Dendritic Cell Cross Talk with Innate and Innate-like Effector Cells in Antitumor Immunity: Implications for DC Vaccination

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ABSTRACT: Dendritic cells (DCs) are key players in the induction of immune responses. Adoptive transfer of autologous mature DCs loaded with tumor-associated antigens is a promising therapy for the treatment of immunogenic tumors. For a long time, its therapeutic activity was thought to depend solely on the induction of tumor-specific CD8+ and CD4+ T cell responses. More recently, DCs were shown to bidirectionally interact with innate and innate-like immune cells, including natural killer (NK), invariant natural killer T (iNKT), and γδ T cells. These effector cells can amplify responses induced by DCs via several mechanisms, including induction of DC maturation and conventional T cell priming. In addition, NK, iNKT, and γδ T cells possess cytolytic activity and can act directly on tumor cells. Therapeutic strategies targeting these innate and innate-like immune cells hence hold potential to improve current DC vaccination protocols.

KEYWORDS: dendritic cells, dendritic cell-based cancer vaccines, natural killer cells, natural killer T cells, γδ T cells

ABBREVIATIONS: APC: antigen-presenting cell; CCL: chemokine (C-C motif) ligand; CCR: chemokine (C-C motif) receptor; CTL: cytotoxic T lymphocyte; CXCL: chemokine (C-X-C motif) ligand; DC: dendritic cell; IFN: interferon; IL: interleukin; iNKT: invariant natural killer T; mDC: myeloid DC; MHC: major histocompatibility complex; moDC: monocyte-derived DC; NK: natural killer; NKT: natural killer T; pDC: plasmacytoid DC; TAA: tumor-associated antigen; TCR: T cell receptor; Th: T helper; TNF: tumor necrosis factor; α-GalCer: α-galactosylceramide

I. INTRODUCTION

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) with the ability to activate and polarize naïve T cells.1 This has made them attractive targets in a vast number of cell-based, antitumor immunotherapeutic approaches. In short, autologous DCs are pulsed ex vivo with tumor-associated antigens (TAAs) and then infused back into the patient, harnessing the patient’s own immune system to eradicate tumor cells. DCs derived ex vivo from either monocytes (moDCs) or CD34+ progenitor cells have been most frequently used as immunotherapy. However, an up and coming strategy is to use naturally occurring DCs that can be isolated from blood. Several DC subsets have been identified in human peripheral blood, generally divided into myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs). Vaccines based on these naturally occurring DCs have shown promising results in melanoma patients,2 as they seem to surpass moDC-based vaccines in prolonging overall survival.

Much has been learned about DC biology and the requirements for inducing antitumor immune responses since the first DC-based vaccination was tested in cancer patients, and the potency of immunotherapy in cancer treatment has recently gained renewed interest.2,3 Tumor cells are typically recognized by CD8+ cytotoxic T lymphocytes (CTLs) and the presence of tumor-specific CD8+...
T cells correlates with improved clinical outcome. Induction of strong CTL responses is mediated by DCs that cross-present antigens on major histocompatibility complex (MHC) class I molecules. Hence, DCs used as immunotherapeutics are usually pulsed with TAAs recognized by CD8+ T cells. To be able to properly activate antigen-specific CD8+ T cells, DCs need to be licensed by interferon (IFN)-γ-producing CD4+ T helper (Th) 1 cells. This was highlighted in a recent study, showing that DCs pulsed with antigens for both CD8+ and CD4+ T cells induce a stronger antitumor response compared to vaccines only targeting CD8+ T cells. Consequently, the CD8+ T cell is not the sole effector cell that is crucial for antitumor responses induced by DC vaccination.

