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Principles of NK Cell/DC Crosstalk: The Importance of Cell Dialogue for a Protective Immune Response

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Key Words
Dendritic cells · Natural killer cells · Lymph nodes · Neoplasm

Summary
A cooperative dialogue between natural killer (NK) cells and dendritic cells (DCs) has been recently described. They help each other to acquire their complete functions, both in the periphery and in the secondary lymphoid organs. In vitro, IL-12, IL-18, IL-15, and type-I IFN are implicated in the activation, proliferation, and cytotoxicity of NK cells by mature DCs, while TNF-α secreted by NK cells is involved in the maturation of DCs. Not completely clarified cell-to-cell contacts are also implicated in this cross-talk. Thus, NK cell activation allows the killing of transformed or infected cells in the periphery, but may also be important for the generation of adaptive immunity. Recent works suggest that they may play a key role in polarizing a Th1 response. This dialogue between DCs and NK cells may be further exploited in immunotherapy aimed to boost immune response.

Schlüsselwörter
Dendritische Zellen · Natürliche Killerzellen · Lymphknoten · Neoplasie

Zusammenfassung
Ein kooperativer Dialog zwischen natürlichen Killerzellen (NK-Zellen) und dendritischen Zellen (DCs) ist kürzlich beschrieben worden. NK-Zellen und DCs helfen einander, ihre vollständige Funktionsfähigkeit zu erlangen – in der Peripherie und in den sekundären lymphatischen Organen. In vitro sind IL-12, IL-18, IL-15 und Typ-I-IFN in die Unterstützung der Aktivierung, Proliferation und Zytotoxizität der NK-Zellen durch reife DCs eingebunden, während die Sekretion von TNF-α durch NK-Zellen in den Reifungsprozess der DCs involviert ist. Zell-Zell-Kontakte, die noch nicht völlig verstanden werden, sind an diesem Dialog ebenfalls beteiligt. So erlaubt die Aktivierung von NK-Zellen die Abtötung transformierter oder infizierter Zellen in der Peripherie, kann aber ebenso von Bedeutung sein für die Generierung der adaptiven Immunantwort. Neuere Arbeiten legen nahe, dass NK-Zellen eine Rolle bei der Th1-Polarisierung spielen können. Der Dialog zwischen NK-Zellen und DCs kann im Rahmen immuntherapeutischer Ansätze verstärkt genutzt werden, um die Immunantwort zu fördern.

Introduction

In immunity, the different types of cells act not only displaying their own protective functions, but also interacting with each other to optimize the response against a pathogen. Recently the relationship between natural killer (NK) cells and dendritic cells (DCs) has been described.

DCs are critical for triggering the immune response [1]. At an immature stage, they act as sentinels in peripheral tissues, continuously sampling the environment thanks to their high ca-

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pacity for endocytosis, sensing the presence of pathogens, and secreting chemokines and cytokines to amplify the response. Upon activation by danger signals, they mature acquiring new molecules, which allows them to migrate into lymph nodes to induce T cell response [1].
NK cells are able to kill cells according to a mechanism regulated by several activating and inhibiting receptors [2]. They can detect the loss of MHC molecules or presence of molecules induced upon a stress. This feature confers them a potential role in cancer immune surveillance and in the control of viral infections [3].
In peripheral tissues and secondary lymphoid organs, DCs and NK cells can establish a dialogue for a bi-directional activation using different mechanisms which involve soluble factors and cell-to-cell contacts [4, 5]. Here we want to address the question of how this dialogue can influence adaptive immunity.

