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ORIGINAL ARTICLE

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The role of natural killer cells in tumor control—effectors and regulators of adaptive immunity

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Abstract Natural killer (NK) cells are the primary effector cells of the innate immune system and have a well-established role in tumor rejection in a variety of spontaneous and induced cancer models. NK cell function is regulated by a complex balance of inhibitory and activating signals that allow them to selectively target and kill cells that display an abnormal pattern of cell surface molecules, while leaving normal healthy cells unharmed. In this review we discuss NK cell function, the role of NK cells in cancer therapies, the emerging concept of bi-directional cross-talk between NK cells and dendritic cells, and the implications of these interactions for tumor immunotherapy.

Keywords Immunotherapy · Immunosurveillance · Cytokine · Cytotoxicity · Dendritic cells

Natural killer cells—an introduction

The immune system can be broadly divided into two arms, innate and adaptive immunity; however, it is becoming increasingly clear that these two components are tightly interwoven. The innate immune system consists of cellular components such as granulocytes, monocytes/macrophages, natural killer (NK) cells, and dendritic cells (DCs), as well as physical barriers and chemical factors. The adaptive immune system consists of B cells and $\alpha\beta^+$ T cells, while $\gamma\delta^+$ T cells, NK1.1$^+$ T (NKT) cells, and other regulatory T cell subsets interface both innate and adaptive immunity depending on their environment. This review discusses the importance of NK cells in the context of tumor immunity, and in particular focuses upon the emerging evidence that these cells not only mediate direct anti-tumor effector functions, but can also regulate innate and adaptive immune responses.

NK cells are defined as a unique subset of lymphocytes that do not express rearranged recognition receptors (e.g., T or B cell receptors), rather expressing a myriad of immuno-

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globulin (Ig)-like receptors and C-type lectin receptors (CTLRs) that deliver a finely tuned balance of inhibitory and activating signals [5, 10, 98]. NK cells generally display a large granular lymphocyte morphology and comprise ~5–20% of peripheral lymphocytes (spleen, liver, peripheral blood). NK cells are present at lower frequencies in the thymus, lymph nodes, and bone marrow (BM), and the latter is the site of mainstream NK cell development in the adult [56]. NK cells were first identified through their ability to rapidly respond to and kill leukemic cells without prior activation [52]. They were subsequently demonstrated to recognize cells expressing abnormal or down-regulated major histocompatibility complex (MHC) class I molecules [58], and more recently to recognize infected or transformed cells expressing "stress-induced" proteins [3]. We now appreciate that a number of different receptor types are expressed by NK cells and these either inhibit self-reactivity (via binding MHC class I-like ligands) or activate NK cell function. These receptors allow the NK cell to discriminate normal healthy cells from transformed or pathogen-infected cells, and regulate NK cell effector functions that control pathogen infection and tumor initiation and spread. Thus, NK cells can mediate spontaneous effector function [11, 85], but unlike T cells are not capable of "memory" when re-exposed to the same antigenic challenge. Hence, the primary role of NK cells is in immediately limiting or eliminating foreign or dangerous challenges to the host.

What makes an NK cell and are they all the same?

A working model of NK cell development has recently been described in both mice and humans [13]. A population of $CD122^{+}NK1.1^{-}DX5^{-}$ committed NK cell progenitors that lack cytotoxic activity have been identified in adult BM [105]. IL-15 secreted by the BM stroma is essential for NK cell development in humans and in mice, and plays a role in driving the final maturation process [51]. The first defining NK cell markers expressed by immature NK cells are NK1.1 in C57BL/6 mice and CD161 in humans; both belong to the C-type lectin NK-cell receptor protein 1 (NKR-P1) family [4]. In the mouse these cells do not express DX5 or Ly49 receptors and, in an analogous fashion, human immature NK cells are CD56 negative and do not express the killer cell Ig-like receptor (KIR) family of receptors [14]. These cells are also functionally immature and do not exhibit perforin-dependent cytotoxicity or produce cytokines like IFN-γ, but can be induced to mature by BM stroma in vitro [74]. A linear differentiation pathway, type 2–type 0–type 1, for human NK cell development has now been proposed by Perussia and colleagues [59], based on NK cell cytokine and cytotoxicity profiles, and the individual stages of murine NK cell development have been similarly defined on the basis of functional, phenotypic and proliferative capacities [53]. However, in both humans and mice these pathways remain merely models, founded largely on in vitro experimental data. Mature NK cells may be very heterogeneous and much remains to be learned about the specific developmental steps that lead to NK cell maturation. Two distinct human NK cell subsets have been identified, which can be distinguished by cell surface density expression of CD56. $CD56^{bright}$ NK comprise about 10% of human blood NK cells, whereas $CD56^{dim}$ NK that express high levels of FcγRIII (CD16) comprise approximately 90% of the population [55]. $CD56^{bright}$ and $CD56^{dim}$ NK represent two functionally distinct subsets of NK cells in humans; $CD56^{bright}$ have relatively poor cytolytic capacity, but the capability to produce large amounts of cytokines, and $CD56^{dim}$ exhibit enhanced natural cytotoxicity, but produce

significantly less cytokines [14]. Currently, the equivalent phenotypic and functional mouse NK cell subsets remain undefined [13].

