REVIEW

Killer dendritic cells and their potential for cancer immunotherapy

**Nicolas Larmonier · Jennifer Fraszczak ·
Daniela Lakomy · Bernard Bonnotte ·
Emmanuel Katsanis**

Abstract Known for years as the principal messengers of the immune system, dendritic cells (DC) represent a heterogeneous population of antigen presenting cells critically located at the nexus between innate and adaptive immunity. DC play a central role in the initiation of tumor-specific immune responses as they are endowed with the unique ability to take up, process and present tumor antigens to naïve CD4+ or CD8+ effector T lymphocytes. By virtue of the cytokines they produce, DC also regulate the type, strength and duration of T cell immune responses. In addition, they can participate in anti-tumoral NK and NKT cell activation and in the orchestration of humoral immunity. More recent studies have documented that besides their primary role in the induction and regulation of adaptive anti-tumoral immune responses, DC are also endowed with the capacity to directly kill cancer cells. This dual role of DC as killers and messengers may have important implications for tumor immunotherapy. First, the direct killing of malignant cells by DC may foster the release and thereby the immediate availability of specific tumor antigens for presentation to cytotoxic or helper T lymphocytes. Second, DC may participate in the effector phase of the immune response, potentially augmenting the diversity of the killing mechanisms leading to tumor elimination. This review focuses on this non-conventional cytotoxic function of DC as it relates to the promotion of cancer immunity and discusses the potential application of killer DC (KDC) in tumor immunotherapy.

N. Larmonier (✉) · E. Katsanis (✉)
Department of Pediatrics, Steele Children's Research Center,
University of Arizona, 1501 N. Campbell Ave.,
PO Box 245073, Tucson, AZ 85724-5073, USA
e-mail: nrlarmon@email.arizona.edu

E. Katsanis
e-mail: katsanis@peds.arizona.edu

N. Larmonier · E. Katsanis
Department of Immunobiology,
University of Arizona, Tucson, AZ 85724, USA

N. Larmonier · E. Katsanis
BIO5 Institute and Arizona Cancer Center,
University of Arizona, Tucson, AZ 85724, USA

J. Fraszczak · D. Lakomy · B. Bonnotte
Faculty of Medicine, INSERM UMR 866,
IFR 100, Dijon, France

Introduction

The primary objective of cancer immunotherapy is to promote tumor eradication through the activation of innate and adaptive immune responses. The unique property of dendritic cells (DC) to act as professional antigen presenting cells (APC) has positioned them as central players in the orchestration and control of the complex interacting cellular networks that govern these immune responses. Such a cardinal role has also been largely the basis for the utilization of these cells as promising tools to induce and sustain anti-cancer immunity [1, 2]. DC represent a heterogeneous population of cells with specific subsets defined by their anatomic distribution, phenotype, mode of antigen presentation and cytokine production profile [3–5]. They continuously gather antigens in peripheral tissues, process them and, following migration to the secondary lymphoid organs, present them on major histocompatibility complexes (MHC) Class I or Class II to CD8+ cytotoxic (CTL) or CD4+ helper (Th) T lymphocytes, respectively [4, 6].

The resulting clonal expansion and activation of CTL and Th cells by DC represents a critical event for the elimination of the source of antigens. The type of DC subset, the level of activation of these cells and the nature of the cytokines they produce dictate the differentiation of effector T lymphocytes toward a defined polarized subset (Th-1, Th-2, Th-17…) [4–8]. DC also modulate the function of numerous other immune cells, including NK, B or NKT cells [9–12]. Although critical for the development of adaptive immune response, DC may also contribute to the mechanisms of immune tolerance, thus playing a central role in the control of autoimmunity [13–17]. These so-called tolerogenic DC may silence or anergize effector T lymphocytes [14, 18, 19], induce FoxP3$^+$ regulatory T cells (Treg) or drive the differentiation of anergic IL-10-secreting immunosuppressive Tr-1 cells [20–23].