DC/NK Cell Interaction in Peripheral Tissues

NK Cell Recruitment

The triggering of the immune response generally takes place in the peripheral tissues. The PAMP (pathogen-associated molecular pattern) of the micro-organism surface can be recognized by PRR (pattern recognition receptor) expressed on DCs. PAMPs are danger signals for DCs and are able to activate them to secrete chemokines to attract other cells of the immunity. During inflammation, activated myeloid DCs (mDCs) and plasmacytoid DCs (PDCs) can secrete different chemokines such as MIP-1β/CCL4, RANTES/CCL5, fractalkine/CX_3CL1, IL-8/CXCL8, and IP-10/CXCL10 [6–8]. The different NK cell subsets are able to migrate in response to these chemokines, according to the receptors they express. $CD56^{low}$ CD16+ NK cells express CXCR1 and CX_3CR1 [9, 10], receptors for IL-8/CXCL8 and fractalkine/CX_3CL1 respectively. This last chemokine would mediate the firm adhesion of cells to the endothelium and subsequent migration to IL-8 [11]. The $CD56^{bright}$ NK cells express CCR5, CXCR3 and CXCR4 which allow them migrate in response to RANTES/CCL5, MIP-1β/CCL4, ITAC/CXCL11, and IP-10/CXCL10 [9]. Thus, DCs would attract the two types of NK cells in the site of inflammation, rendering their encounter possible. This hypothetical interaction is supported by in vivo observations, where NK cells have been found in close contact with DCs either in lesions of atopic eczema / dermatitis syndrome or in Gleevec-induced lichenoid dermatitis in gastrointestinal stromal tumor (GIST) bearing patients [12, 13].

NK Cell Activation by DCs

Besides chemokines, other molecules are implicated in the NK cell/DC dialogue, which leads to the activation of the both populations.

Several in vitro studies show a central role of IL-12, IL-18 and type-I IFN in the triggering of NK cell functions. IL-12 seems to be important to induce the secretion of IFN-γ by NK cells in several systems: lipopolysaccharide(LPS)-activated monocyte-derived DCs, splenic DCs [5], or poly(I:C)-stimulated myeloid DCs [14]. IL-18 may act in synergy with IL-12 to induce the secretion of IFN-γ but also to enhance cytotoxicity, at least in the human CD34+-derived DC system [15]. Type-I IFN are other cytokines that have been shown to induce cytotoxicity of NK cells [16, 17]. Although all types of DCs can secrete type-I IFN, the main producers of these cytokines are PDCs, particularly when activated through Toll like receptor 7 (TLR7), and TLR9 by virus components [18]. Nevertheless NK cells may be activated in an IL-12, IL-18 and type-I IFN independent manner. In fact, IL-12- and IL-18-deficient mice are able to induce IFN-γ secretion by NK cells. In mice, this capability might be under the control of IL-2 secreted by bone marrow-derived DCs activated by bacterial components [19].
IL-15 produced by mature monocyte-derived DCs appears to be particularly important to stimulate NK cell proliferation. Interestingly, this effect may require the membrane-bound form as the proliferation was abrogated by physical separation [5].
Despite the large mass of data showing the role of soluble factors in NK cell activation, an early study in mice suggest the involvement of cell-to-cell contact [20]. Transwell separation of the two populations could abrogate DC-dependent NK cell cytotoxicity induction [20]. The contact through synapse may be necessary for the polarized secretion of IL-12 or of other cytokines by DCs toward NK cells [21], and for ligand-receptor interaction [22].
Likewise, it is probably through such synaptic formations that NK cells may kill DCs. Several groups observed that NK cells recognize and lyse monocyte-derived DCs in vitro [23–26] in a cell-to-cell dependent manner. It has been described that the NK cell:DC ratio is a critical factor to induce NK-mediated DC death. Whereas a low ratio (1:5) leads to DC maturation, a higher NK cell:DC ratio (5:1) causes killing of immature DCs by the autologous NK cells [24]. Interestingly, DC subsets display different susceptibilities to lysis by NK cells; human PDCs were not lysed by IL-2-activated NK cells, whereas mDCs isolated directly from blood underwent only a limited lysis [14].
Moreover mature DCs are protected from NK cell lysis by acquiring a higher expression of HLA-I molecules [27]. They particularly up-regulate HLA-E, which protects them from NK cell lysis. On the contrary, immature DCs are lysed because they do not express sufficient number of HLA-E molecules to induce an efficient inhibition signal through NKG2A/CD94 [28]. Beside the inhibitor receptors, NK cell-activating receptors (NCRs) play a primary role in DC targeting. The activating receptor NKp30 appears to be an important candidate during this interaction, since the single blocking of this NCR inhibits NK cell-mediated immature DC lysis [23]