NK cell effector function

NK cells possess a variety of effector mechanisms enabling them to mount a potent anti-tumor response. NK cells use two major mechanisms to induce target cell apoptosis, the granule exocytosis pathway [99] and the death receptor pathway [87]. While the perforin/granzyme granule exocytosis pathway has been extensively studied, less is known about NK cell death receptor function. Activated NK cells can also produce a variety of cytokines and chemokines that can have a direct effect on tumor growth as well as activating other immune effector cells. The major proteins involved in the granule exocytosis pathway are perforin, a membrane-disruption protein, and a family of structurally related serine proteases known as granzymes [92]. The importance of NK cell perforin in controlling experimental tumors has been well documented [89, 95, 101], whereas granzymes A and B do not appear to be important for controlling tumor growth [18, 86]. The death receptor pathway is largely mediated by members of the tumor necrosis factor (TNF) superfamily. The key apoptosis-inducing members of this family are Fas ligand (FasL), TNF-α, TNF-related apoptosis-inducing ligand or Apo-2 ligand (TRAIL/Apo2L) and lymphotoxin-α (LT-α). They induce apoptosis of target cells by engaging receptors containing cytoplasmic death domains. TRAIL is normally expressed by a small subset (~25–35%) of liver NK cells [83, 96], although expression can be induced on NK cells by cytokines such as IL-15 and IFN-γ. TRAIL also appears to be regulated in vivo on NK cells by IFN-γ [96]. In experimental models, TRAIL has been demonstrated to contribute to NK cell-mediated immune responses controlling tumor growth and specifically liver metastases [16, 83, 96]. TRAIL has also been shown to play a role in immune surveillance of methylcholanthrene (MCA)-induced carcinogenesis effected by NK cells and IFN-γ [16, 97]. IFN-γ also plays an important role in TRAIL-mediated immune surveillance of spontaneous tumor development in $p53^{+/-}$ mice by controlling TRAIL expression on effector cells [97]. These studies strongly imply that NK cell TRAIL is an important immune effector molecule in preventing tumor initiation, growth and spread. The significance of other TNF family members in NK cell-mediated tumor control is less obvious, although clearly in some situations FasL plays a role [77].

Upon activation, NK cells can produce a variety of cytokines (IFN-γ, GM-CSF, G-CSF, M-CSF, TNF-α, IL-5, IL-10, IL-13 and others [69]) and chemokines [72], which can have a direct effect on tumor growth, induce inflammatory and anti-viral responses, regulate hematopoietic cell differentiation and prime immune effector cells that are crucial for subsequent adaptive immune responses. The best studied of these effector cytokines in the context of anti-tumor immunity is IFN-γ [44, 94]. As well as being an important cytokine for activating both innate and adaptive immune responses, IFN-γ can also directly affect tumor cells, decreasing proliferation and metabolic activity in a wide range of tumor cells and inhibiting angiogenesis by tumor cells through induction of CXC chemokines, such as IFN-inducible protein-10 (IP-10) and monokine induced by IFN-γ. IFN-γ produced by NK and NKT cells is essential for the anti-angiogenic effects of α-galactosylceramide (α-GalCer) on tumor growth, and NK cell depletion partially inhibits angiogenesis in vivo [42]. IFN-γ produced by NK cells might also play a role in the regulation of killing by death receptors by down regulating anti-apoptotic proteins [45], or by up-regulating expression of