The current vision of cancer immunosurveillance relies on a multi-stage process tightly enforced and regulated by DC and which requires the initial release of tumor antigens [2, 24, 25]. A principal source of available antigens is generated in the form of apoptotic or necrotic debris that result from spontaneous tumor cell death or from tumor cell killing by macrophages, NK, NKT or other cytotoxic innate immune cells or by chemotherapeutic drugs [26–29]. The initial step consists of the capture of tumor antigens by immature DC attracted to the tumor site. DC subsequently enter a maturation and activation phase, process the captured antigens into peptides and present them to T lymphocytes on MHC Class I or Class II molecules. Following activation, tumor-specific CD8$^+$ CTL, the dominant effector killer cells, leave the secondary lymphoid organs and home to the tumor site where they can eliminate tumor cells by multiple killing mechanisms extensively reviewed in [24]. CD4$^+$ T helper lymphocytes primed by DC may further support CTL responses and provide help to other cytotoxic innate and adaptive immune cells such as NK or macrophages [29, 30]. Activated NKT cells that recognize tumor-derived glycolipids associated with CD1d expressed by DC may also lead to tumor cell destruction [31]. Thus, DC can theoretically orchestrate the immune attack against cancer at virtually all of its stages (initiation, maintenance and regulation, activation of diverse effector anti-tumoral cells). As such, they represent strategic targets for immune intervention strategies and have successfully been used in animals and humans to induce specific anti-cancer immunity after being loaded with tumor antigens [2, 32–39].

Although the direct elimination of tumor cells has primarily been attributed to highly specialized killer cells such as CTL, NKT, NK, or macrophages, many studies conducted mainly in rodents and humans have highlighted the capacity of multiple DC subsets to exert direct cytotoxic activity against cancer cells in vitro and in vivo [24, 25, 40–42]. These killer DC (KDC) may foster the release of tumor antigens and consequently their rapid acquisition and presentation to T lymphocytes. Additionally KDC by virtue of their direct tumoricidal activity may augment the diversity and thereby the efficiency of the cytotoxic effector mechanisms responsible for tumor destruction (Fig. 1). In this review we examine the cytotoxic property of DC as it pertains to their role in the promotion of cancer immunity and the potential interest of these multitasking cells for cancer immunotherapy.

KDC are heterogeneous in phenotype, tissue distribution, function and mode of target cell killing

Multiple subsets of KDC have been described in rodent and human with conflicting results with regard to the mode of induction of their cytotoxic activity (spontaneous or induced) and to their mechanism of target cell killing [24, 25, 40–42] (Table 1).

KDC described in vivo

Native mouse KDC

In mouse, initial evidence indicating that DC may harbor cytotoxic properties came from a study by Suss et al. [43]. A CD11c^{high}CD8α^+Fas-ligand(FasL)$^+$ splenic DC subpopulation was identified with the capacity to kill CD4$^+$ T lymphocytes in a Fas/FasL-dependent manner without additional stimulation [43]. In line with this study, FasL expression by mouse Langerhans DC that became capable of triggering Jurkat cell apoptosis was reported, but required activation of the cells with CD40 ligand [44]. A non-conventional population of CD11c^{int}CD11b^{int} DC expressing inducible nitric oxide synthase (iNOS) and producing large amounts of TNF-α was described in the spleens of mice infected with *Listeria Monocytogenes* [45]. This DC subset critically contributed to innate responses controlling bacterial infection but was not capable of priming naïve T cells [45].

Mouse NKDC and IKDC

Multiple reports have documented the existence in mouse of hybrid NK-DC cells that may first behave as killers and then acquire APC properties leading to the efficient priming T cell immune responses. This concept of NKDC, IFN-producing KDC or IKDC has raised numerous questions and has been an area of extensive controversy [46–65]. Initially characterized phenotypically by the co-expression of DC and NK markers (CD11c and NK1.1, respectively), these cells were shown to produce high levels of IFN-γ following exposure to tumor cells or activation with CpG and IL-4 or
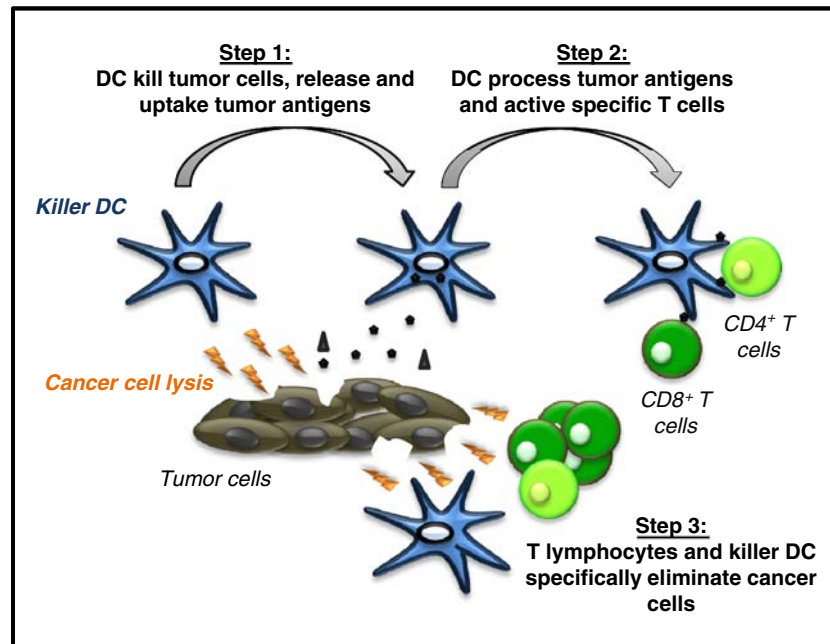
Fig. 1 Role of KDC in immunity against cancer. KDC initially kill directly cancer cells by multiple mechanisms, leading to the production of dead tumor cells or cellular debris that represent an ideal source of antigens available for immediate uptake by KDC. In a second phase, KDC process tumor-specific antigens and associate them with MHC Class I or Class II molecules, mature and migrate to the lymph nodes where they activate tumor specific CD4$^+$ T helper cells and CD8$^+$ CTL that in turn migrate to the tumor site. The final step involves the massive elimination of tumor cells by CTL and other killer cells such as macrophages supported by activated CD4$^+$ T lymphocytes, with the participation of KDC as cytotoxic effectors