Although advancements are being made in optimizing the critical factors in DC vaccination, objective responses in melanoma patients vaccinated with DCs are still rare. This has been partly explained by the immunosuppressive microenvironment formed in many tumors. The use of check-point blocking antibodies in combination with DC-based immunotherapy is, hence, a promising new strategy to combat the inhibition of T cell effector functions. In addition, NK cells also respond to inflammatory cytokines, such as interleukin (IL)-12, IL-15, IL-18, and type I IFNs. NK cells are crucial in antitumor immune responses, as they are able to kill MHC-Ilow tumor cells that escape lysis by CTLs. In addition, tumor cells expressing normal levels of MHC-I molecules can also be killed by NK cells if they express elevated levels of stress-induced ligands that are recognized by activating receptors on the NK cell. Furthermore, activated NK cells appear to function as weak APCs. They can upregulate MHC class II and co-stimulatory molecules, including CD86, CD80, and OX40L, enabling them to induce CD4+ T cell proliferation and Th1 polarization. Moreover, a mouse study suggested the possibility that NK cells are able to cross-present antigens to CD8+ T cells.

II. NATURAL KILLER CELLS

NK cells play a central role in innate immunity and are able to react quickly to viral infections and tumor development. Activated NK cells secrete high levels of IFN-γ and tumor necrosis factor (TNF)-α, providing stimulation to additional immune cells, and possess cytolytic activity. The killing of target cells is mediated via release of perforin and granzyme or via receptor-mediated cell death. This process is regulated by a range of activating and inhibitory receptors expressed by the NK cells. In addition, NK cells also respond to inflammatory cytokines, such as interleukin (IL)-12, IL-15, IL-18, and type I IFNs. NK cells are crucial in antitumor immune responses, as they are able to kill MHC-Ilow tumor cells that escape lysis by CTLs. In addition, tumor cells expressing normal levels of MHC-I molecules can also be killed by NK cells if they express elevated levels of stress-induced ligands that are recognized by activating receptors on the NK cell. Furthermore, activated NK cells appear to function as weak APCs. They can upregulate MHC class II and co-stimulatory molecules, including CD86, CD80, and OX40L, enabling them to induce CD4+ T cell proliferation and Th1 polarization. Moreover, a mouse study suggested the possibility that NK cells are able to cross-present antigens to CD8+ T cells.

A. DC-INDUCED NK CELL ACTIVATION

DCs and NK cells provide signals that support each other’s activity (Fig. 1). This additional stimulation is essential for generating beneficial effects during DC immunotherapy. Studies using B16 melanoma-bearing mice showed that in vivo depletion of NK cells before DC vaccination abolished the beneficial effect of the vaccination. DCs can activate NK cells through both soluble and contact-dependent factors. A major contact-dependent factor is IL-15 presented in trans, complexed to IL-15Rα on the DC surface. This factor enhances NK cell survival, proliferation, and activation. OX40-OX40L interactions between NK cells and pDCs have been shown to enhance IFN-γ
secretion by NK cells. Other contact-dependent signaling is mediated by engagement of NK receptors, such as NKG2D, NKp30, and NKp46. Recently, a tetraspanin-like membrane glycoprotein named IFN regulatory factor 3-dependent NK-activating molecule, was discovered to mediate cross talk between NK cells and polyinosinic-polycytidylic acid-treated DCs in mice, leading to IFN-γ secretion.

Pro-inflammatory cytokines released by activated DCs have the ability to activate NK cells. IL-12 is mainly produced by mDCs and stimulates NK cells to secrete high levels of IFN-γ. In turn, IFN-γ increases IL-12 secretion by DCs, thus forming a positive feedback loop. IL-18 was reported to prime NK cells to respond more strongly to IL-12. Recently, the role of DC-derived IL-27 in antitumor immune responses was explored. This IL-12 family cytokine has been shown to induce NK cell activation and increase tumor susceptibility to NK-mediated killing. A study by Wei et al. showed that IL-27 from DCs induces chemokine (C-X-C motif) ligand (CXCL) 10 production in myeloid-derived suppressor cells, leading to recruitment and infiltration of NK cells. NK cell-derived IFN-γ in turn boosts this CXCL10 production. Thus, DCs and NK cells can work together to modulate the tumor microenvironment in assisting in tumor rejection.