Table 1 NK cell receptors and their ligands

Receptor family	Species	Receptor	Ligand	Signal type	Function
KIR	H	KIR2DL, KIR3DL	MHC class I[a]	INH	Detect changes in
		KIR2DS, KIR3DS	MHC class I	ACT	MHC class I
Ly49	M	Ly49A, C, G, I[a]	MHC class I	INH	
		Ly49D	MHC class I	ACT	
		Ly49H	CMV m157	ACT	Viral infection
NKG2	H and M	NKG2A/C/E-CD94	H: HLA-E	INH	Detect changes in
			M: Qa-1b[a]		MHC class I
		NKG2D	H: MICA/B^{+}	ACT	Detect upregulation
		Associated with two	ULBP		of stress associated
		different adapters	M: Rae1		molecules
		DAP-10 and DAP-12	MULT-1[a]		
			H60		
NCR	H	NKp30	Unknown	ACT	Killing iDC
		NKp44	Unknown		Co-stimulatory
		NKp46	Influenza HA		Viral infection
		NKp80	Unknown		Co-stimulatory
	M	NKp46 only	Unknown	ACT	
NKRP1	H	NKRP1A	Unknown	ACT	
	M	Nkrp1b and d	Clr-b^{-a}	INH	
		Nkrp1c (NK1.1)	Unknown	ACT	
		Nkrp1f	Clr-g[a]	ACT	
Others	H and M	CD27	CD70^{+a}	ACT	Co-stimulatory
	H	CD56	Unknown		
	M	CD49b(DX5)	Unknown	ACT	Co-stimulatory
	H and M	CD16 (FcγRIII)	Ig Fc region	ACT	ADCC
	H and M	2B4	CD48[a]	ACT	Co-stimulatory
	M	KLRG-1	Unknown	INH	

$^{+}$ or $^{-}$ denotes up or down-regulation of expression following DC maturation. Reviewed in [54, 60, 112] (*H* human, *M* mouse, *ACT* activating, *INH* inhibitory)
[a] Constitutively expressed on DC

caspases that are essential for the execution of death receptor-mediated apoptosis [37]. The role of other NK cell cytokines in tumor control is less well studied.

Recognition and control of NK cell activation

NK cell function is controlled by a balance of NK cell inhibitory and activating signals. NK cell inhibitory receptors bind to self-MHC class I molecules, whereas NK cell activating receptors bind to ligands expressed on stressed, transformed, and virus-infected cells. Three inhibitory receptor families exist: the KIRs [107], which are expressed in humans; the Ly-49 lectin-like homodimers [98, 111], which are expressed in mice; and the CD94-NKG2 lectin-like receptors, which are expressed in both mice and humans (Table 1). A wide variety of NK cell activating receptors exist, many of which have short cytoplasmic domains and interact with transmembrane signaling adaptor molecules to activate NK cell function (Table 1). The NK cell activation state is, therefore, determined by the balance of positive and negative signals to the NK cell. The heterodimeric CD94/NKG2 receptors are conserved throughout the species and recognize the non-classical MHC class I molecules HLA-E (in humans) and Qa-1b (in rodents) (Table 1). NK cell activity is inhibited by the

CD94/NKG2A-Qa-1b interaction. NKG2D is a type II disulfide-linked dimer that contains a lectin-like extracellular domain, but is only distantly related to the other NKG2 family members [43]. NKG2D has been identified as an activating receptor and recent studies have shown that NKG2D has two isoforms, a long form NKG2D-L, which is highly expressed in naïve NK cells, and a short form, NKG2D-S, which is up-regulated in NK cells after activation [21]. NKG2D recognizes various ligands expressed on stressed, abnormal or cancerous cells. In vivo rejection of NKG2D ligand-expressing tumor cells is mediated by NK cells and $CD8^+$ T cells [9, 20], is largely perforin-dependent and appears independent of death receptor signaling or cytokine production [40]. NK cells can also be directly activated to induce antibody-dependent cellular cytotoxicity (ADCC) by engagement of their low-affinity cell surface FcγRIII receptors with the Fc portion of antibodies bound to target cell-associated antigens. NK cells in combination with activating cytokines demonstrate high levels of ADCC against tumor cells in the presence of some monoclonal antibodies (mAbs) reactive with tumor antigens [66]. It is now emerging that clinically promising mAbs specific for tumor antigens may mediate their effects in part via NK cells and ADCC. This is stimulating more interest in how to mobilize, expand and activate NK cells in humans. NK cell co-receptors also interact with ligands expressed on target cells and provide co-stimulatory signals to the NK cell that range from enhancement of proliferation and cytokine production to the triggering of cytotoxicity. In each case expression of the ligands makes tumor targets more sensitive to NK cells in vitro and in vivo [32, 33, 49, 50, 67]. For several of these co-receptor:ligand interactions, the mechanisms of NK cell-mediated rejection of tumors have also been defined [49, 50]. Finally, although NK cells express many surface activation receptors (as outlined above), in the periphery they also quickly become activated and proliferate upon stimulation with a variety of cytokines such as IL-2, IL-12, IL-15, IL-18, IL-21, IFN-α and IFN-β.