IL-12 and IL-18 [53, 57]. Their expansion was proposed to be fostered by Flt3-ligand [50]. When isolated from mouse spleen, liver, lymph nodes and thymus and appropriately activated, NKDC were reported to exert cytotoxic effects against tumor cells and to subsequently present antigens to naïve T cells [50, 53]. They have thus been involved in anti-tumor immune responses [50, 53, 57, 58], but also in the control of infection [59]. This concept of a unique hybrid NK-DC cell type unifying phenotypic and functional properties of NK and DC has, however, been questioned based on the fact that the markers used to identify these cells (CD11c and NK1.1) may not unambiguously distinguish them from classical NK cells [24, 42, 66] and possible cross-contamination by conventional DC or NK may have occurred in these studies.

One particular subset of NKDC termed interferon producing killer dendritic cells or IKDC has recently been the focus of a more refined characterization [47–49, 52, 54, 60–65]. IKDC were phenotypically defined as CD11c$^+$B220$^+$ NK1.1$^+$CD3$^-$CD19$^-$Gr1$^-$Ly6C$^-$ cells. Non-activated IKDC were characterized by the absence of MHC Class II or co-stimulatory molecule expression. Following activation with Toll-like Receptor Ligands (primarily TLR3, TLR4 or TLR9 ligands), cytokines or after contact with tumor cells or virus-infected cells (but not normal cells) IKDC were reported to up-regulate MHC Class II, CD40 and CD86 and to produce large amounts of IFN-γ or IFN-α,

or IL-12 [49, 54]. IKDC killing activity was documented as transient and associated with the overexpression of NKG2D and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). The loss of IKDC cytotoxic function coincided with acquisition of MHC class II, costimulatory molecule expression and antigen presenting function [24, 25, 49, 54]. IKDC were proposed to play an important role in tumor immunosurveillance through cancer cell killing by TRAIL or/and perforin-granzyme-dependent mechanisms [54, 62–65] and with a major role of IL-15 to support their expansion and function in vivo [52, 60]. However, compelling evidence has recently revealed that these cells may not belong to the DC lineage but are rather a subset of NK [46, 55, 67]. In addition, and inconsistent with previous studies, IKDC appeared to be very poor APC, did not produce IFN-α [46], were absent in Rag2$^{-/-}$IL2Rγ$^{-/-}$ mice [46, 55] and did not express PU.1, a transcription factor involved in hematopoiesis and expressed by DC but not by NK cells [46].

Rat KDC detected in vivo

In rat, a subset of spleen and thymus DC that express the NK marker NKR-P1 was identified more than 10 years ago [68]. Freshly isolated splenic DC upregulated NKR-P1 after culture and were able to kill the NK-sensitive YAC-1 target cell by a Ca^{2+}-dependent mechanism [68]. The same