Immature DC selection by NK cells has also been described in mice, where TRAIL(TNF-related apoptosis-inducing ligand)-signaling pathway has been observed as an effector mechanism for injected DC killing [29]. Like in humans, mature but not immature DC were spared by NK cells in this model [29]. This DC editing may be of primary importance in transplantation. In vivo experiments showed that T cell-mediated graft-versus-host disease (GVHD) could be prevented by treating mice with alloreactive NK cells. The protection may result from the elimination of recipient immature DCs which could not prime cytotoxic lymphocytes (CTLs) [30].

DC Activation by NK Cells

In peripheral tissues, the bi-directional cross-talk has been proposed to play a relevant role in the mechanisms leading to the selection of DCs with maximal capability of T cell priming [4, 31]. In particular, recent studies have demonstrated that, during the immune response, DC maturation can be mediated by NK cells [14, 17, 24]. This might be interesting when the absence of pathogen-related molecules or inflammation does not drive to DC maturation and to an effective antigen presentation
Recent studies shed light on the molecular mechanisms that regulate this specific part of the NKcell/DC cross-talk. It has been found that at low NK cell:DC ratio (1:5) NK cell/DC interaction induces cytokine production (especially TNF-α and IL-12) by DCs, and this stimulating effect may depend on cell-to-cell contact as well as TNF-α released by NK cells [24]. The physical interaction between these cells might allow the engagement of NCRs that recognize ligands on DC surface. It has been recently reported that NK-mediated DC maturation depends on the triggering of NKp30 on NK cells which in turn secrete TNF-α and IFN-γ [32]. When they used neutralizing monoclonal antibodies against TNF-α, IFN-γ and NKp30 they could not detect DC maturation, demonstrating that these factors may represent different players of the same event.
In the same way, NK cells may help the maturation of PDCs. It has been shown that IL-2-activated NK cells, in association with sub-optimal concentrations of synthetic oligodeoxynucleotides expressing unmethylated CpG motifs, were also able to induce the maturation of PDCs isolated from blood [14]. The molecular mechanisms of this help are not yet understood, but it was found that cytokine secretion and CD83 up-regulation on PDCs are dependent on cell-to-cell contacts [14]. In mice, NK cells may activate bone marrow-derived DCs through the TREM2(triggering receptor expressed on myeloid cell 2)-signaling pathway, promoting up-regulation of CD86 molecules [33].