NK cell control of tumor initiation and spread

While there is no doubt that the major evolutionary force that has shaped host development of the NK cell lineage is pathogen infection, a defining feature of NK cells is their ability to respond to self cells that express an abnormal pattern of MHC class I molecules and/or stress-induced ligands [20, 58, 101]. The importance of NK cells in anti-tumor immunity has been established in a number of experimental tumor models in mice. Most of these studies have relied on NK cell depletion using antibodies, either polyclonal anti-asialo GM1 antibodies or the anti-NK1.1 mAbs, prior to tumor transplantation or initiation, to demonstrate more aggressive tumor growth in the absence of NK cells [57, 84, 89, 101]. However, it is often argued that these antibodies delete other subsets of leukocytes, particularly when the immune system is perturbed by infection. The development of more selective and temporal approaches for the elimination of all mature NK cell function will be invaluable to the field.

Given these limitations, it has been difficult to ascertain whether NK cells naturally protect against tumor initiation. The first informative studies in this area made use of *beige* mice that develop a low level of spontaneous lymphoma [38]; however, they also have a defect in cytoplasmic granule formation that affects many leukocyte compartments, and NK cell cytokine secretion remains essentially intact. More recent studies have demonstrated that mice depleted of NK cells are more susceptible to spontaneous MCA-initiated tumor growth [84]. Many other lymphocyte cell types, including invariant NKT cells

and $\gamma\delta^+$ T cells have also been implicated in host protection from sarcoma induction in this model [31, 90]; however, it is likely NK cells are the major effector population. Recently, a spontaneous resistance/complete resistance (SR/CR) mouse was identified by Cui and colleagues [17], which can reject a number of experimental tumor cell lines. Tumor resistance in these mice was associated with an infiltration of innate leukocytes including NK cells, neutrophils and macrophages into the peritoneum. Breeding experiments revealed the trait was an age-dependent dominant gain of function mutation, suggesting the existence of a novel age-dependent host resistance mechanism against cancer, possibly involving NK and other innate cells. We have recently discovered that mice deficient for both the NK cell effector molecule perforin and β2-microglobulin (β2m) (that enables target cell expression of MHC class I, CD1d, and some Fc receptors) develop a higher rate and earlier onset of spontaneous B cell lymphomas than perforin-deficient or wild-type mice [95]. Interestingly, NK cells and $\gamma\delta^+$ T cells are both capable of rejecting these spontaneous β2m-deficient B cell lymphomas in a perforin-dependent manner when they are transplanted into wild-type mice. Collectively, these data suggest that NK cells may naturally detect and eradicate spontaneously forming tumors in the mouse, and evidence is emerging to support a similar role for NK cells in human malignancy.

For example, NK cells isolated from cancer patients exhibit impaired NK cell cytotoxicity, proliferation, and response to IFNs, and patients with higher levels of NK cell activity post-treatment are reported to remain cancer-free longer than patients with low NK cell activity [103, 104]. However, many such studies are correlative and generally fail to determine whether cancer leads to suppression of NK cell function or vice-versa. Patients lacking NK cells have been described; however, these patients are extremely susceptible to viral infection and die at an early age [1]. Familial hemophagocytic lymphohistiocytosis patients who possess perforin mutations and lack NK cell cytotoxic activity have also been described, but also die at a young age unless rescued by a BM transplant [93]. So, although perforin-deficient mice develop spontaneous B cell lymphoma [91], a clear link between perforin mutations and lymphoma has not yet been made in humans. It is difficult to ascertain from these studies whether defective NK cell function contributes to the development of such B cell lymphomas and whether these tumors arise as a result of failed surveillance or immune dysregulation.

Enhancing NK cell function in cancer therapy

Given the efficacy of NK cells in selectively killing abnormal cells, a variety of approaches have been taken to try and selectively augment NK cell responses to tumors.

Several therapeutic cytokines primarily act via NK cells (such as IL-2, IL-12, IL-15, IL-18, IL-21 and IFNs) and many studies have shown that activation of NK cell differentiation and function leads to more efficient elimination of tumor growth [6, 39, 88, 100]. The adoptive transfer of NK cells further demonstrates the ability of NK cells to mount a therapeutic anti-tumor response [110], and suggests that NK cells can be utilized in controlling human malignancy. Antibodies reactive with tumors, and cytokines (such as type I IFN and IL-2) have also been used successfully in the treatment of chronic myelogenous leukemia (CML), melanoma, and renal cell cancer [73, 80]. Emerging evidence suggests that NK cells may be critical for effective responses following such therapies, IFN-α and rituximAb treatment of human CML in particular [66, 68]. In addition, Velardi and col-

leagues [75] have demonstrated that BM transplantation using haploidentical, but KIR-mismatched, donors induced NK cell alloreactions that could mediate a graft-versus-leukemia effect and prevent tumor relapse in patients.

