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Dendritic cell tumor killing activity and its potential applications in cancer immunotherapy

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Abstract

Universally viewed as the sentinels and messengers of the immune system and traditionally referred to as professional antigen presenting cells, dendritic cells (DC) play a fundamental role in antitumor immunity. DC are uniquely equipped with the ability to acquire, process and present tumor-derived antigens to T lymphocytes. They can drive the differentiation of naïve T cells into activated tumor-specific effector lymphocytes. DC also dictate the type and regulate the strength and duration of T cell responses. In addition, they contribute to NK and NKT cell antitumoral function and to B cell-mediated immunity. Besides this cardinal role as orchestrators of innate and adaptive immune responses, many studies have provided evidence that DC can also function as direct cytotoxic effectors against tumors. This less conventional aspect of DC lifestyle has however raised controversy as it relates to the origin of these cells and the induction, regulation and mechanisms underlying their tumoricidal activity. The possible impact of the cytotoxic function of DC on their antigen presenting capability has also been the focus of intensive research. In this review, we examine these questions and discuss the biological significance of this non-traditional property and possible strategies to exploit DC killing potential in cancer immunotherapy.

Keywords

Cytotoxic dendritic cells; tumor immunity; dendritic cell-based cancer vaccines

I-INTRODUCTION

Since their original discovery by Steinman *et al.*,¹ DC have been the focus of extensive research to understand their ontology and functions in the regulation of immune responses. It soon became clear that DC are endowed with the unique ability to function as professional antigen presenting cells (APC) critical for the development of adaptive immunity.^{2, 3} This primordial characteristic has been the foundation for the development of DC-based vaccines. In the realm of cancer immunotherapy, the prospect of exploiting DC to stimulate protective antitumor immunity has instigated interesting studies in animals and human. This body of

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research demonstrates some promise but also limitations, underscoring the need for further advance in the field.^{4–12}

DC encompass multiple heterogeneous cell subsets defined by a specific phenotype, anatomic localization and specialized functions dictated by their cytokine production profile.^{2, 3, 13, 14} Strategically distributed in the body, DC are the sentinels of the immune system constantly surveying their environment for the presence of invading pathogens and sensing for tissue damages. They constantly acquire antigens by phagocytosis or pinocytosis and migrate to lymphoid organs where they initiate specific T cell responses. The endocytosed antigenic proteins, delivered into the antigen processing machinery of DC, are converted into small peptides that are associated with MHC Class I or Class II molecules and exported at the DC surface for presentation to CD8⁺ or CD4⁺ T lymphocytes, respectively. To efficiently drive the clonal proliferation and activation of CD4⁺ T helper (Th) or CD8⁺ cytotoxic T (CTL) cells, DC need to express lymphocyte co-stimulatory molecules such as CD80, CD86, CD40, OX40L, or ICOSL and produce inflammatory cytokines. Typically, T cell fate decisions are directed by the nature of the DC subset, the level of maturation and activation of the DC and the type of cytokines they produce. The type of T lymphocyte response that eventually develops (for instance Th-1, Th-2, Th-17 or Th-9) is dependent on the DC type.^{2, 3, 14–16} DC are also capable of presenting glycolipids to NKT cells on CD1d molecule and of controlling the activation of NK and B cells.^{17–20} Importantly, DC also play an essential role in the mechanisms of self-tolerance, thereby preventing autoimmunity. In absence of maturation and activation signals, DC lacking co-stimulatory molecules can indeed present self-antigens acquired from apoptotic cells to potentially auto-reactive T lymphocytes, resulting in T cell deletion or anergy.^{21–23} These immature, “tolerogenic” DC can additionally participate in the generation of regulatory T lymphocytes (Treg) or IL-10-secreting Tr-1 cells with suppressor activity.^{24–30}

The notion that arising malignant cells can be recognized and eliminated by the immune system before they develop into clinically apparent tumors, a process known as tumor immunosurveillance, was initially formulated by Burnet and Thomas.^{31, 32} This seminal concept, now supported by compelling experimental evidence, has undergone substantial evolution and refinements leading to its integration into a much broader process, called cancer immunoediting, extensively discussed by Schreiber *et al.*^{32–38} Cancer immunoediting more accurately depicts the complex cross-talks between the immune system and tumors and their outcome as it takes into account the observation that immune cells can not only eliminate tumor cells, but they can also select for immune-resistant cancer escape variants.^{33, 34, 39} This process typically results from three phases, referred to as the 3 “Es” of cancer immunoediting: elimination (the equivalent of tumor immunosurveillance), equilibrium and escape. DC, by virtue of their APC and immune regulatory functions, can intervene at least at two of these stages. During the first phase, the local inflammation generated around neoplastic lesions by modification of the surrounding tissues leads to the recruitment of innate immune cells such as neutrophils, macrophages, NKT or $\gamma\delta$ T cells which in turn promote the attraction of immature DC through chemokine production. A main initial source of tumor antigens available for uptake by these immature DC is provided by the death of a fraction of the tumor cells induced by innate immune cells or the cytokines they produced, such as IFN- γ , or resulting from the anti-angiogenic effects of these molecules.^{32–34, 39} The ingested tumor-derived material is processed into peptides and presented to T lymphocytes on MHC Class I or Class II molecules after homing of the DC to the draining lymphoid tissues. Tumor-specific CTL expanded and activated by DC express specific chemokine receptors leading to their migration to the tumor site where they participate to cancer cell elimination. This CD8⁺ response is further facilitated by DC-activated tumor-specific CD4⁺ Th lymphocytes. DC and Th cells also support the activation of NK, NKT and macrophages. When successful, the elimination phase represents the