**FIG. 1:** DC-NK cell cross talk. Mature DCs can activate NK cells through various pro-inflammatory cytokines and contact-dependent factors. In turn, NK cells release IFN-γ that stimulates IL-12 production in DCs. Cross talk with DCs enhances antitumor activities of NK cells via (1) increased IFN-γ secretion, which inhibits tumor growth and activates NK cells and αβ T cells, and (2) enhanced killing of tumor cells, which generates the release of tumor antigens. Other antitumor activities of NK cells are (3) antigen-presentation to CD4+ T cells, and (4) interactions with iDCs, which results in either apoptosis or maturation of those DCs. A-NKR, activating NK receptor; I-NKR, inhibitory NK receptor; iDC, immature DC.
Although mDCs are able to induce NK cell-derived IFN-γ secretion, enhancement of cytolytic activity is strongly dependent on type I IFNs, which are produced in high amounts by activated pDCs. pDCs are furthermore able to recruit NK cells to malignant tissue, and have been shown to induce proliferation of cytolytic NK cells. This suggests that mDCs and pDCs have differential roles in their cross talk with NK cells and act in synergy to modulate NK cell function.

B. NK CELL-INDUCED DC ACTIVATION

Reciprocally, activated NK cells have been shown to act as an endogenous adjuvant and stimulate DC functions via various mechanisms. First, ligation of cell surface receptors and secretion of TNF-α and IFN-γ induces maturation of DCs, which includes upregulation of co-stimulatory molecules and secretion of pro-inflammatory cytokines and chemokines. Secreted chemokines, including CXCL9, CXCL10, CXCL11, and chemokine (C-C motif) ligand (CCL) 5, attract CD8+ T cells and additional NK cells, further enhancing the antitumor immune response. Additionally, activated NK cells themselves secrete CCL3 and CCL4 to further attract immature DCs. Secondly, tumor cell lysis by NK cells also provides DCs with tumor antigens that can be presented to naïve T cells. This NK cell-mediated tumor cell killing will additionally promote cross-priming of tumor-specific CTLs, thus boosting a tumor-specific Th1 response. Lastly, NK cells are able to kill immature DCs in a process called DC editing. This killing mechanism involves engagement of activating receptors Nkp30 and DNAM-1. Mature DCs are protected from NK cell-induced lysis by upregulation of MHC class I complexes, including HLA-E, which is recognized by the CD94/NKG2A inhibitory receptor. By selective killing of immature DCs, NK cells select for the more immunogenic, mature DCs. This is favorable in a cancerous setting, as it prevents immature DCs from inducing immune tolerance. Indeed, NK cell-mediated DC editing was recently shown to promote the induction of antitumor CTL responses in vivo. Whether NK cells induce maturation or killing of immature DCs seems to partially depend on the ratio between both cell types. At low NK:DC ratios, DCs are mostly stimulated, while at high NK:DC ratios, NK cells mainly induce lysis of immature DCs.

C. NK CELLS IN DC VACCINATION

Due to the bidirectional nature of DC-NK cell cross talk, DC vaccination not only leads to short-term NK cell activation, but also promotes increased NK cell responsiveness after termination of the initial immune activation phase. Long-term innate immune activity is likely caused by host DCs and CD4+ T cells that continue the generation of active NK cells after initial induction by vaccine DCs. The interplay between pDCs, mDCs, and NK cells during vaccination with Toll-like receptor-activated pDCs was demonstrated in a study using the murine B16 melanoma model. In this model, adoptively transferred CpG oligodeoxynucleotide-activated pDCs produced large amounts of CCL3, CCL4, and CCL5 chemokines, leading to CCR5-based recruitment of NK cells. Through secretion of type I IFNs and ligation of surface receptors, pDCs activated NK cells, which in turn killed tumor cells. The authors proposed that released TAAs were subsequently captured by host mDCs, that matured through interactions with NK cells and pDCs. Activated, tumor antigen-presenting mDCs would then ultimately cross-prime antigen-specific T cells in tumor draining lymph nodes.