Another attractive new cytokine therapy under consideration is IL-21, a close relative of IL-2 [81]. Exposure to IL-21 induces a terminal differentiation program in NK cells, enhancing their cytotoxicity and IFN-γ production, but limiting their further expansion in response to IL-15 [6, 48]. IL-21 has been shown to induce potent anti-tumor effects that were mediated predominantly through the enhanced activity of NK cells and $CD8^+$ T cells [6, 64]. Despite these promising advances, suggesting that NK cells can be harnessed to reject mouse and human cancers, the systemic administration of cytokines is associated with significant toxicity—particularly for cytokines such as IL-2 and IFN-α that non-specifically activate a broad range of different immune cell types.

Reciprocal NK cell:DC interactions promote immunity

Interestingly, many of the cytokines that are known to enhance NK cell function are naturally produced by DCs in response to microbial infection [2]. Indeed, there is a growing body of evidence that activated DCs can potently enhance NK cell function. Firstly, DCs are a major source of IL-15, a cytokine essential for NK cell development, that also promotes NK cell survival and proliferation [102]. Depending on the DC-activating stimulus, DCs also produce IL-12 and IL-18, cytokines that enhance NK cell cytokine production, or type I IFNs that promote NK cell cytotoxicity and proliferation. In addition, although T cells remain the major source of IL-2, it has recently been shown that DCs also secrete small amounts of IL-2 following microbial stimulation [35]. Therapeutic approaches that stimulate DCs to produce NK cell-activating cytokines may provide a more refined means of NK cell activation, minimizing the toxic side effects of systemic cytokine administration, with the added advantage that approaches combining activation of NK cells and DCs have the potential to augment the initiation of adaptive immunity to tumors.

DC subsets and function

The activation of tumor-specific T cells is the major goal of many immunotherapy strategies, and DCs are key players in the induction of T cell immune responses, acting as professional antigen-presenting cells and bridging the gap between innate and adaptive immunity. Immature DCs (iDCs) readily take up antigen through receptor-mediated endocytosis, pinocytosis and macropinocytosis and process these antigens for MHC presentation [62]. However, to activate naïve T cells, DCs must first complete a maturational program initiated either by direct exposure to pathogens or through specific interactions with other immune cells [2]. DCs express a variety of pattern recognition receptors, known as Toll-like receptors (TLR1–9) that are triggered by pathogen-associated molecular structures common to large groups of pathogens, such as double-stranded viral RNA, or bacterial cell wall components [61]. iDCs encountering such pathogen-associated danger signals cease endocytosis and up-regulate the expression of antigen-presenting MHC class I and class II molecules, as well as co-stimulatory molecules CD40, CD80, CD86 and CD83 that are critical for the activation of naïve T cells [62]. In the absence of appropriate DC

Table 2 Major mouse and human dendritic cell subsets

Murine DC subsets	Phenotype	Localization/function	Equivalent human DC subsets
CD11b$^+$	CD11c^{hi}CD11b$^+$ CD8a-CD205-	- Marginal zones of spleen - low levels of IL-12p70 - Induce Th2 CD4$^+$ T cells	Myeloid DC: CD11c^{high}CD123^{dim}CD1c$^+$ In vitro: MoDC or DC-1: CD14$^+$ blood monocytes cultured with GM-CSF+IL-4 - Induce Th2 CD4 T cell responses
CD8α^+	CD11c^{hi}CD11b$^-$ CD8α^+CD205$^+$	- T cell areas of spleen - high levels of IL-12p70 - induce Th1 CD4$^+$ T cells - Cross-present exogenous antigen	No functional equivalent identified but as in mouse, splenic CD11b$^-$ DC produce highest amounts of IL-12p70 In vitro: no culture method available
Plasmacytoid	CD11c^{int}CD11b$^-$ CD8$\alpha^{+/-}$CD205$^-$ CD45RA$^+$	- Potent IFNα producers, - moderate IL-12p70 - Less efficient T cell stimulation	Plasmacytoid DC: CD11c$^-$CD123^{bright}CD1c$^-$ In vitro: DC-2: IL-3R$^+$ PBMC cultured with IL-3 - produce IFN-α on stimulation - Induce Th1 CD4 T cell responses

Reviewed in [79]

maturation signals, cognate interactions between T cells and iDCs result instead in the induction of tolerance [65].

