Chapter 3

Highlighting the Role of DC-NK Cell Interplay in Immunobiology and Immunotherapy

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Abstract

Dendritic cells (DCs) and natural killer (NK) cells are both part of the innate immune system, also playing crucial functions in the regulation of adaptive immune responses. In recent years, numerous works have demonstrated that DCs and NK cells mutually influence each other with major consequences in the type and effectiveness of elicited immune responses. Among other effects, DC-NK crosstalk can result in NK cell activation and DC maturation or deletion, depending on its activation status. In this chapter and after a brief overview of DCs and NK immunobiology, we focus on the process of DC-NK crosstalk, highlighting the relevance of rationally exploring this interplay in the development of more effective cancer immunotherapies.

Keywords: dendritic cells, natural killer cells, DC-NK crosstalk, cancer, immunotherapy

1. Introduction

Dendritic cells (DCs) are a heterogeneous population of innate immune cells with unique capacity to process and present antigens to naïve T cells. They are, therefore, responsible for the orchestration of the adaptive immune responses, promoting either immunity or tolerance to self-antigens [1]. Given these unique characteristics, DCs have for long been used in clinical approaches, particularly to boost antitumor immune response during cancer treatment [2].

The results gathered from more than 20 years of experimentation and almost 350 clinical trials demonstrated that DC-based antitumor immunotherapy is safe and with relevant clinical outcomes [3].

One of the key observations from these experiments is that the success of DC-based vaccines relies not only on the capacity of these cells to polarize and activate T lymphocytes but also on their ability to bidirectionally interact with natural killer (NK) cells. In fact, it is known that an optimal antitumor immune response depends on a complex interplay between $CD8^+$ T cells, $CD4^+$ T cells and NK cells [4]. NK cells are large granular innate immune cells with cytotoxic functions. They are crucial for the initial defense against viral infections, destroying infected cells and also for the elimination of tumor cells or foreign and endogenous cells under stress [5]. The complex cell-to-cell crosstalk between DCs and NK cells has major consequences on the modulation of immune responses. Therefore, it is expected that the rational design of new DC-based immunotherapies encompasses this required interplay with NK cells in order to synergistically evoke a superior clinical outcome [6].

In this chapter, we focus on several key aspects: the DC and NK immunobiology, the mechanisms and consequences of DC-NK bidirectional crosstalk, and the potential and relevance of DC-NK crosstalk in cancer immunotherapy.

2. Dendritic cells

2.1. Dendritic cells at a glance

DCs were first described by Paul Langerhans in 1868, being erroneously characterized as part of peripheral nervous system. It took almost 100 years to reveal the real functions of these cells. In 1973 and subsequent years, Ralph Steinman and Zanvil Cohn demonstrated by a series of elegant experiments that DCs are crucial regulators of both innate and acquired arms of the immune system [7, 8]. These cells have the unparalleled ability to polarize naïve T lymphocytes into their different effector or regulatory subsets and are potent enhancers of NK cell cytotoxicity [9, 10] as well as fundamental accessory cells in the production of primary antibody responses [11]. They also take part in the preservation of tolerance to antigens, with thymus DCs helping in the shaping of the T cell repertoire through the deletion of autoreactive lymphocytes [12, 13].

Classically, DCs are found in an immature state at locations of possible antigen entry such as the skin and mucosal surfaces (e.g., genitourinary, gastrointestinal, and respiratory systems), as well as in the connective tissue of all solid organs and even in fat tissue, retina, and brain [8, 14–18]. Upon encountering a potential threat, DCs capture and process it, displaying the resultant antigens on major histocompatibility complex (MHC)-I or MHC-II molecules. During this process, DCs engage a program, termed maturation, characterized by several morphological, phenotypical and functional changes that strongly increase their immunogenic profile. Then, mature DCs enter draining lymphatic vessels and migrate to lymph nodes where they present processed antigens to naïve T lymphocytes, generating a specific immune response [19].

2.2. Dendritic cell subsets and characteristics

DCs are composed of a very heterogeneous family of innate immune cells with characteristics frequently overlapping between subpopulations, making hard to define an unambiguous classification. Hence, in 2014, a novel classification system was created, first centered on DC ontogeny and then on their location, function, and phenotype [20].