To date, NK cell responses have only been monitored in a few DC vaccination trials. Several of these studies reported a correlation between total NK cell numbers, activated NK cell numbers, or NK cytotoxic activity, with favorable clinical outcome. A study on nine patients with different tumor types even suggested that NK cell responses may correlate more closely with clinical responses than T cell responses. It is therefore desirable to implement the monitoring of NK cell responses in future clinical DC vaccination trials. To harness the beneficial effects of NK cells for DC vaccination, the right choice of DC stimuli is important. mDCs or moDCs both efficiently activate NK cell cytokine production, and should preferen-
DC Cross Talk with Innate and Innate-like Effector Cells

favorably be stimulated to produce maximal amounts of IL-12, whereas pDCs should produce high amounts of IFN-α to enhance the cytolytic effect of NK cells. An additional important consideration is where a DC-based immunotherapeutic should be administered for maximal effect of the DC:NK cell cross talk. Injection of activated and chemokine secreting DCs into the tumor site enables increased recruitment of NK cells that can both lyse tumor cells to provide an additional source of tumor antigens, and edit the repertoire of injected DCs so that only the most appropriately equipped cells migrate to the neighboring lymph nodes. Alternatively, DCs injected directly into tissue lymph node might activate, and get further activated by, local NK cells, which via increased IFN-γ production help the DCs to induce a strong Th1 adaptive immune response. Furthermore, as NK cells can induce DC maturation and Th1 polarization, they might be used to improve DC maturation in vitro in clinical settings. Hence, simultaneous transfer of autologous NK cells and DCs might improve antitumor immune responses by augmenting the chance that DCs and NK cells can interact due to increased NK cell numbers in close proximity of DCs.

III. INVARIANT NATURAL KILLER T CELLS

Natural killer T (NKT) cells are lymphocytes that express both T cell and NK cell lineage markers. This population makes up about 0.1% of human lymphocytes and it serves an important function in recognizing glycolipid antigens presented by APCs on the MHC-I-like CD1d molecule. Two NKT cell subsets are distinguished based on the expression of their T cell receptor (TCR): an invariant subset (iNKT cells, type I NKT cells) and a variant subset (type II NKT cells). iNKT cells represent approximately 80% of total NKT cells in vivo and express a TCR composed of a Vα24Jα18 α-chain and a Vβ11 β-chain. Most studies have focused on the more abundant iNKT cells, leading to their further subdivision into Th1-, Th2-, or Th17-like subsets, depending on chemokine receptor, cytokine, and transcription factor expression.

In contrast to conventional αβ T cells, which only recognize highly specific peptide antigens, iNKT cells respond to a variety of both endogenous and exogenous antigens. The nature of the endogenous glycolipids recognized by iNKT cells is still not well understood and only a few have been identified. Interestingly, iNKT cells have been shown to recognize several bacterial glycolipid antigens. A strong inducer of iNKT cell responses is an antigen originally derived from α-galactosylceramides found in the marine sponge Agelas mauritianus. A synthetic version of α-galactosylceramide (α-GalCer) is predominantly employed for iNKT cell activation, and has been used in the majority of clinical studies targeting iNKT cells.

iNKT cells can be activated via CD1d/glycolipid-TCR engagement and/or signaling via pro-inflammatory cytokines such as Type I IFNs, IL-12, IL-18, IL-23, or IL-25. Depending on iNKT cell subset, microenvironment, and CD1d-ligand, the contribution of either mechanism appears to be variable and signaling via cytokines might be equally, or in some situations, even stronger than TCR engagement. In addition, iNKT cells were shown to induce receptor-mediated killing using activating receptors similar to classical NK cells. Activated iNKT cells possess several antitumor effector functions. Tumor cells expressing CD1d molecules can be lysed directly by iNKT cells through perforin/granzyme-, FasL-, or TNF-related apoptosis-inducing ligand (TRAIL)-based mechanisms. In a similar fashion, iNKT cells have been shown to actively kill tumor-associated macrophages (TAMs), which are known to suppress immune cell function and contribute to tumor regression. Furthermore, recognition of CD1d expression together with CD40-CD40L interactions enables iNKT cells to induce conversion of myeloid-derived suppressor cells and, in the presence of tumor-induced serum amyloid A1, tumor-associated neutrophils to more immunostimulatory phenotypes. A highly important antitumor mechanism of the iNKT cells is their production of large amounts of TNF-α and IFN-γ upon activation, which in turn can activate additional effector cells, such as NK cells and γδ T cells. Taken together, iNKT cells can support both innate and adaptive immunity against tumor cells.
and may reverse immunosuppression in the tumor microenvironment. Care should be taken however, as it recently came to light that in some tumors iNKT cells might acquire immunosuppressive functions and may control DC populations in tumor and draining lymph nodes, as demonstrated in a mouse model of aggressive breast cancer.\textsuperscript{76}