complete cancer immunoediting process. However, some malignant cells may acquire additional mutations which confer them with increased resistance to immune detection and elimination.^{39–44} During the equilibrium phase, the immune system exerts a selective pressure on developing tumors leading to the elimination of the most sensitive cancer cells but also, as a corollary, to the selection of these resistant tumor cell variants, eventually leading to the escape phase. At this last stage, uncontrolled tumors commonly exhibit the ability to actively suppress the immune system through the production of immunosuppressive factors or cells as discussed in section III. Some of these mechanisms of tumor-induced suppression result in the negative modulation of DC function leading to the generation of tolerogenic DC. These phenotypically and functionally impaired DC are capable of inducing the anergy or deletion of tumor-specific T lymphocytes and have been involved in immunosuppressive Treg expansion.^{39, 45–47} Thus, although DC play an essential role as orchestrators of the cooperative interactions between the multiple cellular actors involved in the immune attack against tumors during the first phase of cancer immunoediting, these cells can also contribute to the last escape stage by participating to the mechanism of tumor immune tolerance.

Recent studies have lent support to the notion that, besides their role as potent inducers and controllers of immune responses, DC may also be endowed with a direct tumoricidal function. This less conventional characteristic of DC has however received limited attention and has raised many questions as it relates to the actual ontology of so-called “killer DC”, the acquisition of their cytotoxic activity and the underlying mechanism(s) of tumor cell killing. We here review these aspects, further discuss the influence of DC tumoricidal potential on their APC function and their ability to promote or inhibit anti-cancer immunity. We speculate on the integration of this non-traditional property into the broad concept of cancer immunoediting. Possible options to harness and implement DC tumor killing activity in cancer immunotherapeutic strategies are evaluated.

II-PRINCIPAL ASPECTS OF DC CYTOTOXIC FUNCTION

DC endowed with cytotoxic activity have commonly been referred to as “killer DC” (KDC) although these cells do not constitute a separate, dedicated subset. Rather, multiple heterogeneous subpopulations, naturally occurring *in vivo* (native) or generated *in vitro* from specific precursors have been described.^{48–114} A similar degree of diversity and plasticity has been observed as it relates to the modalities of induction of KDC cytotoxic function and to the effector mechanisms underlying tumor cell killing. Because the different subsets of KDC identified in mouse, rat and human have been extensively reviewed previously,^{59, 61–63, 70, 72} (Table I) this section will only summarize the main findings and the principal characteristics of these cells.

A. Identification of mouse and rat KDC

Studies in mouse and rat were the first to highlight the possibility that DC can function as cytotoxic effector cells. An initial report indicated that a mouse splenic DC subpopulation characterized as CD11c⁺CD8α⁺ induced apoptosis of T lymphocytes by a FasL-dependent mechanisms.⁷³ Additional studies later demonstrated the capability of native mouse DC such as Langerhans cells to mediate killing of tumor cells or to play a direct cytotoxic role in the control of bacterial infection.^{74, 75} The notion that native rat DC are endowed with potent tumoricidal activities was initially tackled in a report demonstrating that a subset of splenic DC expressing CD103 spontaneously up-regulated NKR-P1 during overnight culture and kill the NK-sensitive YAC-1 cells by a Ca²⁺ dependent mechanism.⁴⁸ In line with this study, KDC were further characterized in the secondary lymphoid tissues as CD4[−]CD11b⁺NKp46[−]CD103⁺CD200⁺MHCII⁺ and determined to be distinct from the NK cell lineage.^{58, 76, 77} The induction of tumor cell apoptosis was exclusively mediated by

immature DC that did not require exogenous activation and depended on a direct cell to cell contact.⁵⁸ The exact mechanism of cytotoxicity was not elucidated but a possible role for the perforin-granzyme B system or the death receptor family members FasL, TRAIL, or TNF- α was excluded.⁷⁷ These studies also demonstrated that immediately after killing of tumor cells KDC were capable of phagocytosing the resulting apoptotic bodies.^{58, 63} Following maturation these DC became potent APC while exhibiting reduced cytotoxic activity. These early studies sparked a series of investigations focusing on the ability of different DC subpopulations to operate as cytotoxic effectors against cancer cells and on the mechanisms underlying this property.