DC-NK Cell Interaction in Secondary Lymphoid Organs

During maturation, mDCs and PDCs undergo several modifications. They acquire dendritic morphology, express costimulatory molecules and increase the expression of MHC class I and class II molecules [1, 18]. They down-regulate some chemokine receptors, but acquire CCR7 [34], which allows them to migrate into the lymph node where they encounter T cells, and probably also NK cells. In fact CD205+ mDCs and NK cells have been shown to co-localize in the T cell zone of human lymph nodes [5]. Similarly, PDCs can be recruited into inflamed lymph nodes around the HEV in the T cell zone [35] and may probably interact with NK cells, although this has not been demonstrated yet.
Recent studies have revealed the composition of NK cell population in lymph nodes [5, 36]. The main NK cell population of lymph nodes is composed by $CD56^{bright}$ NK cells, whereas the $CD56^{dim}$ NK cell subset is predominant in the blood [5]. Moreover lymph node NK cells lack perforin, KIR, and CD16 molecules [36]. The presence of NKG2A/CD94+ NK cells in lymph node may allow the selection of immature DCs that express insufficient amounts of HLA molecules [27] and particularly of HLA-E molecules [28]. These data suggests a possible role of NK cells in preventing tolerance, because they eliminate immature DC, which can induce T cell inhibition [37, 38]. Furthermore, lymph node $CD56^{bright}$ NK cells are endowed with specific skills. When cultured in the presence of LPS-activated mDCs, they can proliferate and secrete large amounts of IFN-γ [5], while the $CD56^{dim}$ cells could less. IFN-γ production by $CD56^{bright}$ cells is highly dependent on IL-12 since IL-12-blocking antibody could abrogate this phenomenon [5]. Interestingly, the proliferation of this population involves IL-15 expressed by DCs, which seems to act in a membrane-bound form [5]. Similarly to their myeloid counterpart, PDCs can induce blood $CD56^{bright}$ NK cell proliferation in a cell-to-cell contact manner [16], but their effects on lymph node NK cells remain to be explored. NK cells can be resident or recruited [39]. The proliferation induced by mature mDCs may explain the maintenance of this NK cell subpopulation in lymph node. The resident population of NK cells in lymph node might be derived from blood $CD56^{bright}$ NK cells, which express CCR7, which allows their migration to SLC/CCL21 [9]. Indeed, blood and lymph node $CD56^{bright}$ NK cells share the similar features, such as secretion of large amounts of IFN-γ after activation [40], and low cytotoxic capabilities in a steady state.
At the same time, mature DCs in lymph node induce the activation of T cells, which then secrete large amounts of IL-2, to enhance their own proliferation but which might also induce the activation of the $CD56^{bright}$ NK cells to become cytotoxic. An in vitro study has shown that after IL-2 activation, lymph node $CD56^{bright}$ NK cells express perforin and lyse NKG2D ligand-positive cells [36]. In humans, lymph node NK cells has been detected in the T cell area [36]. In addition, a mouse model study suggests that NK cell activation by CD4 cell-derived IL-2 may be possible in vivo [41]. Once IL-2-activated, lymph node NK cells might be able to kill not completely mature DCs or might enter the blood stream as they are not anchored by CCR7 and CD62L to the secondary lymphoid or-

gans [36]. Nevertheless, this hypothesis remains to be verified in an in vivo system.
Thus, mature DCs might play several roles toward CD56^{bright} NK cells in lymph nodes, inducing i) their proliferation by IL-15 expression, regulating NK cell homeostasis, ii) their differentiation into killer cells by enhancing IL-2 production by T cells and iii) their capacity to secrete IFN-γ.

Role of NK Cells in the Polarization of the Adaptive Immune Response

Several works have shown the participation of NK cells in the generation of CTLs. In mixed lymphocyte culture, it has been demonstrated that the generation of human allogeneic CD8+ T cells required the help from NK cells [42]. Moreover, when under-activated DCs could not activate T cells, the presence of NK cells could license these DCs to prime CD8+ T cells [43]. Several in vivo studies comfort the relevance of these in vitro results. In the mouse model, Martin-Fontecha et al. [39] have shown that NK cells provide an early source of IFN-γ that is necessary for Th1 polarization. They demonstrated that DC-dependent recruitment and activation of NK cells in lymph nodes correlated with the induction of IFN-γ-secreting CD4+ T cells while the absence of recruited NK cells led to a defect in Th1 cell generation. In the mouse model, turning off NK cells was sufficient to skew the immune response toward Th2 [44]. Furthermore, IFN-γ produced by NK cells may compensate the absence of CD4+ T cell help in the generation of CD8+ T cells, at least in *Toxoplasma gondii*-infected CD4–/– mice [45].
In addition to this help, several recent studies suggest a direct T cell activation by NK cells. In certain conditions of activation, NK cells may express molecules like DR, CD80, CD86 and OX40L so that they could directly activate T cells [46, 47]. Nevertheless, their contribution in the direct activation of T cells in vivo remains to be demonstrated.