Concerning ontogeny, all DC subpopulations are derived from a common hematopoietic CD34+ stem cell precursor that originates multiple intermediate precursors, which differentiate into several DC subsets in a process that is highly dependent on hematopoietic cytokines and growth factors [8, 14, 21]. There are three major subpopulations of DCs: plasmacytoid DCs (pDCs) characterized by the expression of CD123, CD303, CD304, FCER1, ILT3, and ILT7; classical DCs 1 (cDC1) that express CD141, CLEC9A, XCR1, and CADM1; and classical DCs 2 expressing CD1c, CD11c, CD11b, CD2, FCER1, SIRPA, ILT1, and CLEC4A [2, 22, 23].

cDCs1 are 10 times less frequent than cDCs2 and can be found in the blood, lymph nodes, tonsil, spleen, bone marrow, and in non-lymphoid tissues such as skin, lung, intestine, and liver. Functionally, they are characterized by a high capacity to cross-present antigens via MHC class I to $CD8^+$T cells, promoting their activation to cytotoxic T lymphocytes (CTLs) [24]. Although they secrete low amounts of IL-12 when compared to cDCs2 [25, 26], they are highly effective in promoting Th1 cell polarization and NK cell activation. This is in part due to the expression of the chemokine receptor XCR1, which enables cDCs1 to closely interact with XCL-producing cells such as activated T lymphocytes and NK cells [25–28]. cDCs1 are particularly equipped for the recognition of viral and intracellular antigens: they express TLR3, TLR9, and TLR10, also being major producers of type III interferons IFN λ 1–3 [29].

Regarding myeloid cDCs2, they are the major DC population being found in blood, spleen, skin, lung, and intestine. They express a large panel of pattern recognition receptors (PRRs) namely TLRs 2, 4, 5, 6, and 8, NOD-like receptors (NOD2, NLRP1, NLRP3, and NAIP), and lectin receptors such as DEC205, CLEC4A, CLEC6A, CLEC7A, CLEC10A, CLEC12A, and also the asialoglycoprotein receptor. Once activated, cDCs2 produce high amounts of IL-12 and also secrete IL-1, IL-6, IL-8, IL-10, IL-23, and tumor necrosis factor-alpha (TNF-α) [25, 30]. The different blood and tissue resident cDCs2 orchestrate a wide range of immune responses against viral, bacterial, fungal, and helminthic infections as a consequence of their capacity to polarize naïve T cells toward Th1, Th2, Th17, Th22, and CTL effector populations [30–32].

pDCs present a further limited distribution, being mainly found in blood and T cell areas of lymphoid organs. This DC subset is specifically tailored to sense and respond to viral infections [2, 8, 14, 33]. Indeed, pDCs highly express TLR7 and TLR9, the sensors for single-stranded RNA and double-stranded DNA, respectively [34], and upon activation, they produce high quantities of type I and III interferons, TNF-α, IL-6, and granzyme B [35]. Due to their intrinsic capacity to cross-present antigens to $CD8^+$ T cells, they also play a relevant role in antitumor immunity [36].

Finally, Langerhans cells, the main DCs found in the skin epidermis, are CD45+, MHC-II+, positive for Langerin, and have a low expression of CD11c and a high expression of CD1a.

They are exclusively derived from embryonic precursors and are able to self-renew locally [37]. At a functional level, Langerhans cells can induce immunogenic or tolerogenic responses upon specific maturation stimuli and cellular microenvironment and are particularly effective in antigen cross-presentation [38].

The phenotypical and functional characterization of human DC subsets is an exciting area that remains in continuous evolution. It is conceivable that this increased knowledge in DC immunobiology will empower their use in the design of more rational and effective immunotherapies.

3. NK cells

3.1. NK cell immunobiology

NK cells represent 5–15% of the circulating lymphocytes and play a pivotal role in host defense against pathogens and cancer [5]. Although recognized for their spontaneous killing ability of virus-infected or transformed cells without prior immunization, these cells also play an important role in the modulation of immune responses through the secretion of multiple cytokines and chemokines [39]. Additionally, a growing body of evidence suggests that NK cells can mediate antigen-specific immunological memory, associated with adaptive immunity [40].