Multiple studies have further documented that DC generated *in vitro*, essentially from bone marrow cells cultured with GM-CSF and IL-4, can exhibit tumoricidal activity, but considerably variable results were obtained in terms of cytotoxic mechanisms.^{56, 78–82, 115} A role for the death receptor ligands FasL, TRAIL, or TNF- α , or the involvement of NO have been described. We have demonstrated that in rat and mouse, the cytotoxic activity of DC generated from bone marrow was not mediated by perforin/granzyme B, FasL, TNF- α , or TRAIL, but depended on peroxynitrites, the main metabolite of NO.^{54, 68, 69, 72} In these studies, the killing ability of DC generated from iNOS or gp91 knock-out mice was significantly reduced.^{68, 69} Independent groups have also reported on the essential role of NO in both mouse⁵⁶ and rat⁸³ bone marrow-derived DC-mediated apoptosis of tumor cells. Huang et al. demonstrated that preconditioning tumor cells with a NO donor, promoted the down-regulation of the anti-apoptotic protein survivin, and sensitized lymphomas to Fas-L-dependent DC-mediated killing.⁷⁹ With regard to the mode of induction of DC cytotoxic function, some groups have suggested that DC killing potential may be spontaneous in rat^{58, 76} and mouse.^{56, 79} By contrast, we and other groups have demonstrated that non-stimulated immature DC are not capable of killing tumor cells but that their cytotoxic activity can be induced upon activation with specific stimuli such as the TLR4 ligand LPS, the TLR2 agonist Pam3Cys-SK4,⁶⁹ or IFN- γ .^{68, 69} This discrepancy may be partly explained by the specific DC subset studied and the culture conditions utilized.

An important, yet not fully addressed question in the field relates to the molecular regulation of DC killing activity. Recently, in an effort to unravel the regulatory signaling pathways involved in the regulation of mouse bone marrow-derived DC cytotoxic function we have partly deconstructed the molecular machinery controlling iNOS in DC generated with GM-CSF and IL-4 (IL-4 DC) or IL-15 (IL-15 DC). We demonstrated that LPS-induced iNOS expression required the activation of the transcription factors NF- κ B and ISGF3 only in IL-15 DC and that distinct signaling pathways such as AP-1 may be required in IL-4 DC. IFN- γ induced iNOS expression in IL-4 DC by a STAT-1 dependent mechanism. However in IL-15 DC, the cooperation of PIAS-1 and STAT-3 prevented iNOS expression in response to IFN- γ stimulation (Hanke *et al*, submitted manuscript). These results further underline that depending on the considered DC subset, the mechanisms underlying the cytotoxicity function of these cells are highly variable and are likely to depend on the genetic program specifically activated in a given DC subtype. In addition, differences in DC culture conditions (composition, type of cytokines) and mode of isolation may influence the signaling pathways controlling the expression of defined effector cytotoxic molecules, partly explaining the divergent results reported in the current literature.

The proof of principle that rodent KDC can effectively impair tumor growth *in vivo* and promote the survival of tumor bearing animals has been provided in different studies. For instance, splenic rat KDC allowed to kill an osteosarcoma cell line and to acquire tumor cell debris *in vitro* promoted tumor regression when administered to rats bearing the same osteosarcoma.⁷⁶ This KDC vaccine was found to be ineffective in CD8⁺ T cell-depleted animals, suggesting that KDC were capable of efficiently cross-presenting tumor antigens

obtained from target cells to specific CTL.⁷⁶ Other reports have found that NKG2D agonist antibodies promoted the tumoricidal function of rat CD103⁺ DC *in vitro* and hindered tumor progression *in vivo*.⁸⁴ However, the specific contribution of DC cytotoxic activity was not clearly determined. In another study, bone marrow-derived DC genetically modified to express IL-12 and IL-18 acquired tumoricidal activity and were capable of impairing the growth of an established sarcoma in a CD4⁺ and CD8⁺ T cell-dependent manner when injected into the tumor beds.⁸⁰ The direct contribution of pDC in the elimination of tumor cells following treatment with imiquimod has additionally been recently outlined in a mouse melanoma model.¹¹⁶

