Decidual-Like NK Cell Polarization: From Cancer Killing to Cancer Nurturing

Adriana Albini¹ and Douglas M. Noonan¹,²

Summary: Natural killer (NK) cells accumulate at the fetal–maternal interface and represent 70% of immune cells in the decidua (dNK) at first-trimester pregnancy; they are immune-tolerant toward the semiallogenic fetus and are “nurturing” and nonkilling NK cells. A subset of NK cells in patients with cancer have features in common with dNK, which include expressing CD56, CD9, CD49a, and CXCR3, being poorly cytotoxic and proangiogenic, and mimicking the decidual nurturing role. In the oncologic patient, several factors can “decidualize” NK cells, turning them into immune-suppressant, growth-promoting proangiogenic cells. Here, we suggest ways to sharpen their blunted blades and intercept and curb their cancer-nurturing attitudes to restore their cytotoxic capabilities.

THE NURTURING NATURE OF TUMOR-INFILTRATING NK CELLS

Reproduction is the highest priority of any living organism, even more than nutrition and individual survival, as it is necessary to maintain the continuity of the species. Biological mechanisms involved in reproduction are therefore very powerful drivers of cell function. The maternal–fetal interface undergoes dynamic changes to allow the semiallogenic fetus to grow and develop in the uterus, despite being recognized as alien by the maternal immune cells.

In contrast to other immune-privileged districts, such as the retina, in the first-trimester decidua about 40% of all cells are immune cells. They are responsible for fetal protection from pathogens (1). However, they establish and maintain tolerance for the embryo in the early decidua, and they direct the placental remodeling of uterine vasculature and trophoblast invasion (1). Group 1 innate lymphoid cells (ILC), which include natural killer (NK) cells and ILC1, contribute to defending the host against cancer and infections. The majority of the immune cells in the decidua belong to the NK family (approximately 70%), and the NK cell in the decidua (dNK) is not a killing but a “nurturing” cell, which produces large amounts of cytokines and is poorly cytotoxic (Fig. 1A; refs. 1, 2).

When a tumor starts growing, it expresses signals that make it appear to our defense-immune cells as if it were a fetal growth (Fig. 1B). Here, we want to discuss the hypothesis that NK cells in tumor patients become polarized toward a decidual “nurturing” phenotype: they are tricked into dedifferentiating to help cancer growth instead of killing cancer cells. NK cells that undergo such phenotypical modification should not be seen as “bad” NK, but rather “confused” about their role. The aptitude of NK cells to convert to a fetus nurturing–like phenotype is a warning for us: NK cell–based therapies must take into account the “vocation” of NK cells toward their role in species preservation.

MAJOR NK TYPES IN PERIPHERAL BLOOD AND DECIDUAL NK CELLS

The CD56dimCD16− NK-cell subset accounts for about 90% to 95% of peripheral blood NK (pNK) cells (3). These cells show high levels of cytolytic granules (containing perforin and granzymes), and they are CD11b−CD27dim. NK cells express numerous inhibitory receptors such as KIRs and the CD94–NKG2A complex, which are apt to inhibit NK activity when lysis is not requested and to sustain a quiescent state. CD56dimCD16+ NK cells deploy their cytotoxic capabilities when they encounter cells expressing high levels of activating ligands and low levels of inhibitory ligands (mostly class I MHC), such as virally infected cells and tumor cells (the “missing self” mechanism), and when mediating antibody-dependent cell cytotoxicity (3). Major NK-activating receptors are NKG2D, NCR, and DNAM1. Another peripheral blood NK-cell subset is CD56brightCD16− (about 5% to 10% of peripheral blood NK), which is CD11b+CD27+. These cells are poorly cytotoxic, and they can produce cytokines, including TNFα, and GM-CSF. The CD56dimCD16+ NK-cell subset is considered mature or “terminally differentiated.” By contrast, the CD56brightCD16− subset is considered immature (3). What is the possible role of a nonkilling killer? The poor cytolytic CD56brightCD16− cells are considered to be endowed with immune-regulatory functions on account of their high capacity for secreting cytokines.

dNK cells can be considered a third type of NK; they show a CD56brightCD16− KIR− phenotype and are characterized by the two cell markers CD9 and CD49a (Fig. 1A). CD9 is a member of the tetraspanin family, which is associated with various integrin adhesion receptors, regulating cell migration and invasion. CD9 is upregulated by TGFβ (4, 5). CD9 is also present in exosomes. CD49a constitutes the α-subunit of

¹Scientific and Technology Pole, IRCCS MultiMedica, Milan, Italy. ²Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy.