NK cell activity is tightly regulated by a complex array of germline-encoded activating and inhibitory receptors randomly generated during NK cell differentiation and maturation [41]. The integration of the signals transmitted by these receptors forms the basis of NK cell reactivity to their targets and determines the magnitude of NK cell–mediated cytotoxicity and cytokine production. The inhibitory receptors such as killer immunoglobulin-like receptors (KIRs) and the lectin-like CD94/NKG2A heterodimer recognize self-molecules of the major histocompatibility complex (MHC) class I expressed in almost all healthy cell types and protect themselves from NK cell-mediated killing [42]. Paradoxically, the engagement of MHC class I molecules maintains NK cells in a state of responsiveness to subsequent activation, a property referred to as NK cell licensing [43]. During the course of tumorigenesis or viral infection, cells often decrease or even lose the expression of MHC class I molecules and upregulate the expression of a wide spectrum of stress-induced surface ligands that are recognized by activating receptors in NK cells, including the natural cytotoxic receptors (NCRs: NKp46, NKp30, and NKp44), the C-type lectin receptors (CD94/NKG2C, NKG2D, NKG2E/H, and NKG2F), DNAM-1, and killer immunoglobulin-like receptors with a short cytoplasmic tail (KIRs, KIR-2DS, and KIR-3DS) [41, 44]. When multiple of these activating receptors are simultaneously engaged and reach a threshold that surpass the inhibitory signals, NK cells are shifted to an activated phenotype, exerting their cytolytic activity against target cells [41, 45]. Several cytokines have been described to activate and promote NK cells antitumor activity. The common gamma chain (γc) family of cytokines, like IL-2, IL-12, IL-15, IL-18 and IL-21, are the most well-recognized ILs to boost NK cell antitumor activities and have been used to improve the proliferation, differentiation and effector function of NK cells [46].

Regarding their cytolytic activity, the perforin/granzyme pathway is the main mechanism used by NK cells to kill target cells. Upon activation, NK cells polarize the lytic granules to

the immunological synapse formed with the target cell and release the membrane disrupting perforin that forms transient pores on the target cell membrane, allowing the entrance of granzymes, a family of serine proteases, that trigger an apoptotic-like cell death [47]. The death receptor pathways involving FasL and TRAIL are also employed by NK cells on target cell-induced apoptosis through a perforin-independent mechanism. Death receptor members of the TNF-α family such as FAS and the death receptor 5 (DR5) are usually upregulated in tumor cells and transduce apoptotic signals upon binding to their cognate ligands FasL and TRAIL on NK cells, resulting in a classical caspase-dependent apoptosis [48]. NK cells are also mediators of the antibody-dependent cellular cytotoxicity (ADCC), another type of granule-mediated cell death that occurs when the Fc receptor expressed by NK cells (FcγRIII or CD16) binds to the Fc portion of IgG1 antibodies-coated target cells [49]. This interaction results in a strong activation signal that overcomes the inhibitory signals, leading to a downstream cascade of activation events with the release of cytolytic granules and inflammatory mediators.

In addition to the direct cytotoxic mechanisms, NK cells also act as immunomodulatory cells engaged in reciprocal interactions with DCs, macrophages, and T cells through the release of various cytokines, chemokines and growth factors, which might augment or dampen immune responses [50].

4. DC-NK cell interplay

Currently, it is clear that DCs and NK cells have a crucial role in modulating innate and adaptive immune responses through a complex cell-to-cell crosstalk (**Figure 1**). Indeed, DC-mediated activation of NK cells contributes to the development of potent innate immunity, whereas, in turn, activated NK cells provide signals for DC activation, maturation, and cytokine production, promoting adaptive immunity [6]. This DC-NK crosstalk occurs, *in vivo*, in the lymph nodes [51, 52], at the sites of inflammation, in peripheral tissues such as the skin and mucosa [53] and in solid tumor microenvironments [54].

4.1. Activation of NK cells by DCs

Several studies have demonstrated the potential of DCs to influence the function of NK cells. Seminal works revealed a reduction of NK cell-dependent antitumor effects in mice depleted from CD8α DCs, suggesting a direct role of DCs in NK cells activation [55]. The triggering of NK cells by DCs seems to be dependent on both cell-to-cell contact and soluble factors. Accordingly, in human and animal studies, NK cells activation by DCs was significantly disrupted by transwell separation, reinforcing a major contribution of cell-to-cell contact to this close communication [55, 56]. Further studies have demonstrated that DC-produced IL-12 is also crucial for NK cells activation, namely for their production of IFN-γ [57]. This process comprises the formation of stimulatory synapses between DCs and NK cells, which promote the polarized secretion of pre-assembled stores of IL-12 by DCs toward NK cells [58]. Furthermore, Poly(I:C)-treated DCs and IFN-α-treated DCs also induce NK cells to secrete IFN-γ by the binding of activating NK cell receptor NKG2D to its specific ligands, such as MHC class I-related chains A and B (MICA/B) [59, 60]. Another relevant interaction that results in NK cell cytotoxicity and IFN-γ release occurs between CXC3CL1 expressed on DCs and CXC3CR1 on NK cells [61].