Special mention should be made of interferon-producing killer dendritic cells (IKDC).^{49, 85–103} Originally characterized in the mouse by a B220⁺CD11c⁺NK1.1⁺CD49b⁺ phenotype with a variable MHC Class II expression profile, these cells were described as hybrids exhibiting characteristics of both NK cells and plasmacytoid DC. IKDC were reported to exhibit potent anti-tumoral effects through TRAIL and perforin/granzyme-dependent mechanisms. It was proposed that when appropriately stimulated IKDC could first function as NK cells by virtue of their cytotoxic property and then following contact with tumor cells could up-regulate MHC Class II and behave as APC endowed with the ability to activate naive CD8⁺ and CD4⁺ T lymphocytes. In addition, it was documented that these cells could produce both IFN- γ and IFN- α .^{90, 98, 100–103} The notion that this hybrid NK-DC cell type unifying killing and APC functions belongs to the DC lineage has however been subjected to intensive debate and more recent studies have eventually provided evidence that IKDC merely originate from NK progenitors.^{85, 91, 104–106} Importantly, the APC potential of these cells has also been questioned.^{85, 91} IKDC were consequently redefined as B220⁺ NK cells and were considered as a subset of activated NK. However, a recent study has again challenged this notion, proposing that B220⁺NK cells, *alias* IKDC, may be an intermediate in NK development which therefore may more accurately be referred to as “pre-mNK”.¹⁰⁶

B. Human KDC

Numerous independent studies have documented the cytotoxic activity of different subsets of native and *in vitro* generated human DC. Similar to the results obtained in rodents, the tumoricidal function of human KDC can be triggered by a variety of agents. The mechanisms of cancer cell killing primarily involved the death receptor ligands, perforin/granzyme B and NO. The requirement for TRAIL in the cytotoxic activity of native plasmacytoid DC (pDC) activated through TLR-7 or -9 and of blood CD11c⁺ DC stimulated with IFN- α or IFN- γ has been documented.^{50, 51, 107} Recent studies have further highlighted the possibility that human pDC producing type I interferons may also function as tumor cell killers.¹¹⁷ Immature CD4⁺HLA-DR⁺Lin⁻ DC have also been shown to trigger apoptosis of different cancer cells, but not normal cells, by four different TNF family member ligands (TRAIL, TNF, FasL, and Lymphotoxin (LT)- $\alpha_1\beta_2$).^{52, 108} Another human DC subset, termed slanDC (characterized by the 6-sulfo LacNAc modification of PSGL-1 and identified using the mAb M-DC8) have also been reported to kill different tumor cell lines by a TNF- α -dependent mechanism.^{65, 109} Furthermore, a plethora of studies have demonstrated that monocyte-derived DC generated *ex-vivo* with GM-CSF and IL-4 or IFN- α or with different cytokine combinations can kill cancer cells by TNF- α , TRAIL, FasL, peroxynitrites or granzyme B acting alone or in cooperation.^{52, 53, 64, 67, 71, 110, 111} In these studies, immature non-activated DC were the primary cytotoxic effectors and their killing activity was reduced or not modified following activation with CD40L, IFN- α , - β , or - γ , or LPS.^{52, 108, 112, 113} or was induced or enhanced with LPS,^{71, 114, 118} IFN- α ,⁶⁴ IFN- β ,¹¹⁰ IFN- γ ,¹¹⁴ CD40L,^{67, 118} or double stranded DNA.⁶⁷ We have recently reported that LPS-activated DC generated from human CD14⁺ blood monocytes killed cancer cells by a

peroxynitrite-dependent mechanism.⁷¹ Additional DC subsets, such as CD34⁺-derived DC,¹¹⁰ or cord blood monocyte-derived DC,⁵⁵ were further described for their capability to eliminate cancer cells by TRAIL-dependent mechanisms following stimulation with IFN- β ,¹¹⁰ IFN- γ or LPS.⁵⁵ Of considerable importance, in the vast majority of these studies, the killing activity of DC was selectively directed against malignant cells while non-cancerous cells were spared. The mechanistic basis explaining normal cell resistance to DC killing activity has not been determined and is still being explored.

In a clinically relevant study, Stary *et al.* demonstrated that administration of patients with basal cell carcinoma with the TLR7/8 agonist imiquimod induced the recruitment to the tumor site of both CD123⁺HLA-DR⁺ pDC and CD11C⁺HLA-DR⁺ mDC.⁵⁷ These two DC subtypes exhibited direct tumor killing activity. However, mDC were detected only at the tumor periphery and expressed perforin and granzyme B, while pDC were identified primarily within the tumor beds and expressed TRAIL.⁵⁷ Further analysis demonstrated that purified mDC and pDC killed perforin-sensitive K562 and TRAIL-sensitive Jurkat cells, respectively, after stimulation with TLR7/8 agonists.⁵⁷

A substantial number of independent studies have thus provided experimental support for the existence, *in vivo* of different subsets of DC endowed with direct cytotoxic activities and for the possibility to generate such KDC *ex vivo*. However, the physiological significance of native DC cytotoxic activity as it relates to cancer development is still an open question.