Corresponding Author: Adriana Albini, IRCCS MultiMedica, Polo Scientifico Tecnologico, Viale Fantoli 16/15, 20138 Milan, Italy. Phone: 39-348 2308475; Fax: 39-0255406570; E-mail: albini.adriana@gmail.com

Cancer Discov 2021;11:28–33doi: 10.1158/2159-8290.CD-20-0796

©2020 American Association for Cancer Research.
Figure 1. Nurturing NK cells and cytotoxic ones: a double-edged sword in pregnancy and antitumor action. NK cells in various states: A, CD56brightCD16−, CD9+, CD49a+ dNK cells nurturing in the reproductive system, helping embryo implant and fetal development. dNK cells produce proangiogenic factors (including VEGF, CXCL8, angiogenin, and galectin-1), MMP9, and glycodelin. B, CD56brightCD16−, CD9+, and CD49a+ dNK-like cells, displaying protumor activities, nurturing in cancer. dNK-like cells produce proangiogenic factors (including VEGF, CXCL8, and angiogenin), MMP9, and TIMP1. C, CD56dimCD16+ cytotoxic dNK cell activation responsible for miscarriage and pregnancy loss. Cytotoxic dNK had increased perforin, granzyme B, and IFNγ expression. D, CD56dimCD16+ cytotoxic activities active in antitumor behavior. Cytotoxic NK cells release perforin and granzyme B and destroy the cancer cells. In bold are the factors produced by the NK cells. The figure images were adapted and modified from Servier Medical Art (Creative Commons license).
the αβ1 integrin receptor (VLA1), which binds collagen IV enriched in the basement membrane. Single-cell sequencing of the human early maternal–fetal interface identified three types of dNK cells (dNK1, dNK2, and dNK3), all expressing CD9 and CD49a (1).

dNK cells produce large amounts of proangiogenic cytokines in vitro, including VEGF, CXCL8, and angiogenin (2, 5), and large amounts of IFNγ. They are critical for decidual vascularization and spiral artery formation (Fig. 1A). They are proangiogenic—the addition of dNK cells to tumor cell xenografts has been seen to significantly enhance tumor growth through angiogenesis (2). dNK cells secrete the matrix metalloproteinases MMP9 and MMP2, which are important for the breakdown of extracellular matrix required for vascular remodeling and trophoblast invasion (6, 7). It is believed that dNK cells are also critical in maintaining immune balance, and that they act as an immune suppressor (1). Various data suggest that dNK cells serve as pivotal sentinel cells that control local inflammation and maintain tolerance at the maternal–fetal interface (1). dNK cells that lose the killer phenotype provide a microenvironment compatible with the embryo (Fig. 1A), which supports a healthy pregnancy (1, 2). CD56brightCD25+ decidual NK cells are reported to traffic toward the maternal–fetal interface in early stages of pregnancy, and their migration is mediated by CXCR4 and CXCR3, the chemokine receptors for the ligands CXCL12 and CXCL10, respectively (1).