Murine splenic DCs can be broadly categorized into three phenotypically and functionally distinct subsets (Table 2). Human DC subsets are less well defined than those of mice due to the limited availability of tissues for their isolation, and most information about human DC subsets and function is derived from in vitro cultures (Table 2). Different DC subsets express defined subsets of TLR [23, 46], so different types of pathogen stimuli selectively activate particular DC subsets, inducing either Th1- or Th2-promoting cytokines, thus directing adaptive immune responses along an appropriate path [71]. The triggering of particular TLR, through the introduction of molecules that mimic pathogen-induced danger signals, is likely to be a useful way to invoke both innate and adaptive immune mechanisms to tumors and to manipulate the type of immune response. Some examples of TLR ligands already on trial in anti-tumor therapies include synthetic oligonucleotides containing unmethylated CpG sequences that mimic bacterial DNA (TLR-9), bacterial cell wall components such as BCG (TLR-2/4), polyI:C a mimic of viral RNA (TLR-3), and imiquimod, a synthetic ligand for TLR-7 [78, 108]. These molecules specifically trigger the maturation of DCs and other innate immune cells expressing the appropriate TLRs. For example, in humans, TLR-9 is expressed exclusively on plasmacytoid DCs and induces type I IFN production by these cells, whereas TLR-2 and 3 are expressed on myeloid DCs and induce IL-12 production [46]. Although most of these studies have focused on enhanced T and B cell responses to tumors, it has recently been shown that human NK cells also express a number of TLRs (TLR2, 3, 5 and 9), and can thus be directly activated by exposure to their ligands [12, 82]. Interestingly direct activation of NK cells through TLR signaling appears to only be optimal in the presence of DC-derived cytokines such as IL-2 and IL-12, suggesting that NK-DC cross-talk may facilitate TLR-mediated activation of NK cells. The dual activation of NK cells and DCs may contribute significantly to the success of therapies employing TLR ligands to augment tumor immunity.

Mechanisms of NK cell and DC communication

The first demonstration that DCs could enhance NK cell function in vivo arose from the observation that increasing DC numbers, through adoptive transfer of autologous BM-DC or Flt3L treatment, led to enhanced NK cell-mediated rejection of class I-deficient tumors [29]. Surprisingly this enhanced NK cell function was not dependent on the production of type I IFNs or IL-12, and appeared to be restricted to the CD8α^+ DC subset. Subsequently, NK cell-DC interactions have been documented in a variety of tumor and disease settings with evidence emerging of complex bi-directional cross-talk between the two cell types [15, 25, 114]. In vitro co-cultures of human NK cells and DCs [28, 34, 70] support a role for both cytokines and direct cell contact in the enhancement of NK cell proliferation and function by DCs, and provide some useful insights into the underlying molecular interactions. While cytokines such as IL-12 and IL-18 promote optimal NK cell cytokine production, even in the absence of these cytokines, NK cells still become activated following co-culture with DCs, up-regulating activation markers such as CD69 and CD25 and producing low levels of IFN-γ. One recent study has suggested that the production of IL-2 by DCs may be more important than DC-derived IL-12 and IL-18 in driving IFN-γ production by resting NK cells [36]. However, inhibiting all of these cytokines alone or in combination does not completely abrogate the enhancement of NK cell function following exposure to DCs, suggesting that cell-surface interactions are also important. The nature of the molecules involved in these cell surface interactions remains poorly defined, and it is likely that a combination of cell-surface and cytokine signals are required for optimal NK cell activation. iDCs, while producing little cytokine, constitutively express a number of NK cell receptor ligands whose expression is further regulated upon DC maturation (Table 1). It remains possible that there are also changes in as yet undefined NKR ligands expressed on DCs, such as those for the NCR and NKRP1 families of receptors, or expression of pathogen-derived ligands (eg. m157) in infected DCs that interact with NKR (eg. Ly-49H) [22].

While both iDCs and mature DCs (mDCs) can be shown to augment NK cell function, one of the more intriguing aspects of the NK cell-DC interaction is that, unlike most normal healthy cells, iDCs appear to be uniquely susceptible to NK cell-mediated lysis, while mDCs are protected. It is thought that the susceptibility of iDCs to NK cell cytotoxicity is due to constitutively lower levels of MHC class I expression on iDCs than on other normal tissues, leading to a lack of NK cell inhibition. mDCs, on the other hand, are protected from NK cell-mediated killing through the up-regulation of MHC class I and related molecules, particularly HLA-E (Qa-1b in mice) [26, 106]. However, evidence is now emerging that death of iDCs is not an obligatory outcome of interaction with NK cells. Under certain conditions, NK cells are able to induce or at least augment the maturation of iDCs, causing the up-regulation of co-stimulatory molecules and increased production of IL-12 in response to DC-activating stimuli. Again both soluble factors, notably TNF-α and cell-cell contact are involved [34, 70]. One of the key factors that appeared to modulate the outcome of killing versus maturation of iDCs by NK cells was the NK cell:DC ratio. At low NK cell:DC ratios DC responses were dramatically enhanced, whereas at high NK cell:DC ratios DC function was inhibited due to potent killing of iDCs by activated NK cells [70]. Together these studies highlight the complexity of NK cell-DC interactions and reveal a potential new role for NK cells as regulators of adaptive immunity. In vivo, the outcome of NK cell-DC interactions is likely to be determined by a variety of factors,