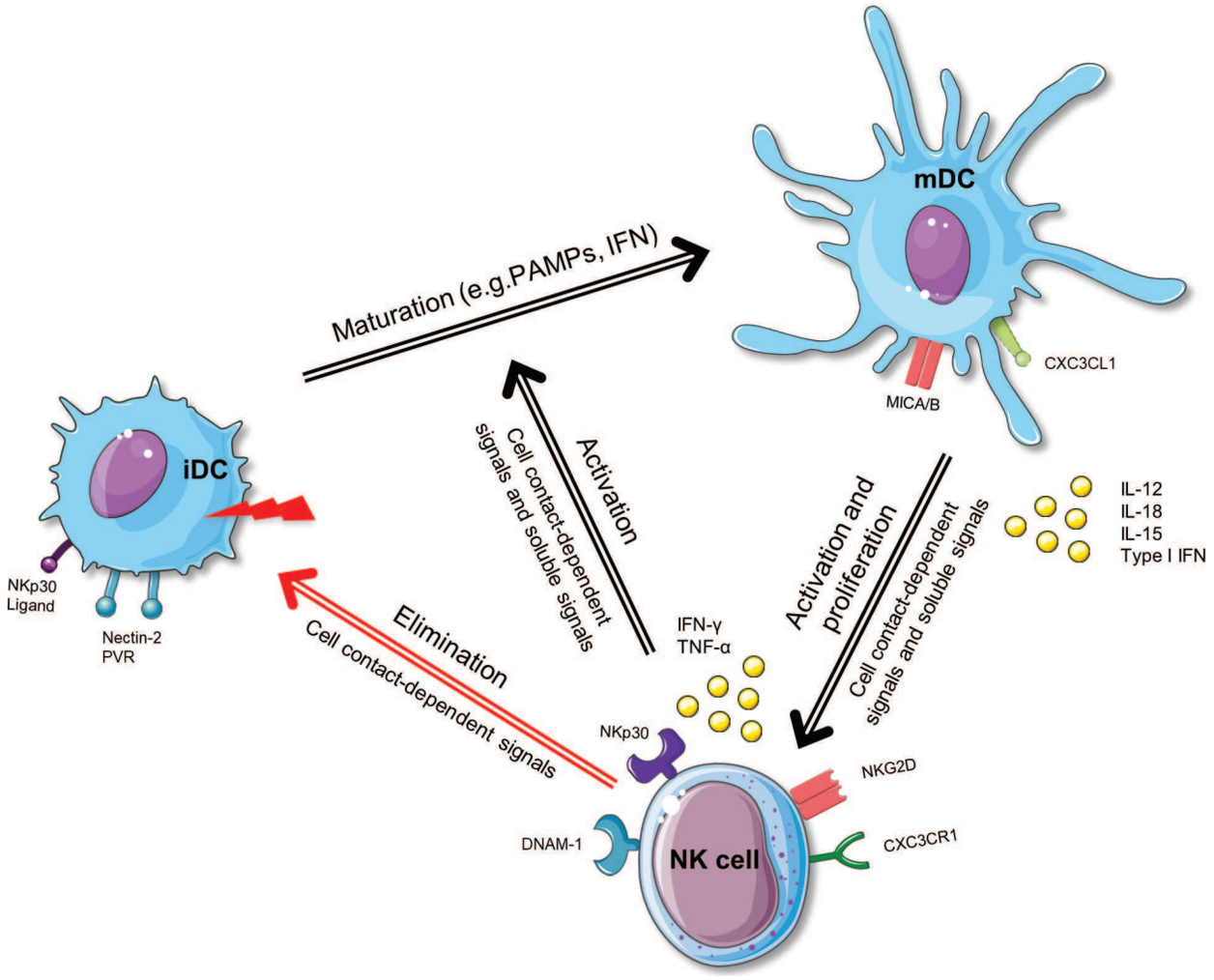

Figure 1. Dendritic cell-natural killer cell interplay. iDCs can undergo maturation by exposure to several stimuli, pathogen-associated molecular patterns (PAMPs), and IFN. The resulting mDCs secrete IL-12, IL-18, IL-15 and type I IFN, which in turn induce the proliferation and activation of NK cells, leading to further secretion of IFN-γ. NK-DC interaction by NKG2D with MICA/B and CXC3CR1 with CXC3CL1 can also lead to NK cell activation. Thus, activated NK cells are able to increase DC maturation, dependent on cytokines like IFN-γ and TNF-α, as well as the interaction of NKp30 receptor on NK cells with its ligand on DCs. On the other hand, activated NK cells can also eliminate MHC-I low-expressing iDCs by cell contact-dependent interactions of NKp30 receptor with NKp30 ligand and DNAM-1 with Nectin-2 or PVR. iDCs, immature dendritic cells; IFN, interferon; mDCs, mature dendritic cells.

IL-15 can be considered as an additional and important cytokine involved in the process of DC-mediated activation of NK cells. This cytokine can be found bounded to DCs membrane and is able to induce NK cell proliferation, survival, and to enhance their cytotoxic functions [57, 62]. Additionally, in DCs, transmembrane TNF, as well as membrane-bound IL-15, can enhance NK cells proliferation, CD69 expression, and IFN-γ secretion [63]. Both signaling mechanisms are mediated by cell-to-cell contact via simultaneous engagement of DCs transmembrane TNF and membrane-bound IL-15, with their respective NK cell receptors, leading to its activation. Furthermore, NK cells proliferation is also dependent on the interaction between CD40 and B7 molecules on DCs with CD40L and CD28 on NK cells, respectively [64].

IL-18 expressed by immature and mature DCs has also been implicated in NK cells activation. DC-derived IL-18, as well as IL-12, is involved in the upregulation of NK cell cytotoxicity [56].

Similar to IL-12, IL-18 seems to be delivered in secretory lysosomes at the NK/DC synaptic cleft, leading to NK cells activation [65]. In case of regulatory DCs, their insufficient production of IL-18 is involved in the restrained IFN-γ secretion by NK cells, downregulating NK cells activation [66]. In addition, studies performed in mice revealed that both IL-18 and IL-12 are also involved in the expansion of $Ly49H^+$ NK cells promoted by $CD8\alpha^+$ DCs [67].