A. DC-iNKT CELL CROSS TALK

Similar to DC cross talk with NK cells, bidirectional interaction between iNKT cells and DCs plays an important role in the iNKT cell-mediated antitumor responses (Fig. 2). There are several immunosuppressive factors within the tumor microenvironment with the

![Diagram of DC-iNKT cell cross talk](image-url)

**FIG. 2:** DC-iNKT cell cross talk. Both mature and immature DCs may activate iNKT cells through secretion of pro-inflammatory cytokines and/or contact-dependent factors, including presentation of glycolipids on CD1d and ligation of various activating NK receptors. In turn, iNKT cells mature DCs mainly through CD40-CD40L interactions, while IFN-γ stimulates IL-12 production in DCs. Cross talk with DCs enhances antitumor activities of iNKT cells via (1) increased secretion of IFN-γ, which inhibits tumor growth and activates NK and αβ T cells, and TNF-α, which can activate γδ T cells, and (2) enhanced direct killing of tumor cells, which generates the release of tumor antigens. Other reported antitumor activities of iNKT cells are (3) direct killing of TAMs, and (4) conversion of MDSCs and TANs to more immunostimulatory phenotypes, in which MDSCs function as APCs, while TANs secrete more IL-12 and less IL-10. A-NKR, activating NK receptor; iDC, immature DC; MDSC, myeloid-derived suppressor cell; TAN, tumor-associated neutrophil.
ability to block DC maturation. As a consequence, immature DCs can take up tumor antigens, but are unable to activate naïve T cells. However, immature DCs presenting glycolipid ligands on CD1d molecules can activate iNKT cells, since in contrast to conventional T cells, iNKT cells do not require stimulation via co-stimulatory molecules for activation, although co-stimulation may still play a role in iNKT cell responses. Engagement of CD40 on DCs by CD40L on iNKT cells in turn promotes IL-12 production in DCs, which reciprocally stimulates the release of TNF-α and IFN-γ from iNKT cells and further enhances the DC maturation process. Among the lymphocyte populations, iNKT cells are the first to respond to infections, and cross talk between CD1d expressing, pro-inflammatory cytokine-producing mature DCs and iNKT cells potentiates the ability of DCs to stimulate naïve T cell responses.

Unlike mDCs, pDCs do not express the CD1d molecule, and hence cannot present glycolipids to iNKT cells. However, pDC-derived cytokines, mainly TNF-α and IFN-α, are important for the activation of NKT cells. In addition, activated pDCs can interact in a contact-dependent manner with iNKT cells via ligation of OX40L with OX40 on the iNKT cell surface. In this way, CpG oligodeoxynucleotide-stimulated pDCs license iNKT cells to respond effectively to iNKT ligand-presenting mDCs. Reversely, OX40-OX40L interactions with iNKT cells stimulate IFN-α production by pDCs. Thus, DC-iNKT cross talk is not restricted to mDCs.

In addition to direct cross-activation, iNKT cells can also indirectly contribute to DC function. Since iNKT cells can kill CD1d-expressing tumor cells, iNKT cell-mediated tumor cell lysis has been shown to provide DCs with tumor antigens and to induce adaptive responses. DC editing can, under certain conditions, also be mediated by iNKT cells, when NK cell-mediated killing is inhibited by high HLA-E expression.

### B. iNKT CELLS IN DC VACCINATION

Several reports have shown that iNKT cells can be activated by a combination of DC-derived cytokines and CD1d-presentation of self-antigens, or by DC-derived cytokines alone. This suggests that iNKT cells can amplify responses induced by mature DCs. Indeed, greater numbers of circulating or tumor-infiltrating NKT cells have been reported to correlate with improved clinical outcome in patients with various types of cancer.