III- KDC IN TUMORIGENESIS AND CANCER IMMUNITY: DR. JEKYLL OR MR HYDE?

Why highly specialized APC such as DC have acquired an effector cytotoxic activity during the process of evolution is still unclear. It has been proposed that immature non-activated KDC may participate in the mechanisms of peripheral self-tolerance by virtue of their ability to kill self-reactive T lymphocytes under specific conditions.^{63, 73, 119–121} In addition, as molecules associated with bacteria and viruses, such as LPS, TLR-7, TLR-9 or type I IFN can trigger their cytotoxic activity, KDC have been proposed to play a dual role in the control of infection. Indeed, they may eliminate infected cells and present the acquired antigens to specific CTL thus triggering protective immunity, but they may also mediate apoptosis of activated T cells, thereby contributing to pathogen escape from immune responses.^{50, 63, 67, 107, 122–124} In this section we discuss the implications of these versatile KDC in the development of tumors.

As outlined previously, DC have been integrated in the cancer immunoediting model based on their function as initiators and regulators of immune responses. However, how the killing activity of DC may fit in this immunoediting picture has yet to be formally addressed. We envision that KDC cytotoxic function may contribute to each of the 3 phases and may either foster tumor elimination or promote tumor escape depending on the activation status of the cells (Figure 1). Firstly, KDC may play an essential role during the different steps of the Elimination stage. Together with neutrophils, macrophages, NKT or $\gamma\delta$ T cells, KDC may participate in the initial killing of tumor cells, which provides a source of antigens and promotes the recruitment of additional inflammatory cells to the tumor site. It is noteworthy that IFN- γ , a primordial cytokine produced during the elimination phase, can also trigger the cytotoxic function of DC. Conceivably, the tumoricidal function of DC may thus be induced as these cells arrive in the tumor beds. Importantly, the direct killing of cancer cells by KDC allows for the rapid uptake of immediately available cancer-derived antigens in the form of debris or apoptotic bodies before their clearance by macrophages or neutrophils, thereby promoting the acquisition of tumor-derived material in a more efficient manner. Whether KDC may induce an immunogenic type of cancer cell death which may enhance

antigen uptake and processing still needs to be delineated as discussed in section IV hereafter. The possible release of “danger signals” by dying tumor cells may further promote the recruitment and activation of innate immune cells and augment the production of pro-inflammatory cytokines such as IFN- γ . Following killing of cancer cells DC can switch function to their traditional role as messengers and present processed tumor-derived antigens to CD4⁺ or CD8⁺ T lymphocytes leading to the activation of adaptive immunity. Finally, KDC by participating, together with CTL, Th and NK cells, in the direct elimination of tumor cells may contribute to increase the diversity and thereby the efficacy of the immune cytotoxic effector mechanisms resulting ultimately in the elimination of neoplastic foci. The tumoricidal activity of DC may thus be of considerable significance for a successful tumor immunosurveillance process. Secondly, it is possible that during the equilibrium phase and later during the escape stage, the cytotoxic activity of DC may be subverted by cancer cells to escape immune detection and elimination. Indeed, immature KDC that have killed tumor cells, but that are blocked in their maturation by cytokines and factors present in the tumor environment may present acquired tumor antigens in absence of co-stimulation or pro-inflammatory cytokine secretion, leading to apoptosis or anergy of T lymphocytes or to the generation of immunosuppressive Treg. In this case, the tumoricidal property of DC, used to efficiently gather antigens, may promote their tolerogenic function. The possibility that KDC directly mediate killing of CD4⁺ or CD8⁺ T cells may also blunt anti-tumor responses. As the maturation status of DC dictates the outcome of the response (anti-cancer immunity versus immune suppression), it would therefore be essential to define the conditions under which tumor cell killing by KDC *in vivo* results in their full maturation or conversely in the inhibition of their maturation. Such conditions may be partly driven by the type of tumor cell death (immunogenic or not) inflicted by KDC and therefore may depend on the nature of the cytotoxic mechanism (TRAIL, peroxynitrites for instance) triggered in a defined tumor-infiltrating DC subset. Thirdly, in instances where KDC cytotoxic function is associated with the production of peroxynitrites, these cells may serve as a source of reactive nitrogen intermediates (RNI) and reactive oxygen species (ROS) which are known to induce DNA damage.¹²⁵ It is therefore possible that KDC may enhance the genetic instability that contributes to the emergence of new tumor escape variants during the equilibrium phase. Conceptually, the direct tumor killing activity of DC may thus have important implications at all steps of cancer immunoediting and a transition of KDC from “Dr. Jekyll” to “Mr. Hyde” may occur during the equilibrium phase.