The role of type I IFN (IFN-α/β) on NK cell activation has also been assessed during NK-DCs crosstalk. Type I IFN, secreted by plasmacytoid DCs [68], is required for NK cell cytotoxicity in response to virus infection [69, 70]. In the context of TLR stimulation, NK cells priming is dependent on the recognition of type I IFN signals by DCs and on the subsequent production and trans-presentation of IL-15 by DCs to resting NK cells [71]. Finally, IL-2 produced by bacterially activated myeloid DCs has also been shown to be required, both *in vitro* and *in vivo,* for NK cells activation and IFN-γ-efficient production [72].

4.2. DC modulation by activated NK cells

Over the last two decades, multiple studies have reported that NK cells play a relevant role in the DCs maturation process, either by killing DCs that did not properly acquire a mature phenotype or through direct DCs stimulation [73]. The process of immature DC lysis is dependent on NK-activating receptors as well as on the amount of MHC class I molecules on DCs [74, 75]. *In vitro* assays have demonstrated that activated NK cells can recognize and kill DCs via NKp30 natural cytotoxicity receptor, suggesting the expression of a still unknown NKp30 ligand on DCs surface. Mature DCs are susceptible to NK cell killing when NK inhibitory signal is blocked by MCH-I antibodies, confirming that mature DCs are naturally spared due to their high expression of MHC class I molecules [76]. In addition, DNAM-1-triggering receptor and its ligands, poliovirus receptor (PVR) and Nectin-2, have been demonstrated to be crucial in NK cell-mediated lysis of immature DCs. DNAM-1 receptor on NK cells cooperates with NKp30 receptor in the NK-mediated elimination of DCs. The degree of contribution of DNAM-1 appeared to correlate with the surface amount of its specific ligands PVR and Nectin-2 on DCs [77]. Other *in vivo* studies have also shown that NK cells efficiently kill injected immature bone marrow-derived DCs, via a pathway dependent on the TNF-related apoptosis-inducing ligand (TRAIL) [78]. Similarly, another study confirmed that NK cells can kill incompletely matured DCs in the context of a viral infection via TRAIL—Death Receptor 4 (DR4) pathway [79]. These findings led to the hypothesis that the killing of immature DCs by NK cells should promote the survival of the most immunogenic DCs, supporting and empowering efficient and protective immune responses. In fact, it has been demonstrated that the killing of immature DCs by autologous NK cells is particularly important for the expansion of cancer-specific CTLs [80].