Table 1 Major subsets of KDC

Subset	Killing mechanism	Induction	Targets	Reference
Mouse				
Bone marrow-derived	Fas-L	Spontaneous	Jurkat	[93]
	NO	Spontaneous	Tumor cells	[96]
	TRAIL	Enhanced by LPS	T cells	[97]
	TNF-α, TRAIL	IL-12, IL-18	Tumor cells	[95]
	FasL, TNF, TRAIL	Spontaneous	Tumor cells	[94]
Spleen CD8α⁺	Fas-L	Spontaneous	CD4⁺ T cells	[43]
Spleen NKDC (CD11c⁺CD49b⁺)	*ND*	LCMV + anti-CD40	RMAS, YAC	[51]
Spleen NKDC (CD11c⁺NK1.1⁺)	Perforin/Granzyme	CpG/IL-18	NK-sensitive cell line	[53, 57]
IKDC (CD11c⁺ NK1.1⁺B220⁺)	TRAIL	Imatinib + IL-2	Tumor cells	[54]
IKDC (CD11cⁱⁿᵗCD49⁺ B220⁺)	Perforin/Granzyme	CpG, listeria	YAC	[49]
Langerhans cells	Fas-L	Anti-CD40	T cells	[44]
Lymph Node DC	Fas-L	Influenza virus	T cells	[118]
Rat				
Bone marrow-derived	NO	NKG2D (anti-NKRP2)	Histiocytic tumor	[73]
	NO	LPS, IFN-γ	Tumor cells	[99]
Spleen CD103⁺	Perforin/Granzyme	Spontaneous	YAC	[68]
			Osteosarcoma	[69]
	TNF-α	NKG2D (anti-NKRP2)	Tumor Cells	[72, 73]
Spleen and Lymph Node (CD103⁺CD4⁺)	*ND*	Spontaneous	Tumor cells, Endothelial cells	[70, 71]
Thymic DC	NO	Spontaneous	Thymocytes	[117]
Human				
Blood CD11c⁺ mDC	TRAIL	IFN-α, IFN-γ	Tumor cells	[76]
	TNF-α	LPS, IFN-γ, IL-15	Tumor cells	[79]
	Perforin/Granzyme	TLR7/8 (Imiquimod)	K562	[80]
Blood M-DC8⁺ mDC	TNF-α	IFN-γ	Tumor cells	[84]
Blood (CD4⁺HLADR⁺Lin⁻)	TNF-α, TRAIL FasL, LTα1β2	Spontaneous	Tumor cells, Endothelial cells	[77, 78]
Blood pDC	TRAIL	TLR7/8 (Imiquimod)	Jurkat	[80]
		Influenza virus CpG, R848	A549, Mel	[75]
		HIV, IFN Imiquimod	T cells	[74]
CD34-derived	TRAIL	IFN-β	Tumor cells	[102]
Monocyte-derived	TRAIL	Measles virus	MDA231	[104]
		CD40-L	Tumor cells	[104]
		IFN-α, IFN-γ	Tumor cells	[109]
		LPS, IFN-γ	Tumor cells	[112]
		IFN-β	Tumor cells	[102]
		dsRNA	MDA231	[104]
		Spontaneous	Tumor cells	[103]
	TNF-α, TRAIL FasL, LTα1β2	Spontaneous	Tumor cells	[78]
	TNF-α	LPS, IFN-γ	Tumor cells	[79]
		CD40L	MDA231	[104]

team later characterized splenic and lymph node KDC as CD4⁻NKp46⁻CD11b⁺CD103⁺CD200⁺MHC Class II⁺ and established that these cells were not related to the NK lineage [69–71]. Freshly isolated immature rat KDC triggered apoptotic death of NK-sensitive but also and more impor-tantly NK-resistant tumor cell lines, further arguing against a NK-type lineage [70, 71]. Interestingly KDC killing activity was spontaneous (no exogenous activation was required), restricted to immature cells, did not involve the perforin-granzyme system and was not dependent on the

death receptor family molecules Fas-L, TRAIL or TNF-α or on caspase activation, but required cell contact [70]. Following killing of tumor cells, these KDC were capable to specifically take up the resulting apoptotic bodies [70]. Interestingly, KDC activation resulted in the loss of their tumoricidal and endocytic ability [70]. A more recent study provided some relevance of these findings in vivo as it pertains to tumor growth control [69]. In this report, splenic KDC driven to kill and take up an osteosarcoma tumor cell line in vitro were shown to promote tumor regression when administered to rats bearing the same osteosarcoma. Since the beneficial effects of KDC vaccination were abrogated in CD8 T cell depleted animals, the authors suggested that following killing and capture of cancer cells, KDC were capable to efficiently cross-presenting tumor antigens to specific CTL [69]. It has independently been reported that rat CD103+ DC from spleen or lymph nodes of tumor-bearing rats expressed NKR-P2 (NKG2D). Agonistic NKG2D antibodies promoted the tumoricidal activity of KDC in vitro and hindered tumor progression in vivo, while KDC killing potential was impaired by blocking the interaction of this molecule with its ligand [72]. NKR-P2/NKG2D engagement led to KDC activation evidenced by the upregulation of MHC Class II, NKR-P2 and costimulatory molecule expression, production of cytokines such as TNF-α and secretion of high amounts of nitric oxide (NO) leading to apoptosis of tumor cells [72, 73].