Targeting iNKT cells by injecting α-GalCer in vivo in various murine cancer models has shown promising results, both when used in therapeutic as well as prophylactic settings. However, early clinical trials of direct α-GalCer injection in cancer patients did not show signs of clinical improvement, although the treatments were well tolerated. Mouse studies subsequently showed that the initial activation of iNKT cells by injected α-GalCer is followed by a contraction phase of massive apoptosis, ultimately resulting in long-term iNKT cell anergy. Also, multiple injections of α-GalCer can polarize the adaptive immune response towards a Th2 phenotype, which is unwanted in cancer immunotherapy.

To overcome these shortcomings, researchers explored the employment of ex vivo loaded DCs to stimulate iNKT cells. Experiments using α-GalCer-pulsed DCs in mouse models showed in vivo inhibition of tumor growth, and the induction of long-term iNKT cell responses which, similar to NK cells, seemed to be dependent on continuous interactions with host DCs and CD4+ T cells. Furthermore, α-GalCer-loaded DCs do not induce the strong iNKT cell anergy observed during α-GalCer administration. Strikingly, human in vitro studies could confirm these results, as α-GalCer-pulsed DCs were shown to expand and activate patient-derived iNKT cells, paving the way for patient trials. Several clinical studies using α-GalCer-pulsed DCs have since been performed, confirming the safety and feasibility of this approach. Immunological responses, including iNKT cell proliferation, their accumulation in the tumor microenvironment, and their increased IFN-γ production were detected, with one study reporting a correlation between IFN-γ responses and increased patient survival.

While the use of immature DCs loaded with α-GalCer induced only moderate iNKT cell responses, stronger responses were achieved.
when matured DCs loaded with α-GalCer were administered.\textsuperscript{104} In addition, synergistic action of Toll-like receptor ligands and CD1d-binding glycolipids on DC maturation and iNKT cell activation was reported.\textsuperscript{81,85} Vaccination with ex vivo matured DCs loaded with both TAA and CD1d agonist might therefore be a promising strategy to enhance antitumor immune responses.

A recurring problem with iNKT cell-based therapies is the low number of these cells in humans, especially in cancer patients with decreased frequencies of iNKT cells.\textsuperscript{57,58,89} Accordingly, methods for adoptive transfer of activated iNKT cells expanded in vitro have been developed.\textsuperscript{60,108,109} Clinical trials combining this approach together with the transfer of α-GalCer-pulsed DCs have generated promising results, with several patients showing tumor regression.\textsuperscript{9,110}

**IV. γδ T CELLS**

Like NKT cells, γδ T cells are lymphocytes that share properties with both NK cells and conventional αβ T cells. Although often characterized as part of the adaptive arm of the immune system, γδ T cells do not differentiate to memory cells to the same extent as αβ T cells and they display several innate effector functions. In peripheral blood of healthy individuals, γδ T cells represent up to 5% of the T lymphocytes and can dramatically expand following bacterial infections.\textsuperscript{111} γδ T cells express a TCR composed of a γ- and a δ-subunit in contrast to conventional αβ TCRs. γδ TCRs are less diverse than αβ variants and less stringent in their antigen specificity. Depending on the particular γ- and δ-chains expressed, γδ T cells can be divided into specific subsets.\textsuperscript{111,112} The majority of human peripheral blood γδ T cells express the Vγ9Vδ2 TCR, while other TCR-variants are mainly expressed by their tissue-resident counterparts. The Vγ9Vδ2 T cells recognize phosphoantigens, which are non-peptidic phosphorylated molecules produced in high levels by microorganisms, stressed cells, and tumor cells.\textsuperscript{113–115} Unlike conventional αβ T cells, γδ T cells do not require antigens to be presented on MHC molecules to get activated.\textsuperscript{116} The receptor presenting phosphoantigens is still not characterized, but lipid antigens presented on CD1 molecules are recognized by tissue residing Vδ1-expressing γδ T cells.\textsuperscript{111,112}

