrophages as scavengers preventing dissemination of circulating and live tumor cells has recently emerged. Indeed, the pentaspanin integrin associated protein CD47 was found to be overexpressed on myeloid leukemia and migrating hematopoietic progenitors (and also solid tumors), resulting in reduced uptake by signal-regulatory protein alpha (SIRPα) expressing macrophages. The level of CD47 expression correlated with the ability to evade phagocytosis and macrophage-dependent immunosurveillance against developing leukemia [22•,23]. It is likely that an 'eat me' signal, like calreticulin, might be expressed in cis on leukemic cells to engage a counterreceptor mediating efficient uptake (via lipoprotein receptor-related protein 1 (LRP) [24]) by the phagocyte. However, peripheral tolerance is governed by efficient phagocytosis of apoptotic cells and cross-presentation of self-antigens by CD8α$^+$ DC [25]. This subset may not use CD36, DEC205, αvβ3/αvβ5 integrins for the clearance of dying self. Using UV-irradiated thymocytes, Nakayama *et al.* demonstrated that Tim3 expressed on CD8α$^+$ DC is crucial for the uptake of dying cells and prevention of autoimmunity [26]. Using Fas-expressing tumor cells, Qiu *et al.* discovered that blood borne cell associated antigens are cross-presented by marginal zone splenic CD8α$^+$CD103$^+$DEC207$^+$ DC to provide peripheral tolerance [27].

## Tertiary lymphoid organogenesis (TLO): friends or foes?

The tumor microenvironment may contain lymphoid-like structures that could regulate local adaptive immune responses. Tumor infiltrating-bronchus associated lymphoid tissues (Ti-BALTs) have been recently described in non-small cell lung cancers endowed with very favorable clinical outcome [28]. Ti-BALTs appear to be orchestrated around DC-Lamp$^+$ DC that interact with CD4$^+$ T cells of a memory phenotype. The DC/CD4$^+$ T cell clusters were associated with CD8$^+$ T cells of a Th1 pattern (T-bet$^+$) residing in tumor nests and surrounded by B cell follicles containing germinal center DC. While concordant with previous reports showing the association between TLO and autoimmune flare up or atherogenesis, this clinical observation is contrasting with a mouse study unveiling the protumorigenic behaviour of LN paracortex stroma-like areas containing lymphoid tissue-inducer cells and high endothelial venules [29•]. Such structures were induced by tumor cells overexpressing CCL21, a CCR7 ligand promoting recruitment of Treg and MDSC. By contrast, CCL21-deficient tumors induced antitumor immunity. The tolerogenic switch triggered by the CCL21-driven mimicry of the LN stroma was associated with a recruitment of naïve T cells to peripheral sites (as opposed to memory T cells), CCL21 promoting their differentiation into Treg and inducing effector T cell senescence [30]. Moreover, the mouse study failed to mention B cell follicles (in contrast to the former setting) that could elicit a humoral anticancer immune response synergizing with T cell reactivities. Future studies will pinpoint the role of RORγt, LTβ, IL-7/IL-7R and NK22 cells, features associated with lymphoid tissue-inducer cells, in the induction or maintenance of long term protective antitumor immune responses.