Native KDC described in human

In humans, several studies have reported on the cytotoxicity of different subsets of native DC endowed with a wide variety of killing mechanisms and modes of induction. Human blood DC may be classified into two subpopulations: CD11c+HLA-DR+ myeloid DC (mDC) or CD123+HLA-DR+ plasmacytoid DC (pDC). Following exposure to viruses (such as HIV or the influenca virus) pDC stimulated through TLR7 or TLR-9 may acquire TRAIL-dependent killing properties [74, 75]. IFN-α or IFN-γ may also promote TRAIL expression on freshly isolated peripheral blood CD11c+ DC, but not IL-3 receptor α+ (IL-3Rα+) pre-DC, endowing them with the capacity to kill TRAIL-sensitive tumor cells [76]. Additional studies have suggested that native immature HLA-DR+CD4+lin− DC from peripheral blood induced apoptosis of various cancer cells but not normal cells through TNF, Fas-L, TRAIL or Lymphotoxin-α1β2 [77, 78]. TNF-α-dependent anti-tumor effects of blood DC were also described against breast cancer cell lines which were enhanced with LPS, IFN-γ or IL-15 [79]. Of clinical relevance, the seminal work of Stary et al. in patients with basal cell carcinoma demonstrated that a TLR7/8 agonist (Imiquimod) promoted the recruitment to the tumor site of CD123+HLA-DR+ pDC and CD11c+HLA-

DR+ mDC both endowed with cytotoxic activity [80]. Interestingly, mDC were located at the tumor periphery and expressed perforin and granzyme B while pDC were infiltrating the tumor beds and expressed TRAIL [80]. In this study, blood-derived mDC were shown to also express perforin and granzyme B (10–15%) but not TRAIL upon TLR-7/8 stimulation and were capable of killing K562 leukemia cells but not perforin/granzyme-resistant Jurkat cells [80]. Conversely, expression of granzyme B was detected in the cytoplasm of a majority of unstimulated pDC isolated from the peripheral blood but was significantly reduced following activation with TLR7 or TLR7/8 ligands [80]. However, these TLR agonists induced TRAIL expression on blood-derived pDC which gave them the ability to trigger TRAIL-dependent apoptosis of Jurkat cells [80].

CD11c+CD123− mDC are commonly subdivided into three subpopulations based on the expression of CD16, CD1b/c, and CD141 (BDCA-3). The CD16+ subset encompasses cells expressing or not the M-DC8 antigen (M-DC8+ or M-DC8− DC) [81, 82]. Freshly isolated blood M-DC8+ cells have been reported to express FcγRIII and FcγRII and to induce tumor-directed antibody dependent cell-mediated cytotoxicity (ADCC) [83]. These blood myeloid M-DC8+ DC may also exert cytotoxic effects against different tumor cell lines but not against normal cells when activated with IFN-γ [84].

Besides the above mentioned reports, Langerhans cells have additionally been shown to express the death receptor ligand Fas-L [85], but the physiological relevance of this finding remains unclear. Whether other subsets of human DC may be capable of killing target cells remains poorly explored.

These studies thus demonstrate the existence in vivo in rodents and humans of different subsets of DC with a potential cytotoxic activity that, in most cases, is dependent on exogenous activation signals.

Generation of KDC ex vivo

The paucity of DC in vivo has been a key-limiting factor for the application of DC-based vaccines in cancer therapy. This limitation has been overcome by the development of ex vivo differentiation and expansion procedures that allow the generation of these cells in large numbers. The primary source of DC currently used in clinical trials and in most animal studies consists in monocyte-derived DC [33, 86–89]. Other techniques of generating DC include the expansion of CD34+ bone marrow or blood-derived precursors [90–92]. Although relatively limited data are available regarding the cytotoxic function of freshly isolated native DC (see above), numerous studies have reported on the killing activity of ex vivo generated DC, essentially monocyte-derived human DC.