On the other hand, in chronic viral infections, IL-10 produced by NK cells induces contrasting phenotypic changes in DCs; specifically, immature DCs exhibit aberrant resistance to NK cell-mediated elimination, whereas mature DCs had an increased susceptibility to NKG2D-dependent elimination. This process leads to the accumulation of poorly immunogenic DCs in lymph nodes, causing a progressive immune dysfunction [81]. Furthermore, DC lysis by NK cells can also negatively regulate the duration and effectiveness of virus-specific T cell responses *in vivo* by limiting the exposure of T cells to infected antigen-presenting cells, which

negatively impacts the quality of T cell responses and their ability to limit viral persistence [82]. Additionally, it has been shown that in solid organ transplantation, host NK cells kill allogeneic DCs via the perforin pathway. This will limit allogeneic antigen presentation to host lymphocytes, reducing T cell–mediated graft-versus-host disease [83].

Activated NK cells can also improve DCs maturation and activation, enhancing their ability to stimulate T cell responses. When NK cells are cultured with immature DCs in the presence of maturation stimuli, such as lipopolysaccharide (LPS), they strongly enhance DCs maturation, specifically by upregulating the DCs co-stimulatory molecule CD86 and IL-12 production. NK cells activated by IL-2 are also able to induce DCs maturation, improving their ability to stimulate allogeneic naïve $CD4^+$ T cells. These effects of NK cells on DCs maturation are cell contact dependent, although the secretion of IFN-γ and TNF-α is also relevant [84, 85]. These findings show that both effects of NK cells on DCs (DC killing and stimulation) are dependent on NKp30-triggering receptor.

Further studies have shown that NK cell-activated DCs produce higher levels of IL12p70 after subsequent CD40 ligands stimulation, leading to an increase in the induction of T cell responses [86, 87]. The effect of NK cells on DCs is also dependent on the type of NK cell activation; IL-2-primed "effector" NK cells can kill DCs, whereas IL-18-primed NK cells are just prone to enhance the ability of DCs to produce IL-12p70 dependent on CD40L stimulation [88]. In fact, NK cells are able to trigger immature DCs to secrete IL-18 through a $Ca2^+$-dependent and tubulin-mediated recruitment of IL-18-containing secretory lysosomes toward the adhering NK cell. Then, IL18-activated NK cells secrete the pro-inflammatory "danger signal" high-mobility group B1 (HMGB1), which induces DC maturation and protects DCs from lysis, thus favoring the development of adaptive immune responses [65]. Furthermore, human NK cells, exposed to different cytokines, are able to promote distinct pathways of Th1 priming. Specifically, IL-12- or IL-2-activated NK cells induce maturation of DCs capable of priming IFN-γ-producing Th1 cells, whereas IL-18-conditioned NK cells induce Th1 polarization only when co-cultured with both DCs and T cells, which release IL-12 and IL-2, respectively, promoting IFN-γ production. Thus, the local prevalence of IL-12, IL-2, and IL-18 at the inflammatory sites may differentially modulate the NK cell interaction with DCs, leading to different outcomes in naïve T cell polarization [89].

Recently, IL-23 was uncovered as an enhancer of NK cell ability to stimulate DCs. IL-23 induces NK cells activation and displays a synergistic effect with IL-18 for IFN-γ production by NK cells. This cytokine also potentiates the increase of CD86 expression and IL-12 secretion by LPS-treated DCs upon IL-18-stimulated NK cells contact [90].

5. DCs and NK cells in cancer immunotherapy

The main purpose of cancer immunotherapy is to change the balance from tumor escape or equilibrium to cancer cells elimination. Latest developments have been focused at increasing the activation status of the innate and adaptive immune systems, comprising cytokine administration, Car T cells, DCs and NK-based vaccines, checkpoint inhibitors and monoclonal

antibodies engineered to target high-yield elements in oncogenic-signaling pathways [91]. A crucial point for the development of such approaches was the definition of the optimal characteristics of an antitumor immune response. Specifically, it became evident that this response depends on a complex cells interplay involving DCs, $CD8^+$ T cells, $CD4^+$ T cells, and NK cells.