A possible additional role of KDC in cancer has yet to be considered: the implication of these cells in inflammation-promoting tumorigenesis. Chronic inflammation resulting from bacterial or viral infection, exposure to pollutants or obesity has been reported to increase the risk of cancer by different mechanisms.^{125, 126} During many of these inflammatory conditions DC are recruited and activated. In theory, KDC endowed with the capability of producing ROS and RNI could participate in tumor initiation as these molecules can induce DNA damage and inactivate mismatch repair enzymes therefore enhancing mutagenesis and driving normal cells along the tumorigenic path.¹²⁷ Although in some cases such as chronic infections, DC cytotoxic property can be triggered,^{50, 107, 122–124} the actual presence of these cells and the possibility that they may enhance oxidative stress needs to be determined in many chronic inflammatory conditions. In addition, whether the levels of RO and RNI produced by KDC, a relatively sparse population *in vivo*, are sufficient to actually impact target cells is questionable.

The specific contribution of DC cytotoxic function in cancer immunoediting and carcinogenesis is complex and difficult to analyze experimentally given the heterogeneous nature of KDC and the diversity of their mechanisms of toxicity. Comprehensive studies are therefore still needed to address this question. One possibility to address such issues may consist in the evaluation of tumor occurrence and development in conditional knock-out

animals. Specifically inhibiting the expression of different cytotoxic molecules in different DC subsets *in vivo* may provide important information. For example, it would be interesting to determine the susceptibility of animals with iNOS, TRAIL or Fas-L deficiency in the CD11c compartment to develop spontaneous chemically-induced tumors. Similar studies maybe performed in chronic inflammation models.

The cytotoxic function of KDC may thus contribute to the intertwined pro- and anti-tumoral roles of these multifaceted cells but more systematic studies are needed to provide definitive experimental proof of the physiological role of these cells during cancer development.

IV- HARNESSING THE TUMOR KILLING ACTIVITY OF KDC: PROMISES, LIMITATIONS AND CURRENT QUESTIONS

The possibility of harnessing the ability of DC to function as professional APC capable of orchestrating adaptive and innate immunity has led to the development and implementation of DC-based vaccines in cancer immunotherapy strategies. However, the initial evidence that protective anti-tumor immunity can be successfully generated by vaccination with tumor antigen-loaded DC has been undermined by the limited clinical responses observed in cancer patients, dampening enthusiasm for this approach.^{128–136} The prospect of exploiting the non-conventional tumoricidal activity of these cells may therefore represent a novel step toward the development of improved DC-based cancer immunotherapies. To this end, different applications for KDC in cancer immunotherapy can be envisioned.

One approach may consist in the administration of *in vitro* generated KDC generated *in vitro* that are allowed to kill tumor cells, capture and process the derived antigens in culture. As outlined in the previous section the mode of acquisition of cancer-derived antigens by KDC may be of particular efficacy. In line with this notion, further studies are required to determine whether KDC may promote a more immunogenic type of tumor cell death which may enhance antigen uptake, processing and presentation. It is indeed possible that DC-mediated killing of cancer cells is associated with the generation of signals expressed at the surface of dying tumor cells or secreted, such as calreticulin, HMGB1, uric acid or HSP^{137–141} that may enhance DC maturation, cytokine production and ability to induce T lymphocyte activation. It will also be important to determine if KDC-mediated tumor cell death may stimulate DC endocytosis capabilities or activate the antigen presenting molecular machinery, and to define the underlying mechanisms. How conventional DC fed with dead tumor cells isolated from a KDC-cancer cell co-culture compare to the same DC loaded with apoptotic or necrotic cancer cells in term of ability to induce tumor-specific T lymphocyte activation may provide some answers to this question. Of clinical relevance, it will be important to determine the potential advantage of using KDC (purified after killing of cancer cells *in vitro*) as cancer vaccines to treat tumor-bearing hosts over conventional DC (loaded with different approaches such as peptides, RNA, DNA, tumor-derived exosomes or dead tumor cells). The possibility to control the activation status of the DC *in vitro* using cytokines or TLR ligands represents an important advantage of this approach as it avoids the generation of immature tolerogenic DC. A second strategy may entail the systemic or intra-/peri-tumoral injection of unloaded KDC generated *in vitro*. Encouraging results of this approach have been reported.¹⁴² Additional approaches may consist in promoting the tumoricidal activity of DC *in vivo* and/or the recruitment of these KDC to the tumor beds. The study by Stary *et al* using the TLR-7 ligand Imiquinod has provided the framework for such therapies.⁵⁷ Of importance, the efficacy of the strategies outlined above may be augmented by promoting simultaneously the tumoricidal activities of multiple KDC subsets, capable of inducing tumor cell killing by different mechanisms. Lastly, the artificial generation of killer DC by genetic manipulation (induction of TRAIL, FasL or iNOS

expression in conventional DC) may also be considered. The engineered KDC may then be directly injected into the tumor beds or administered after killing of tumor *cells in vitro*.