γδ T cells display potent antitumor activity. Due to increased metabolic processes, tumor cells accumulate phosphoantigens, such as isopentenyl pyrophosphate, which are recognized by Vγ9Vδ2 T cells.\textsuperscript{117} In addition to isopentenyl pyrophosphate, the Vγ9Vδ2 TCR may also recognize mitochondrial F1-ATPase, which is expressed by some tumor cells.\textsuperscript{118} Also, γδ T cells can recognize opsonized tumor cells and stress-induced molecules such as heat shock proteins and NKG2D ligands expressed on tumor cells.\textsuperscript{119–122} Activated γδ T cells can upon recognition directly lyse tumor cells through release of perforins and granzymes, FasL engagement, and TNF-related apoptosis-inducing ligand (TRAIL)-dependent mechanisms.\textsuperscript{123–125} In addition to their direct antitumor effects, activated γδ T cells can boost the activity of surrounding immune cells by secreting large amounts of TNF-α and IFN-γ.\textsuperscript{31,126,127} Vγ9Vδ2 T cells have also been shown to act as APCs and present antigens to αβ T cells,\textsuperscript{111,112} a characteristic that has led to the proposal of using TAA-presenting γδ T cells as a vaccine in cancer immunotherapy.\textsuperscript{128}

However, certain γδ T cell-derived factors may also promote tumor growth.\textsuperscript{129} Vδ1-expressing γδ T cells are negatively correlated with clinical outcome in breast cancer and they can secrete cytokines that suppress DC maturation and T cell effector function.\textsuperscript{130–132} Vγ4+ γδ T cells are reported as the main source of γδ T cell-derived IL-17.\textsuperscript{130} IL-17-producing γδ T cells have a dual role in tumor immunity. As such, in vivo models showed that they can be tumor-promoting,\textsuperscript{133,134} but may also contribute to antitumor effects in other anticancer treatment modalities.\textsuperscript{135,136} For application in cancer immunotherapy, strategies that focus on activation of Vγ9Vδ2 T cells seem to be the most attractive, although other γδ T cell subsets should not be neglected.\textsuperscript{137}

**A. DC-γδ T CELL CROSS TALK**

Naïve γδ T cells can be activated by both mature and immature DCs (Fig. 3). Cytokines produced by mature DCs, such as IL-1β, IL-12, IL-18, TNF-α,
and type I IFNs, have been reported to mediate γδ T cell stimulation.\textsuperscript{126,138–140} In addition to providing cytokines, DCs can induce contact-dependent γδ T cell activation via CD86–CD28 interactions and by presenting γδ T cell ligands, such as Lipid A and phosphoantigens.\textsuperscript{127,141} Lack of maturation does not appear to negatively affect DC–γδ T cell cross talk,\textsuperscript{127,142} and some studies even conclude that immature DCs are more efficient in activating γδ T cells than their mature counterparts.\textsuperscript{126,143} Reciprocally, activated γδ T cells can mature DCs via contact-dependent mechanisms and secretion of soluble factors. Immature DCs co-cultured with activated γδ T cells show upregulation of MHC and costimulatory molecules such as CD86.\textsuperscript{127,144} Moreover, lipopolysaccharide-induced DC maturation is enhanced by co-culture with γδ T cells.\textsuperscript{145} This γδ T cell-induced DC maturation appears to be mediated by TCR-CD1, Fas-FasL, CD40-CD40L, and CD28-CD86 interactions, as well as TNF-α secretion.\textsuperscript{127,146–148} In addition, γδ T cell-derived IFN-γ induces IL-12 secretion by DCs and both cytokines polarize Th1 responses.\textsuperscript{144,149,150} Finally, γδ T cell-induced tumor cell lysis generates tumor antigens that might be taken up and presented.
by DCs, which in turn would promote tumor antigen-specific adaptive immune responses.41

Interestingly, Vγ9Vδ2 T cells may also stimulate NK cells. This costimulation enhances NK cell killing of tumor cell lines,151 but may also affect DC-NK cell cross talk. Antigen-stimulated Vγ9Vδ2 T cells were shown to enhance the DC killing capacity of NK cells via ICOS/ICOSL interactions.152