Given their positioning at the interface between innate and adaptive immunities and their unparalleled capacity to interact and modulate immune effector cells, DCs have been scrutinized and settled as highly desirable and full of translational and clinical potential. Since the 1990s, DCs have been used in more than 350 clinical trials as cellular antitumor vaccines [3]. Currently, there are three approaches exploring DCs in oncologic treatments: (1) non-targeted protein and nucleic acids-based vaccines captured by DCs *in vivo*; (2) direct targeting of antigens to DCs *in vivo*; and (3) vaccines composed of *ex vivo*-generated DCs matured and loaded with tumor antigens [19]. Notwithstanding the good safety profile of antitumor DC-based vaccines, the rate of success in inducing clear therapeutic outcomes is inconsistent [3]. Objective tumor responses are usually above 15% [92], and promising vaccines in early-phase studies [93, 94] often fail to present clinical benefits in pivotal phase III trials [95]. Differentiation of DCs *ex vivo* from blood monocytes followed by their injection back into the patient is by far the most common strategy [1, 8]. This approach suffers from some limitations: very few of the injected DCs migrate to the lymph nodes to present antigens to T cells, and it became evident that monocyte-derived DCs are functionally limited when compared to endogenous DCs subpopulations [96, 97]. *In vivo* targeting of antigens to specific DC subsets, tailoring of *ex vivo* differentiated DCs to particular phenotypes, and the combination of DCs-based vaccines with other antitumor therapies are critical steps for the effective success of new DCs immunotherapies [2–4, 92].

As referred in the earlier section, NK cells can directly eliminate tumor cells and indirectly enhance antitumor adaptive immunity by favoring DCs maturation and by killing immature DCs, thus enhancing immunogenic DC populations that will polarize antigen-specific CTLs [76, 98]. Importantly, the cell debris resultant from NK tumor cell destruction is also an important source of multiple tumor antigens for DCs cross-presentation to $CD8^+$ T cells [99]. Due to these characteristics, NK cells have been clinically explored in recent years in several immunotherapeutic strategies for cancer. There are mainly two therapeutic approaches based on NK cells: the use of NK cell stimulants/modulators to take advantage of endogenous responses and the adoptive cell transfers of fully differentiated and *ex vivo*-activated NK cells [100, 101]. The adoptive cell transfers of autologous NK cells have been tested and well tolerated in the treatment of several cancers, including glioma, lymphoma, and renal cell carcinoma, though clinical responses have not always been observed [102, 103].

6. Future perspectives

In parallel with the growing knowledge on immune cells and cancer biology, cell-based immunotherapies must be tailored to answer the new demands. Whereas initial research focused on generating mainly tumor CTLs responses, it becomes clear that the activation of multiple immune effector cells is the key to success for curative cancer vaccination.

Apart from CTL induction, DC-NK cell crosstalk is of major importance in antitumor immune responses [4]. Efficient DC-vaccine-mediated antitumor immunity has been shown to be strongly dependent on NK cell activity [6]. This was highlighted by experiments where NK cell depletion drastically impacted tumor elimination following DC vaccination [104, 105]. Moreover, data from several clinical trials on DC-based antitumor vaccines indicate that positive outcomes seem to correlate with high levels of activated NK cells in responder patients [106].

Taken together, these data underscore why future research efforts should also focus on optimizing the NK cell–interacting properties of DC vaccines, in addition to improving their T cell–stimulatory capacity. The NK cell–activating character of DC vaccine preparations can be imprinted at multiple levels, such as by (1) tailoring the phenotype of *ex vivo* differentiated DCs, (2) using specific DCs subsets, and (3) targeting endogenous DCs populations that are intrinsically prone to interact with NK cells. The former approach includes the use of DCs expressing high levels of