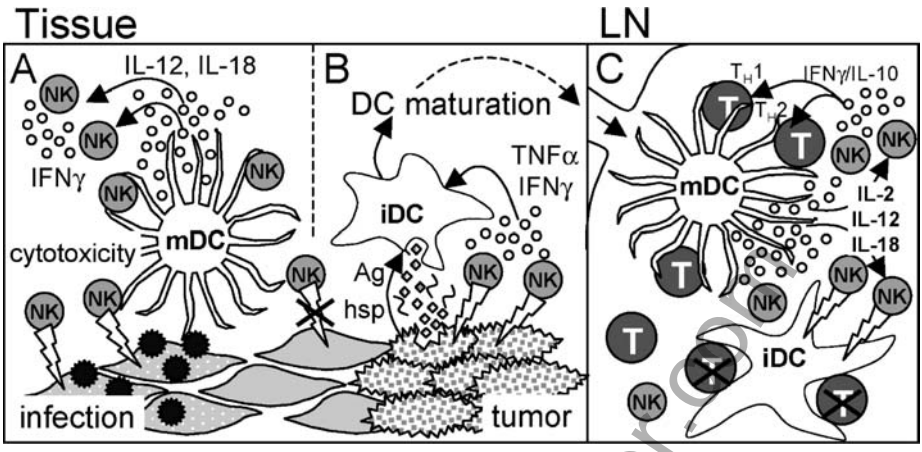

Fig. 1 Outcomes of NK cell and DC interactions at different sites. **A**, **B** NK cell-DC interactions in tissues in situations of infection or tumorigenesis. **C** NK cell and DC interactions in the LN draining these sites. **A** DCs activated by contact with pathogens augment NK cell cytokine production and killing of infected cells, enhancing virus clearance at the site of infection. **B** NK cells activated following detection of transformed cells release cytokines and kill tumor targets. Depending on the relative numbers of NK cells present in the tumor bed, contact between NK cells and iDCs may result in NK cell-mediated DC maturation or death. Maturation of iDCs may be direct, through cell-cell contact or the production of cytokines, such as TNF-α, or indirect, through the release of heat shock proteins from tumor cells killed by NK cells. Killing of tumor cells may also increase the availability of tumor antigens for uptake by iDCs. **C** Cytokines produced by mDCs entering the LN may activate LN NK cells to up-regulate perforin, TRAIL and NCR cell receptors, and to become prolific cytokine producers. This might allow NK cells to selectively kill iDCs already present in the LN, thus focusing T cell responses on foreign antigens presented by newly arrived mDCs. The production of cytokines by LN NK cells might also influence the type of adaptive immune response generated (*NK* natural killer, *DC* dendritic cell, *LN* lymph node, *iDC* immature DC, *mDC* mature DC)

such as the location at which these interactions take place, and the type of activating stimuli encountered by both DCs and NK cells.

Predicting the outcomes of NK cell-DC interactions in vivo

In disease states, the first contact between DCs and NK cells most likely occurs at the site of infection (Fig. 1A). Most human peripheral blood NK cells ($CD56^{dull}CD16^{+}$) express high levels of the chemokine receptors CXCR1 and CX3CR1 [8]. These chemokine receptors facilitate their migration into inflamed tissues in response to IL-8 and fractalkine, where they could interact with tissue-resident DCs also recruited to sites of inflammation and activated by the presence of pathogens. Indeed, direct contact between NK cells and DCs has been observed in vivo in Malassezia-induced skin lesions [7]. Another significant site where NK cells and DCs might come into contact are the lymph nodes (Fig. 1C). As part of their maturation program, DCs up-regulate the CCR7 chemokine receptor, allowing them to migrate to lymph nodes where the cytokines they produce could activate resident NK cells and promote their expansion. Interestingly, a recent study found that, although $CD56^{dull}$ NK cells are the primary mediators of cytotoxicity against tumor targets, the ability to kill immature DCs was restricted to the $NKG2A^{lo}KIR$ (CD^{16+}) subset [19], a phenotype closely resembling that of NK cells found to be enriched in human LN [27].

The phenotypic and functional differences between NK cells that migrate into tissues and those present in the LN might suggest differential immunoregulatory roles for NK cell-DC interactions at these sites (Fig. 1).