DC-NK Cell Interaction in Antiviral Immune Response

NK cells are particularly important in antiviral response [3] because they can rapidly kill infected cells. Diverse types of activating receptors are implicated in antiviral immunity. In murine cytomegalovirus (MCMV) model, the activating Ly49H receptor expressed on NK cells has been shown to be directly involved in the control of viral infection [48]. In human, NK cells can also be directly activated through NCRs. It has been described that CMV-infected cells could express MIC molecules, ligands for NKG2D, in interstitial pneumonia [49]. The expression of NCR ligands can be induced by IFN-α or IL-15 on mDCs which participate in the activation of NK cell killer function [50].
Furthermore, it has been recently found that human NK cells express TLR3 and TLR9 (receptors for viral components) which allow the direct recognition of double-stranded RNA and DNA, respectively [51, 52]. In the presence of IL-12, NK cells activated with suboptimal quantities of poly(I:C), a TLR3 ligand, could be licensed to kill target cells [51]. Thus, NK cells may be able to detect the presence of virus using their TLR, but need soluble factors to be activated, which may be provided by DCs.
In the early phase of infection, one of the most important cells might be PDCs. Human PDCs express TLR7 and TLR9 which recognize single-stranded RNA and DNA respectively. In addition, mouse PDCs express TLR8 which also recognizes single-stranded RNA [53]. These receptors act through Myd88 pathway to induce inflammation and type-I IFN production [54]. In response to viral components, PDCs are able to attract NK cells [8] and secrete large amounts of type-I IFN [35, 55, 56], which is a STAT1-dependent potent activator of NK cell cytotoxicity [14, 16, 57]. PDCs have also been shown to produce IL-12 after CpG ODN and CD40L activation [58].
However IFN-α does not seem to be required in all cases. It has been shown that the lack of IFN-α leads to a high level of IL-12 secretion by DCs [56, 59] that allows normal NK cell and IFN-γ responses in the MCMV model. Thus, these studies suggest a cross-regulation of cytokine response in DC subsets [59]. Furthermore, the requirement for the different cytokines in the course of infection may depend on the type of virus [3, 60]. IL-12 secreted by mDCs seems to be important in the response against MCMV but not against lymphocytic choriomeningitis virus (LCMV) [60]
After recognition of the ligand, NK cells are able to kill the infected cells by inducing apoptosis or necrosis [61]. These infected dead cells can be phagocyted by mDCs, and viral components contained in apoptotic cells can interact with TLR3 leading to mDC maturation. This way of activation may be particularly efficient for viral antigen cross-presentation to CD8+ T cells [62, 63], a mechanism critical against virus which can not directly infect antigen-presenting cells.
As suggested by recent studies, DC/NK cell interaction in the periphery can influence the immune response and may condition the type of response induced in lymph nodes. It has been shown that DCs activated by NK cells could secrete higher amounts of IL-12 which promoted Th1 polarization [31]. Several works in mice demonstrated that NK cell depletion led to a severe defect of antiviral CTL generation [39, 56, 64]. The NK cell contribution might probably be the secretion of IFN-γ in the lymph node [39].
PDCs are also implicated in the generation of antiviral CTLs [65]. Virus-activated PDCs may enter lymph nodes [35] where they secrete IFN-α. They might be able to allow the proliferation of lymph node CD56^{bright} NK cells and activate their cytotoxic functions as it has been demonstrated for their blood counterpart [16].