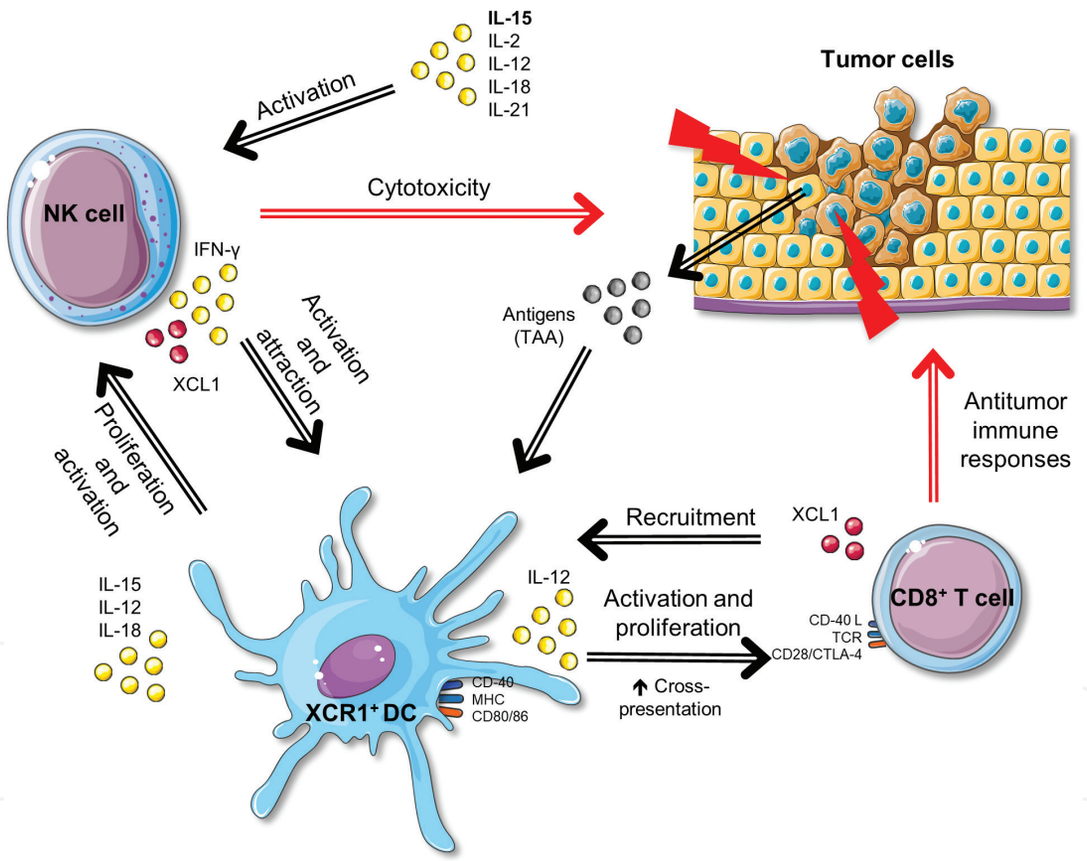

Figure 2. DC-NK cell crosstalk in cancer. NK cells can be activated by DCs, directly by cell contact and/or by DC-produced cytokines such as IL-12, IL-15 and IL18. Activated NK cells can then proliferate and secrete cytokines like IFN-γ, which will stimulate antitumor-acquired immune response. Specifically, IFN-γ induces CD8+ T cells to convert into antigen-specific CTLs and supports the creation of immunological memory against the tumor antigens. Additionally, IFN-γ can also stimulate the polarization of CD4+ T cells into Th1 subset that in turn stimulates CTL differentiation. Furthermore, activated NK cells, additionally to induce DCs maturation, will destroy tumor cells, fueling DCs with tumor antigens that are then cross-presented to CD8+ T cells. The cDCs1 population (CD141+ XCRI+) is particularly effective in this interplay: The expression of XCR1 receptor by this DC subset and of its ligand XCL1 by T and NK cells during infectious and inflammatory responses potentiates the interaction between these cells. Additionally, CD141+ XCRI+ DCs present an exceptional antigen cross-presentation capacity and are producers of high amounts of IL-12 following activation. CD-40 L, CD-40 ligand; IFN-γ, interferon γ; IL, interleukin; TAA, tumor-associated antigens; TCR, T cell receptor; XCL1, chemokine (C motif) ligand; XCR1, chemokine receptor for XCL1.

IL-12 and IL-15 [107] or manipulated to express the receptor XCR1. On the other hand, the other potential strategy is to target endogenous DCs subsets expressing XCR1. By using/targeting XCR1 expressing DCs, we potentiate their interaction with activated $CD8^+$T cells and NK cell, given that these are the main producers of the XCR1 ligand XCL1 (**Figure 2**) [26, 27, 108].

In conclusion, the design of new DC-based vaccination strategies should encompass NK cell-stimulating potency. Additionally, it would be of great value to systematically incorporate NK cells monitoring as an outcome in antitumor DC-based clinical trials.

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Conflict of interest

The authors declare no conflict of interest.

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