## Sensing of tumors by CD8α$^+$DC: tolerance or immunity?

CD8α$^+$ DC have been shown to be a masterpiece of peripheral tolerance to cell associated self/tumor antigens [25]. However, the same DC subset is pivotal for antitumor immunosurveillance against MCA induced sarcoma, which depended upon IFN type I and type II receptors and lymphocytes [31]. Deletion of the transcriptional factor Batf3 ablated development of CD8α$^+$ DC that provoked tumor outgrowth of very immunogenic tumor variants [32•]. Importantly, Reis e Sousa and coworkers identified DC NK lectin group receptor 1 (DNGR-1), a SYK-coupled C type lectin receptor, selectively expressed by CD8α$^+$ DC and involved in sensing and cross-presenting antigens from necrotic cells [33]. Despite the fact that DNGR-1 is an endocytic receptor, its main function may not be phagocytosis but rather to maintain the phagocytic cargo away from lysosomal compartments to allow retrieval of antigens for efficient cross-presentation. The DNGR-1-SYK pathway does not activate DC in response to dead cells and targeting of DNGR-1 with a specific antibody coupled to a tumor antigen requires TLR3 adjuvants to promote antitumor immunity [34••]. The putative human equivalents have been recently reported by several teams, showing that BDCA3$^{high}$DNGR-1$^+$ Lin$^-$DR$^+$ DC, constituting 0.1% of human spleen cellularity, express most of the mouse CD8α$^+$ DC markers (Necl2, CD205, CD207, BATF3, TLR3, IRF8), internalize dead cells and respond to TLR3 and TLR8 agonists to cross-present long peptides [35••]. Whether this novel human DC subset could be exploited for immunological interventions against cancer remains an open conundrum.

Alternatively, bona fide CD8α$^+$ DC residing in the LN can acquire MHC I/peptide complexes generated by cross-presentation from inflammatory monocyte-derived DC [36•]. Such cross-dressing can prime naïve CD4$^+$ and CD8$^+$ T cells against tumor antigens from a necrotic tumor (but not live tumor) that have been uptaken by monocyte-derived DC [37,38].

There are anatomical determinations as to whether CD8α$^+$ versus CD8α$^-$ DC might be more eligible for efficient cross-presentation. It remains unclear how the tumor microenvironment might dictate such events and how cell death (either spontaneous or therapy-induced) might edit the biological features of cross-presentation.

In as much as immunogenic tumors might be frequently invaded by effector memory TILs [39] and since DC also

play a major role in reactivating memory $CD8^+$ T cell responses [40], one wonder whether tumor infiltrating DC (as opposed to LN-residing DC) contribute to the pool of tumor-reactive T cells. Arguing against that hypothesis, memory T cells appear to better respond to LN resident $CD8\alpha^+$ DC than migratory $CD8\alpha^-$ DC located in inflamed peripheral tissues during influenza virus infection (such as virally infected-skin or lung) [41].

It is noteworthy that other inflammatory phagocytes such as neutrophils, diverging ontogenically from DC, may be able to transport tumor antigens from peripheral tissues to lymphoid organs [42], and to cross-present tumor antigens to naïve T cells [43•]. However, neutrophils may contribute to tumor-induced tolerance as suggested by clinical reports associating the presence of intratumoral neutrophils with short recurrence-free survival in localized renal cell carcinoma [44].

## Innate cells providing help for antitumor T cell immune responses

NK cell-killing of target cells is far more efficient at eliciting humoral and cellular immunity than the UV or gamma irradiation-driven cell death modality. The immunogenicity of the NK cell-killing pathway involves TRIF/Myd88 signaling and both type I and II IFNR [45]. Recognition of tumor cells by NK cells can occur via downregulation of MHC class I molecules as well as expression of stress associated ligands or costimulatory molecules (such as CD70 and CD80), all engaging NK cell signaling. The coinciding inflammatory signals with cytotoxicity appear to optimally gear the ensuing adaptive immune response. Indeed, the NK-DC cross-talk plays a dominant role in T cell priming, either by boosting DC maturation (and production of IL-12 and type 1 IFN [46,47]), or by eliminating DC to limit adaptive immunity (as shown in viral infections, [48]). Other innate subsets could do both, kill tumor cells and promote DC maturation. CD1d-restricted NKT cells can induce IL-12p70 production by DC in a CD40-dependent fashion [49] and were shown to promote help for DC cross-presentation to $CD8^+$ T cells in a CCL17/CCR4 dependent manner [50]. Vδ1 γδT cells can trigger DC maturation in a TNFα and CD1c-dependent manner, and synergize with LPS for the induction of naïve $CD4^+$ T cell responses [51].

## Concluding remarks and novel prospects

Immunotherapeutic interventions may have not been very successful so far because of their inability to counteract tumor-induced immunosuppressive pathways and/or of their low capacity to elicit potent and coordinated interactions in the immune network. Resetting the DC/tumor dialogue may be approached in many advantageous ways [52]. A few novel strategies of active or passive immunization will be developed in this conclusion.