Rodent KDC differentiated from bone marrow cultures

Murine bone marrow-derived DC (BMDC) have been reported to exert killing activity against cancer cells [93–98]. In most reports, this tumoricidal function was spontaneously acquired during their differentiation in culture and did not seem to require pro-inflammatory signals [93, 94, 98] but could be enhanced by LPS [97]. DC produced from bone marrow cells cultured in the presence of GM-CSF and IL-4 were shown to express Fas-L and to mediate apoptosis of Jurkat cells [93]. The involvement of the death receptor ligands Fas-L, TRAIL and TNF-α in bone marrow-derived KDC cytotoxic activity has been reported in additional studies [93, 94, 97]. However, in contradiction with these reports, others found that apoptosis of syngeneic and allogenic tumor cells by bone marrow-derived KDC did not depend on death receptors or on IFN-α or TNF-α and did not require cell contact but was partially dependent on NO and on undefined pro-apoptotic factor(s) [96]. Interestingly, our own recent data indicated that mouse CD11c$^+$ BMDC acquired killing capabilities toward tumor cells only when activated with the TLR-4 ligand LPS or the TLR-2 agonist Pam3Cys-SK4. The cytotoxic potential of these KDC was not mediated by Fas-L, TNF or TRAIL, but was dependent on peroxynitrites, the main metabolites of nitrite oxide (NO). Consistent with this observation, we further demonstrated that the tumoricidal activity of BMDC from iNOS or GP91 knock-out mice was significantly reduced. Importantly, after killing of cancer cells KDC were capable of engulfing dead tumor cell fragments and of presenting tumor antigens to specific T lymphocytes (J. Fraszczak et al., manuscript in revision). The interest in using the anti-tumoral potential of KDC generated from bone marrow to treat established cancers was highlighted in a study by Tatsumi et al. [95]. These authors demonstrated that the adenoviral infection of BMDC with IL-12 or IL-18 or a combination of both strongly enhanced TNF-α and Fas-L expression and resulted in improved tumoricidal activity [95]. Intratumoral injection with these genetically modified bone-marrow KDC promoted the regression of sarcoma, which was dependent upon the presence of CD4$^+$ and CD8$^+$ T cells [95]. Beside these studies in mice, we have reported that in rat, KDC generated from the bone marrow were endowed with cytotoxic properties against syngeneic, allogenic and xenogenic tumor cell lines [99]. BMDC killing activity was significantly enhanced with IFN-γ and LPS and was mediated by a NO-dependent non-apoptotic mechanism [99]. It is thus not yet clear, from the aforementioned conflicting reports, whether in rodents the cytotoxic function of KDC generated ex vivo from bone marrow cells is spontaneous or may require some form of exogenous activation.

Human KDC generated from peripheral blood monocytes

Numerous studies have reported that human DC generated from monocytes cultured in vitro may act as potent cytotoxic effectors, with a range of divergent reports describing their mode of killing and requirement for activation of their tumoricidal activity [77, 78, 84, 100–107]. Immature DC generated from peripheral blood monocytes were shown to induce caspase-dependent apoptosis of several cancer cell lines as well as freshly isolated non-cultured cancer cells and endothelial cells but only of a minority of tested normal cells [77]. The basis of normal cell resistance to DC killing activity, however, has not been determined. Interestingly, following activation with CD40L these monocyte-derived DC lost their tumoricidal activity [77]. Consistent with this result immature monocyte-derived DC were observed to be cytostatic and cytotoxic against tumor cells, however, KDC activation with IFN-α, IFN-β, IFN-γ or LPS did not affect their killing potential [100, 101]. Others have reported that the inhibition of the growth of several human tumor cell lines by DC generated from blood monocytes was enhanced by LPS and IFN-γ, but not by TNF-α or CD40L [106]. It has conversely been shown that CD1a$^+$ monocyte-derived immature DC stimulated with soluble CD40L, LPS or both promoted growth inhibition of breast cancer cells [105]. The promotion of DC killing activity by CD40L or double stranded RNA (dsRNA) has been attributed to two different mechanisms [104]. In fact, dsRNA, but not CD40L, were capable of indirectly triggering TRAIL expression by DC through the induction of IFN-α production, while CD40L-mediated KDC cytotoxic function depended on TNF-α and other unidentified mechanisms [104]. Providing further insights into the role of CD40 and CD40L, a study has recently described the tumoricidal properties of immature human myeloid-derived DC exposed to OK432, a penicillin-inactivated lyophilized preparation of Streptococcus pyogenes. The observed killing properties of these KDC were associated with OK432-induced up-regulation of CD40L and depended on the expression of CD40 on tumor cells [108]. As described for their rodent counterparts, the mechanisms underlying the killing activity of human KDC are subjects of controversy. These cells were shown to promote the death of their targets by TNF-α, FasL or TRAIL-independent pathways [100, 103]. These observations were challenged by other reports indicating that TNF, Fas-L, TRAIL or lymphotoxin-α1β2, acting alone or in concert, were involved in the tumoricidal activity of human KDC, which was induced or enhanced after IFN-α, IFN-β or CD40L stimulation [78, 102, 104, 109]. However, it is to be underlined that the role of CD40L added in DC-tumor cell co-cultures is unclear since this molecule may directly affect the survival or growth of some CD40-expressing cancers [110]. Our own