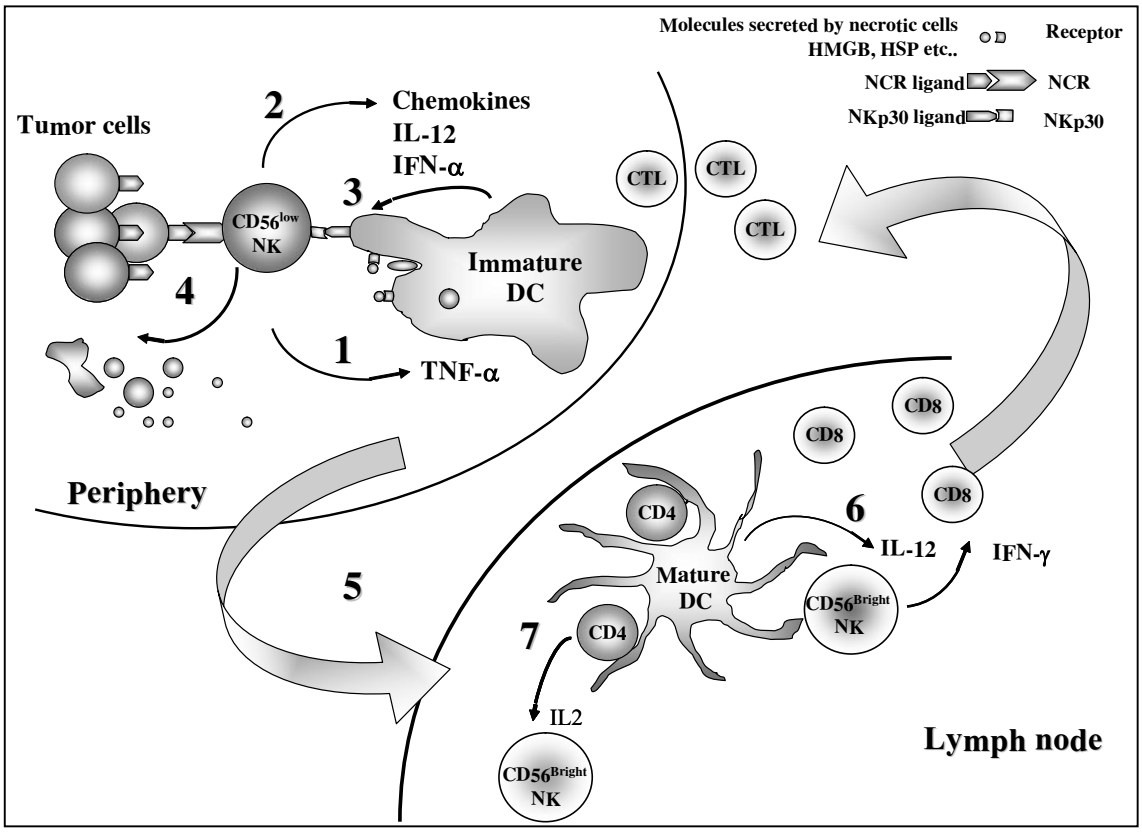

Fig. 1. Possible NK cell/DC cross-talk in cancer immunosurveillance. After recognition of NCR ligands on tumor cells, NK cells may be activated and may secrete TNF-α (1). They also produce chemokines that can recruit other cells of the immune system (2). DCs may be helped to mature by TNF-α released from NK cells, and may produce cytokines like IL-12, or IFN-α, which can induce or strengthen NK cell cytotoxicity (3). On the other way, immature DCs may be eliminated by NK cells after the engagement of NKp30 (3). NK cells are then allowed to lyse tumor cells, providing a source of antigens and danger signals, (like HMGB, uric acid, RNA) for DCs, which phagocyte dead cells and mature (4). Then, DCs express CCR7 and migrate into the lymph node, site of encounter with T cells (5). Mature DCs can secrete IL-12, which may act on CD56^{bright} NK cells inducing the secretion of IFN-γ (6). This cytokine plays a primary role in Th1 response, and promote the development of CTLs, which may migrate into the tumor site. Mature DCs may activate CD4+ T cells to secrete IL-2, which in turn may activate CD56^{bright} NK cells (7). These NK cells might be able to migrate into the periphery to participate to the eradication of tumor cells.