An important recent study has shown that recruitment of NK cells to LN is effectively mediated via CXCR3-dependent signals by DCs activated by TLR4 ligands [59a]. Further, this recruitment provides IFN-γ vital for Th1 priming.

Unlike the destruction of tumor targets, the killing of iDCs by NK cells appears to be largely dependent on death receptor, rather than granule exocytosis-mediated pathways. In vitro co-cultures of DCs with human blood NK cells show that expression of NKp30 is required for the killing of iDCs [24]. Murine NK cells do not express NKp30; however, killing of adoptively transferred iDCs is mediated primarily via the TRAIL death receptor pathway [41]. As mentioned previously, TRAIL can be up-regulated on NK cells by cytokines such as IL-15 or type I IFNs, so one possibility might be that interaction with cytokine-producing mDCs induces the expression of TRAIL on murine LN NK cells allowing them to kill iDCs. How NK cells are activated might have other effects on the outcome of NK cell-DC interactions. In vitro NK cells activated in the presence of different cytokines develop distinct phenotypes with respect to their expression of effector molecules. For example, IL-2-activated NK cells do not express TRAIL, whereas IL-15-activated NK cells express high levels of TRAIL. IL-18-differentiated NK cells up-regulate FasL, and NK cells activated in the presence of IL-21 strongly up-regulate perforin expression and show enhanced cytotoxicity against tumor targets (our own unpublished data and [6]). The activation of NK cells through receptor ligation may also have different outcomes with respect to NK cell function. For example, NK cells activated through recognition of NKG2D ligands, such as Rae1, mediate their effects largely through perforin-dependent cytotoxicity [40], whereas activation of NK cells through specific recognition of CD70 results in critical IFN-γ production [49].

The relative importance of these different pathways of NK cell activation in vivo is not clear. Different NK cell-activating stimuli can clearly have quite distinct outcomes in terms of NK cell function and the ability of NK cells to kill iDCs, parameters that will be of critical importance in the rational design of tumor therapies that aim to exploit NK cell-DC interactions.

NK cells as regulators of innate and adaptive immunity to tumors

One of the major difficulties in generating immunity to tumors is that, being derived from self tissues, they are generally poorly immunogenic and do not display the danger signals necessary for DC activation. NK cells are unique in their ability to detect changes in tumor cells in the absence of inflammatory stimuli and as such can be responsible for rejecting tumors directly [47, 101] or alternatively, they can stimulate components of the adaptive immune system to eliminate tumors [49, 109] (Fig. 1). Firstly, NK cells can facilitate the adaptive anti-tumor immune response by producing IFN-γ and other cytokines to recruit and activate DCs, T cells and B cells [85]. IFN-γ produced by NK cells can directly stimulate the development of CTL and lead to the development of immunological memory against the tumor [50, 89], as well as promoting the development of $CD4^+$ T helper cells that subsequently aid CTL generation [85]. Cytokines produced by NK cells may also regulate B cell production of anti-tumor antibodies [113]. Finally, recent evidence suggests

that activated NK cells can also stimulate the maturation of DCs, licensing them to present antigen to CTL in lymph nodes [15, 34] (Fig. 1B). NK cells mediating primary rejection through the recognition of CD27 [50] or NKG2D [20] ligands expressed on tumor cells may evoke T cell immunity against secondary MHC class I-sufficient tumors. Given the requirement for DCs in the activation of naïve T cells, it is likely that NK cell interactions with DCs are central to this process. In the case of NK cell-mediated rejection of MHC class I^{low} tumors, the involvement of NK cell-DC communication has been demonstrated more directly, with activated IFN-γ-producing NK cells priming DCs to induce protective $CD8^+$ T cell responses [63]. NK cells might also enhance adaptive immunity to tumors in a more indirect way. NK cell-mediated killing of tumor targets could provide increased access to tumor antigens for uptake by DCs. iDCs have been found to phagocytose apoptotic and necrotic cells very efficiently and process antigens for presentation to T cells, and can be matured by exposure to necrotic (but not apoptotic) cells, most likely through the release of heat shock proteins [30, 76]. Therefore, another possibility is that NK cells might kill targets in a distinct way that both facilitates antigen uptake by DCs and triggers their maturation (Fig. 1B). While the links between NK cells and adaptive immunity remain poorly defined at a molecular level, they are clearly important in the generation of effective anti-tumor responses.

Conclusions

Notwithstanding the differences in human and mouse NK cell and DC subsets, studying the interactions of these cells in mouse models of cancer can provide useful insights into the way cells of the innate immune system interact together. A better understanding of the way NK cells and DCs interact will allow us to design strategies that not only harness the potent direct anti-tumor effects of the innate immune system, but also maximize the generation of long-lasting adaptive immunity to tumors.

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