data indicated that human CD14$^+$ monocyte-derived DC generated from the peripheral blood acquired a killing activity against different type of cancer cells following activation with LPS. We established that, similar to our observations in mice, the cytotoxic function of these cells was mediated by a peroxynitrite-dependent, death receptor ligand-independent mechanism (D. Lakomy et al., unpublished data).

Human KDC produced from cord blood, CD34$^+$ cells or from other sources

Besides KDC generated from peripheral blood precursors, immature DC prepared from monocytes isolated from ascitic fluid of patients with ovarian carcinomas were identified as potent tumor cell killers [111]. These cells triggered apoptotic cell death in autologous and allogeneic ovarian cancer cells through a Ca^{2+}-independent, Fas-L-dependent mechanism and were capable of capturing dying tumor cells [111]. In addition, DC generated from CD34$^+$ cells after 8 days in culture were reported to express TRAIL following stimulation with IFN-β while LPS treatment actually reduced the expression of this molecule [102]. It was further determined that this induction of TRAIL partially contributed to the enhanced cytotoxic effects of IFN-β-stimulated CD34$^+$ stem cell-derived DC [102]. Human cord blood monocyte-derived DC were additionally reported to exert selective cytotoxic effects against hematological tumor cells but not against normal cells following activation with IFN-γ or LPS [112]. Both, IFN-γ or LPS stimulation up-regulate the expression of intracellular, but not cell-surface TRAIL [112].

In summary, multiple subsets of KDC have been described with a considerable variability with regard to the modalities of induction of their cytotoxic function and to the mechanisms of tumor cell killing.

Role and therapeutic potential of KDC in cancer: a double edge sword?

Cancer immunotherapy strategies have traditionally been designed to induce specific anti-tumoral T lymphocytes through different vaccination approaches, one of them using DC loaded with tumor antigens of diverse sources [113–115]. In most of these approaches, the interest in DC-based vaccines has centered on the antigen presenting and immunostimulatory function of the DC. However, even if proven clinically safe and efficient to prime and sustain immune responses, conventional DC-based immunotherapy has only sparked moderate enthusiasm because of the relatively limited objective clinical responses that have been observed in cancer patients. Therefore, the non-conventional ability

of DC to act as direct tumor cell killers certainly opens new avenues for the design of future cancer immunotherapies.

Different aspects of KDC properties should be considered with regard to their potential clinical benefits. Firstly, advocating in favor of the implementation of KDC in cancer immunotherapies, these cells could directly participate in tumor cell elimination. Considering the fact that tumors have evolved multiple strategies to resist killing inflicted by NK or CTL through classical pathways (such as perforin/granzyme, Fas-L…), the wide variety of the mechanisms used by KDC to kill their targets may provide a significant advantage insofar as it increases the diversity and therefore the efficiency of cytotoxic effector responses. Secondly, the direct killing of tumor cells by KDC is of considerable relevance for the acquisition of tumor-derived material in a rapid and efficient manner. Indeed, not only it quantitatively fosters the release of available tumor antigens, but the direct elimination of cancer cells allows for their rapid uptake by DC and thus prevents their clearance by scavenger neutrophils or macrophages. Thirdly, following killing and capture of cancer cells debris, KDC are capable of switching their function from killers to messengers capable of processing and presenting or cross-presenting acquired tumor antigens to CD4$^+$ or CD8$^+$ T lymphocytes. The unification of these properties therefore makes KDC highly desirable for the induction of specific anti-tumoral immunity. Finally, the fundamental observation that KDC cytotoxic activity is mainly directed toward tumor cells implies their specific recognition through cell surface receptors (such as NKG2D or other unidentified molecules) and importantly provides these cells with the ability to spare non-malignant cells. One may therefore logically expect relatively limited side-effects associated with the exploitation of the killing potential of KDC in clinic.