### Can we ameliorate the coordination of the multiple DC subtypes?

The cooperative functional pathways existing between various DC subsets have been extensively reviewed [25]. Consequently, a spatiotemporal delivery by poly (lactic-co-glycolic acid) matrices of high levels of GM-CSF containing tumor lysates as well as polyethylenimine-condensed CpG ODN could provide a secondary immunostimulatory site of tumor antigen presentation eliciting efficient $CD8^+$ T cell responses and tumor eradication [53••]. Such matrices could recruit up to $1.2 \times 10^6$ pDC, $6 \times 10^5$ $CD8\alpha^+$ DC and $3 \times 10^6$ $CD11b^+$DC correlating with the local expansion of antitumor CTLs (and the proportional decrease of Tregs) and their recirculation to spleens. Importantly, this local orchestration of a DC network was concomitant with the accumulation of type 1 IFN and IL-12 (and the reduction of TGF-β and IL-10). The prophylactic antitumor efficacy of this vaccine was proportional to the presence of pDC, $CD8\alpha^+$ DC and IL-12.

### How could we best handle $CD4^+$ T cell help?

Naïve $CD4^+$ T cells could become cytotoxic and highly contribute to tumor rejection. Adoptively transferred naïve $CD4^+$ T cell (ACT) specific for self/tumor antigens can differentiate into Th1 and LAMP1/GrzB/Pfr positive cells capable of eradicating large melanoma and inducing autoimmunity. Therapy was independent of prior vaccination, exogenous cytokine support, B or $CD8^+$T or NK or NKT lymphocytes but strongly relied upon common IL-2R γ chain [54]. It appeared that improper DC activation (i.e. low MHC class II and CD86 expression and low sensitivity of DC to respond to and to produce IL-12 and IL-15) will dramatically affect Th1 priming of the $CD4^+$ T cell based-ACT. Low IFN-γ may also contribute to reduced CXCL9 production by the tumor milieu causing a poor recruitment of CXCR3 expressing Th1 $CD4^+$ T cells [55]. Cytokine/antibody immune complexes to IL-15 or IL-2 or IL-7 may mimic the effects of lymphopenia required for such an efficient ACT of naïve $CD4^+$ T cells. These findings highlight the potential of harnessing host DC or *ex vivo* derived DC to better control ACT in the future.

### Can chemotherapy reset tumor immunogenicity?

Certain tumor cell death modalities differentially triggered by routinely administered chemotherapies can be immunogenic, and act as cryptic vaccines [56]. We indeed characterized the molecular pathways associated with an immunogenic cell death during chemotherapy or radiotherapy of cancer. Anthracyclines, oxaliplatin and X Rays, by promoting an ER stress response before apoptosis, induce the exposure of calreticulin (CRT) [57] and the release of high-mobility group box 1 (HMGB1) from dying tumor cells [58]. CRT and HMGB1 play the role of an 'eat me' and a 'danger' signal, respectively, thereby facilitating engulfment and processing of apoptotic

bodies by DC. In addition, dying tumor cells must release ATP to engage P2RX7 on host DC, triggering the activation of the inflammasome plateform Nlrp3, culminating in the release of IL-1β, which in turn elicits tumor-specific IFN-γ producing $CD8^+$ T cells that are indispensable for the success of chemotherapy [59].

TRAIL (and Fas)-mediated tumor cell death is accompanied by CRT exposure on dying tumor cells [60]. Interestingly, anti-DR5 antibody targeting mouse TRAIL promoted tumor clearance through a mechanism involving $CD11c^+$DC [61] suggesting a role for DC in cross-presentation of anti-TRAIL Ab-directed dying tumor cells.

Genetically modified tumor vaccines hold promise in breaking immune tolerance to the tumor by various interesting mechanisms [62] but their GMP manufacturing should take into account the 'cell death-associated molecular patterns' that appear crucial for an appropriate orchestration of immune effectors.

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