From a therapeutic and clinical perspective, the primary interest in KDC relies on the multitasking aspect of these cells unifying killer and messenger functions. In the design of KDC-based therapies, the cytotoxic activity of these cells is likely to be more relevant for the acquisition of tumor antigens than for the actual direct control of tumor growth *in vivo*. Supporting this idea, some studies have indicated that the protective anti-tumoral effects of KDC *in vivo* ultimately require the induction of CD4⁺ and CD8⁺ T lymphocytes.⁸⁰ Therefore, it is essential that KDC can act not only as powerful cytotoxic effectors but can switch function to efficient antigen presenting cells capable of priming T lymphocytes. This important notion that a same population of cells can unify killing and APC activities is still an area of contention and the true antigen-presenting ability of KDC has been intensively debated. We have demonstrated that following killing *in vitro* of B16 melanoma cells expressing the model antigen ovalbumin (OVA), KDC were capable of activating lymphocytes from OT-I (recognizing H-2K^b/OVA_{257–264} complexes) or OT-II (recognizing I-A^d/OVA_{323–339}) mice.^{68, 69} Importantly, these KDC were significantly more efficient at inducing T cell activation than non-cytotoxic DC from iNOS^{−/−} mice. (Hanke *et al.*, submitted manuscript) and⁶⁸ Most KDC expressed both iNOS and H-2K^b/OVA_{257–264} suggesting that the same cells that processed OVA peptides were capable of producing the cytotoxic molecule NO and therefore were equipped with killing abilities. In addition, KDC injected into B16-OVA tumor beds and re-isolated from the draining lymph nodes were more potent at promoting OT-I and OT-II proliferation than their non-killer counterparts. (Hanke *et al.*, submitted manuscript) and⁶⁸ These results therefore established a link between the tumoricidal activity of DC and their competence at presenting antigens from the cancer cells they have killed.

The peculiar ability of some KDC subsets to selectively kill malignant cells while sparing normal cells further reinforces the therapeutic interest in KDC-based therapy as limited side-effects associated with the administration of these cells are expected. The molecular bases explaining this relative specificity have not been delineated. KDC may express inhibitory receptors recognizing defined ligands on normal cells, which may lead to the suppression of their cytotoxic activity. Alternatively, KDC may also recognize, through specific receptors such as NKG2D, specific molecules induced at the tumor cell surface during the process of transformation and leading to the induction of their killing potential. Determining how KDC may distinguish tumor from non-malignant cells remains one of the many challenges in the KDC field.

As underlined in section I, cancer cells can alter the development of anti-tumor immunity by a plethora of mechanisms leading to the establishment of a suppressive environment. It is now well-accepted that this immuno-inhibitory tumor environment represents a major obstacle for cancer immunotherapy strategies including DC-based vaccines. A number of molecules, including TGF- β , IL-10, IL-13, VEGF, IDO, or PGE₂, produced by tumor or stromal cells may exert inhibitory effects on immune system.^{13, 143–149} These tumor-derived factors impair DC differentiation and promote the accumulation of immature tolerogenic DC, as well as myeloid-derived suppressor cells (MDSC)^{47, 147, 150–154} or CD4⁺CD25⁺FoxP3⁺ Treg induced by tumors.^{29, 155–160} Multiple studies in humans and in animal models have demonstrated that associating DC-based therapy with Treg or MDSC elimination or inactivation strategies, and more generally with approaches aimed at overcoming tumor-induced tolerance may enhance the clinical efficiency of DC based cancer vaccines.^{161–163} For instance chemotherapeutic drugs such as cyclophosphamide foster adoptive immunotherapy of established tumor through the elimination/inactivation of immunosuppressive Treg.^{156, 164, 165} We have reported that in an established lymphoma

model, the efficiency of DC pulsed with total tumor cell lysates is significantly enhanced by imatinib mesylate, a chemotherapeutic drug used to treat BCR-ABL⁺ leukemia.¹⁶⁰ Therefore, to reach optimal therapeutic efficacy, KDC-based vaccines may need to be combined with strategies to lessen tumor-induced tolerance.

V-SUMMARY/CONCLUSION

Significant progress in our understanding of DC immunobiology has been the driving force behind the design, optimization and implementation of these cells as vaccines in cancer immunotherapy. However, the modest clinical efficacy of this approach has eroded initial enthusiasm. Arguably, the establishment of tumor-induced immune tolerance represents one of the major challenges encountered in the field, especially in patients with terminal stage disease. The unexpected potential of DC to mediate an effector cytotoxic function has revived the attractiveness of these cells in cancer therapy. Although formal experimental evidences are still awaited, we propose to integrate KDC as a multitasking cellular actor in the cancer immunediting concept with an essential role at different stages of this process. Remaining areas of contentions still persist in the field, which warrant additional investigations. The possibility to generate and manipulate KDC *in vitro* may open new possibilities for the design of more potent anti-cancer vaccines, which may be exploited to improve immunotherapies for cancer patients. The future of KDC-based vaccines may likely consist in their implementation in combination regimens, such as chemoimmunotherapy, designed to concomitantly promote tumor cell killing, trigger adaptive immune responses and eliminate or avert the suppressive effects of tumor-associated factors or immune inhibitory cells.