However, despite these undeniable theoretical advantages of using KDC as powerful killers–gatherers–messengers, one should consider yet another face of these cells: their potential role as inducers of immune tolerance. It has been proposed that immature DC may take advantage of their cytotoxic function to acquire self-antigens from apoptotic normal cells and present them to autoreactive T cells, leading to self tolerance [42, 116]. As previously described some studies indicate that KDC may also promote the killing thus the deletion of T lymphocytes or thymocytes [42, 43, 117, 118]. In cancer, DC are defective in their maturation and immunostimulatory function and thus are capable of inducing T cell tolerance to specific tumor antigens [13, 14, 17, 20–22, 116]. It should therefore be taken into account that tumors may subvert the tumoricidal properties of KDC to escape immune detection and elimination. It is indeed conceivable that immature KDC that had killed cancer cells may, in absence of activation signals, present processed tumor antigens to T lymphocytes without

co-stimulation or pro-inflammatory cytokine secretion, leading to T cell anergy or deletion. Based on this consideration, it is thus critical to clearly determine whether tumor cell killing by KDC may lead to their maturation. This potential deleterious effect of KDC in anti-tumoral immune response may very probably be avoided by appropriately promoting the full maturation of KDC into potent APC using exogenous stimuli such as TLR ligands, interferons or a combination of these molecules as described [76, 80, 84, 95, 99, 102, 104–106, 109, 112].

Several immune intervention strategies may be envisioned to evaluate the potential of KDC in human cancer immunotherapy. First, KDC generated in vitro that are allowed to kill, capture and process tumor cells in culture may be administered as conventional DC vaccines. A second approach may consist in the systemic or intra-/peritumoral injection of KDC generated in vitro. Encouraging results of this approach have been reported by Triozzi et al. [119]. Third, therapies can be designed to promote the tumoricidal activity of DC in vivo and/or the recruitment of these KDC to the tumor site, as evidenced by the seminal work of Stary using the TLR-7 ligand Imiquinod [80]. In all these approaches, the choice of the type of DC activation signal(s) is critical since it may determine the nature of the killing mechanism. This is an important point to consider since tumor cells may develop resistance to specific death pathways. It may therefore, be advantageous to promote simultaneously the tumoricidal activities of multiple KDC subsets (capable of inducing tumor cell killing by different mechanisms) to overcome the emergence of resistant tumor variants. Finally, the genetic engineering of DC to render them cytotoxic (expression of TRAIL, Fas-L…) or to enhance their tumoricidal properties, thus creating a "customized" killer APC, may represent an additional useful approach. However, it is likely that complete tumor elimination will not solely depend on the anti-tumoral activity of KDC but will require the ultimate induction of tumor-specific T lymphocyte activation.

A major obstacle for successful cancer immunotherapy is the expansion of $CD4^+CD25^+$Treg that occurs during tumor progression [38, 120–124]. Tumor-induced Treg compromise the function of anti-tumor effector $CD8^+$ T cells, curtail $CD4^+$ T cell help and impede antigen-presenting cell activity [37, 124, 125]. Studies in humans and in animal models have demonstrated that attempts to disrupt Treg suppressive activity promote anti-tumoral immunity [38, 121, 126, 127]. Therefore, associating KDC-based therapy with Treg elimination or inactivation strategies, and more generally with approaches aimed at overcoming tumor-induced tolerance (inhibition of immunosuppressive molecules or cells such as TGF-β or myeloid-derived suppressor cells…), may further enhance the clinical efficiency of these killer-APC.

Conclusion

Besides their primary role as potent inducers of tumor-specific $CD4^+$ and $CD8^+$ T lymphocytes or of NK cells, numerous reports have extensively demonstrated that DC are also endowed with the capacity to directly kill cancer cells. This concept integrates KDC in a multi-step process in which they may generate their own source of tumor-derived material by virtue of their cytotoxic activity, then switch function to present captured and processed tumor antigens to specific T lymphocytes. This notion of a 'multitasking' cell population that can act at virtually all levels of anti-tumor immune response has sparked considerable interest for the development of novel cancer immunotherapeutic strategies in humans.

However, multiple questions remain unanswered particularly as they relate to the induction and regulation of DC killing function, the kinetics in acquisition of their killing and APC potential and the mechanisms by which they trigger death of their targets. For instance, a major challenge is to establish whether recognition and killing of tumor cells in vivo may drive the differentiation of KDC into powerful APC capable of inducing anti-cancer immunity, or whether immature KDC that had killed tumor cells may behave as tolerogenic APC responsible for effector T cell deletion or Treg induction. It will also be critical to address the mechanism underlying the specific recognition and killing of tumor cells and why, in most scenarios, normal cells are spared by KDC. The outcome of these studies may contribute to further understanding and developing this novel generation of tumor killer cells and expand their application beyond their primary role as inducers and regulators of immune responses against cancers.

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