DC-NK Cell Interaction in Cancer Immunosurveillance

In cancer immunosurveillance, NK cells have the capability to recognize transformed cells by sensing MHC class I molecule level which is often lower on cancer cells [66, 67] and by recognizing ligands for their activating receptors expressed by tumor cells (fig. 1). Tumor cells can express MICA and MICB [68] and ULBP [69] which can be recognized by NKG2D, an activator receptor largely expressed on NK cell subsets. Recently, the Necl-2 (Nectin like molecule) involved in cell adherence, has been shown to be a ligand for CRTAM (class I-restricted T cell-associated molecules) expressed on activated NK cells [70]. CD70 expressed on renal carcinoma cells might participate to NK cell activation by interacting with CD27 expressed on NK cells [71]. Activated NK cells enhance their secretion of chemokines to attract mDCs [72] which in turn can increase NK cell cytotoxicity [20]. The microenvironment of the tumor site is not neutral. It contains high amounts of HMGB1 (high mobility group box 1) [73] which has recently been shown to increase NK cell cytotoxicity [74]. In addition to the secretion of TNF-α after NCR engagement [32], NK cells may provoke the release of danger signals by necrotic cells able to induce DC maturation [75]. Few candidate molecules have been identified so far: HSP [76, 77], uric acid [78], or HMGB1 [79, 80]. For their adjuvant activity, the presence of NK cells might be required [81]. Finally, as in viral infection, lysed cells provide a source of antigens to DCs which can take up dead cells and process antigens for cross-presentation to CD8+ T cells [82] in the lymph nodes.

Several in vivo studies support the importance of this cross-talk in tumor growth control and generation of CTLs. In mice, depletion of NK cells leads to a decrease of tumor-infiltrating DCs and absence of CTL generation [83]. The hypothesis of an IFN-γ help is also supported by the work of Adam et al. [84]. Their results suggest that NK cell-secreted IFN-γ can bypass the CD4+ T cell help in the induction of antitumor CTLs. This may be also essential for the generation of a long-term antitumor T cell response [85].

In the lymph node, mature DCs may interact with both T cells and NK cells. Some works suggest a cooperation between these three types of cells, showing a surprising role of CD4+ T cells in the antitumor activity of NK cells. They showed that

mDC injection could induce a protection which correlated with increased NK cell activity and higher infiltration of NK cells, but not CD8+ cells, in lungs of tumor-bearing mice [41]. The depletion of CD4+ T cells abrogates antitumor NK cell activity. A possible explanation might be that NK cells are activated by IL-2 secreted by DC-activated T cells.

Altogether these mouse model studies support the notion that the cross-talk between NK cells and DCs may be crucial in the control of tumor growth.

However, the few studies that have dealt with the presence of NK cells in tumors show that they are generally rare in patients [86]. Nevertheless consistent with this proposed theory, infiltration of NK cells appears to have prognostic value in lung, gastric and colorectal carcinomas, since a relatively higher level of infiltrating NK cells correlates with a better prognosis [87–89]. Likewise, increased infiltrating myeloid DCs have been shown to correlate with a better prognosis [90]. On the contrary, the presence of PDCs seems to be detrimental, at least in breast cancer [91]. However, the co-localization and interaction of NK cells and DCs at tumor sites have not yet been identified, and investigations in this field are highly required to better understand cancer immunobiology.

In conclusion, from al these recent studies the idea is emerging that NK cells are more than mere killer cells. They can detect the presence of transformed cells and help the maturation of DCs, a critical point on which relies the generation of the adaptive immunity. Moreover, they may dramatically direct the polarization of the adaptive response toward Th1 in the lymph node and in association with DCs may favor the generation of CTLs. This dialogue between DCs and NK cells may be further exploited in immunotherapy aimed to boost immune response.

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