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ABBREVIATIONS

APC	antigen presenting cells
CTL	cytotoxic T lymphocytes
DC	dendritic cells
FasL	fas-ligand
γδT	gamma/delta T cells
GM-CSF	granulocyte macrophage colony stimulating factor
HMGB1	high mobility group box -1
HSP	heat shock proteins
IFN-γ	interferon-gamma
iNOS	inducible nitric oxide synthase
ISGF3	interferon-stimulated gene factor 3
KDC	killer dendritic cells
LPS	lipopolysaccharide
mDC	myeloid DC
MDSC	myeloid-derived-suppressor cells

MHC	major histocompatibility complex
NF-κB	nuclear factor κ B
NK	natural killer cells
NKR-P1	natural killer cell receptor protein 1
NKT	natural killer T cells
NO	nitric oxide
OVA	ovalbumin
pDC	plasmacytoid DC
RNI	reactive nitrogen intermediates
ROS	reactive oxygen species
STAT	signal transducer and activator of transcription
Th	T helper lymphocyte
TLR-	toll-like receptor
Treg	regulatory T lymphocytes
TNF-α	tumor necrosis factor alpha
TRAIL	TNF-related apoptosis-inducing ligand

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Fig. 1. The multifaceted role of KDC in cancer immunoediting

The cytotoxic function of KDC may promote the release and subsequent acquisition of tumor-derived antigens during the first steps of the elimination process. After maturation, KDC can switch function and present processed cancer antigens to tumor-specific CD4⁺ or CD8⁺ leading to their clonal expansion and activation. KDC may also contribute to the equilibrium phase by producing RNI and ROS which may exacerbate the mutational potential of malignant cells and foster the emergence of new populations of immune escape variants. During the escape stage, immature KDC may take advantage of their killing activity to gather cancer antigens. These KDC, impaired in their maturation by the immunosuppressive environment may trigger the anergy or apoptosis of T lymphocytes or promote the generation of suppressive Treg.

Table I**KDC subsets involved in tumor cell killing**

Subset	Mechanism	Induction	Target	Reference
Human				
Blood CD11c ⁺ mDC	TRAIL	IFN- γ , IFN- α	Jurkat, OVCAR3, PC-3, WM793, not normal cells	51
	TNF- α	IL-15, IFN- γ , LPS	Various breast cancer cell lines	53
	Perforin/Granzyme	TLR-7 agonist	K562	57
Monocyte derived	TRAIL	dsRNA, Type I IFN, CD40L	MDA231	67
		Type I IFN	Jurkat	166
		IFN- β	HL60, Reh	110
		Spontaneous	Various hematopoietic tumor cells	167
		Spontaneous	HPV ⁺ keratinocytes	168
	NO	LPS	HT29	71
Blood pDC	TRAIL	TLR-7 agonist	Jurkat	57
		HIV, TLR-7 agonist	SupT1	107
		Virus and TLR-7/TLR-9 agonists	A549, MEL	50
CD34 ⁺ derived	TRAIL	IFN- β	HL60, Reh	110
Cord Blood CD14 ⁺ derived	TRAIL	LPS, IFN- γ	HL60, Daudi, Jurkat, not normal cells	55
Blood CD4 ⁺ HLADR ⁺ Lin ⁻	TRAIL, TNF- α , FasL, LT- $\alpha_1\beta_2$	Spontaneous	Various tumor cell lines	52, 108
Blood slanDC (M-DC8 ⁺)	TNF- α	IFN- γ	Various tumor cell lines, not normal cells	65
Mouse				
Bone marrow derived	NO	Spontaneous	MCA205, MC38, TS/A	56
		IFN- γ	4T1, B16	68
		LPS, Pam3Cys-SK4	B16F10	69
	FasL	Spontaneous	A20, EG7, EL4	79
	TNF- α	IL-12, IL-18	CMS4, MethA (sarcomas)	80
Rat				
Bone marrow-derived	NO	NKG2D Ligand (anti-NKRP2)	AK-5	
		LPS, IFN- γ	PROb	54
Spleen CD103 ⁺	TNF- α	NKG2D Ligand (anti-NKRP2)	AK-5	84
	No role of FasL, TRAIL, TNF- α	Spontaneous	Various tumor cell lines	58, 76, 77