B. γδ T CELLS IN DC VACCINATION

Several clinical trials utilizing in vivo expansion or adoptive transfer of γδ T cells have been performed.153 Clinical activation of γδ T cells is commonly achieved with bisphosphonate drugs, such as zoledronate, which can enhance the expression of isopentenyl pyrophosphate and other phosphoantigens.153 Zoledronate is commonly used to prevent osteoporosis, but has also been shown to have antitumor effects, correlated with increased activity of γδ T cells.8,123 However, repeated doses of bisphosphonate drugs may lead to exhaustion and a decrease in γδ T cell numbers.154 As the levels of γδ T cells are often reduced in cancer patients, and to circumvent the risk of exhaustion, adoptive transfer of ex vivo expanded autologous Vγ9Vδ2 T cells has been tested in a number of clinical studies with varying results.153 Several in vitro studies reported on improved functionality of γδ T cells when co-cultured with zoledronate-treated DCs, leading to the expansion of tumor-specific αβ T cells.155,156 In a recent study, autologous DCs treated with zoledronate were shown to efficiently expand functional Vγ9Vδ2 T cells from cancer patients ex vivo, indicating that DCs can be used to restore the impaired γδ T cell responses in patients.157 Furthermore, injection of activated, TAA-pulsed DCs treated with zoledronate induced detectable TAA-specific responses in two out of three acute myeloid leukemia patients, demonstrating the clinical feasibility of targeting γδ T cells in DC vaccination.158 However, mechanistic insight in how DCs stimulate γδ T cells and how this affects the course of the disease is lacking, and further studies are needed before the efficiency of DC-based γδ T cell-targeting can be evaluated.

V. CONCLUDING REMARKS

DCs have the ability to interact with a great variety of both innate and adaptive immune cells and are often referred to as the bridging element between the two arms of the immune system. In addition to presenting antigens to adaptive immune cells, DCs also stimulate and boost local responses at the site of tumorous growth or infection. This is a major advantage over downstream effector cells when targeting the immune system for immunotherapy, and more insight would be constructive for future trial design.

Both DCs and the effector cells discussed above have been tested clinically as cancer treatments, with mixed results. Importantly, the synergistic effects of cross talk between DCs and innate(-like) effector cells have been described both in vitro and in vivo. Ex vivo matured DCs can induce in situ activation of NK, iNKT, and γδ T cells. These effector cells subsequently promote direct antitumor effects and propagate immune responses by inducing further activation of tissue DCs. From these data, we can conclude that the innate arm of the immune system could play a pivotal role in potentiating DC vaccines, resulting in a stronger and more robust antitumor immune response.

Protocols for ex vivo expansion of iNKT cells or γδ T cells using autologous moDCs as APCs have already been developed, and vaccines based on combined or subsequent administration of effector cells and DCs might promote synergy to induce multifaceted responses. Furthermore, cross talk between co-cultured DCs and NK, iNKT, or γδ T cells enhances DC activation. Hence, in a vaccine setting, these effector cells might as well act as endogenous adjuvants during ex vivo stimulation of DCs, which can be beneficial when potent DC activators like Toll-like receptor ligands are lacking. Alternatively, the in vivo activity of innate(-like) lymphocytes could be boosted prior to administration of DC vaccines, or by ex vivo pulsing the DCs with iNKT cell antigens or γδ T cell antigens in addition to TAA. Activation of innate(-like) lymphocytes in the tissue will increase tumor destruction, leading to elevated levels of tumor antigens and increased uptake by, and maturation of, DCs. Taken together,
this would subsequently induce a broader adaptive antitumor response and a greater chance of disease regression.

To conclude, despite strong evidence for the importance of adaptive lymphocytes in tumor control, their presence in cancer patients does not guarantee protection. This is possibly due to immune suppression and various immune escape mechanisms employed by tumor cells. NK, iNKT, and γδ T cells have the ability to recognize many tumors that escaped surveillance by adaptive lymphocytes and their effector functions are often immediately available. Treatment strategies aiming at either adaptive or innate(-like) lymphocytes have, so far, been developed in parallel with mixed results. In this review, we highlighted synergistic interactions between DCs and innate(-like) lymphocytes that could lead to robust immune activation. Vaccines targeting both adaptive and innate(-like), lymphocytes simultaneously could thus lead to superior tumor control by inducing a strong adaptive immune response for long-term tumor control and an effective innate(-like) lymphocyte response for immediate reduction of tumor burden and protection against escaping tumor variants.

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