TISSUE-RESIDENT NK CELLS IN NON-UTERINE TISSUES AND WOUND HEALING

Plasticity and heterogeneity of NK cells add additional complexity to their functions (3). CD56highCD16−/low NK cells in tissue are different from the CD56highCD16−/low NK cells in the peripheral blood, as reviewed in ref. 3. High expression of CD49a has been described not only in the uterus, but in other tissue-resident NK-cell populations with proinflammatory capabilities as well (3). Human liver-resident NK cells have been previously shown to carry a distinct phenotype compared with peripheral blood NK cells. CD49a expression has been reported to define liver-resident NK cells in mouse models as well as in human liver samples. ILC1 and NK cells have been suggested to regulate liver fibrosis during chronic hepatotropic infections and chronic inflammatory processes (3). As other examples of resident NK, during tissue repair, NK cells are able to stimulate angiogenesis in the myocardium and the cornea (reviewed in ref. 5).

dNK CELL REGULATION AND DYSREGULATION

TGFβ is a multifunctional cytokine belonging to the TGF superfamily. The TGFβ superfamily includes endogenous growth-inhibiting proteins; however, in cancer its immunosuppressive functions dominate, contributing to oncogenesis. The decidual microenvironment is rich in TGFβ, which has been shown to convert pNK cells into a decidual-like phenotype in vitro (reviewed in ref. 5). In cell culture studies, TGFβ-treated pNK cells express CD9, CD49a, and CD103, together with increased levels of CXCR3 and CXCR4 receptors; they produce VEGF and they stimulate trophoblast invasion (5). TGFβ reduces the cytotoxicity of NK cells. In vitro, human pNK cells cultured under hypoxic conditions, with a combination of TGFβ1 and the demethylating agent 5-aza-2′-deoxycytidine, display reduced cytotoxicity and enhanced secretion of VEGFA, and acquire capacity to promote trophoblast invasion (4, 5). CD56brightCD16−/low cells from these cultures express KIR, CD9, and CD49a, together with chemokine receptors and CD151, CD62L, and CD94, in a configuration that is unique to dNK cells. NK cells in a hypoxic environment, a situation occurring in pregnancy, upregulate VEGFA, CXCL8, CXCR4, and CXCR3 (8). dNK cell-inducing stimuli are necessary to maintain a high percentage of CD56brightCD16−/low KIR+ cells. Interestingly, and importantly, this pattern of phenotype is “reversible,” and the reversion of polarization is similar to the one observed when dNK cells are removed from the decidual microenvironment and cultured for a week in the presence of 21% oxygen. IL18 enhances the TGFβ effect on the pNK cells. TGFβ/IL15/IL18 cytokines are also able to shape the phenotype of pNK cells and shift them toward a dNK cell-specific phenotype (1). These major changes are not due to cell proliferation, IL2 treatment, or cell death.

Glycodelin-A is a glycoprotein belonging to the lipothelin family with four isoforms. Glycodelin-A is expressed in large amounts in the decidua and amniotic fluid, and is involved in the maintenance of normal human reproductive activities (9). Glycodelin-A inhibits proliferation of T cells and induces a Th2-type shift in cytokine profile (1, 9). Glycodelin-A interacts with L-selectin to activate ERK signaling cascade for the production of IL6 in monocytes, and it induces a tolerogenic phenotype in dendritic cells and macrophages (1, 9). Glycodelin-A is a modulator of NK phenotype. It can convert CD56highCD16−/low NK cells into dNK-like cells (9), which regulate endothelial cell angiogenesis, via VEGF, and trophoblast invasion. The desialylation of Glycodelin-A, and an anti-L-selectin blocking antibody, inhibits the biological effect of the CD56highCD16−/low NK cells, converting them to dNK-like cells. The dNK cells express also galectin-1 (9). Galectin-1 confers immune privilege in the decidua and is proangiogenic (9).

Dysregulation in cytotoxic and regulatory NK-cell balance is involved in recurrent miscarriage and preeclampsia (Fig. 1C). dNK cells treated with a CD49a-neutralizing antibody increase perforin, granzyme B, and IFNγ expression levels and killing activity, whereas the migration and adhesion of dNK cells are downregulated. dNK cells from women who underwent recurrent spontaneous abortions had lower levels of CD49a and increased perforin, granzyme B, and IFNγ expression when compared with dNK cells from a control group of age-matched healthy women. At the time of miscarriage, women with recurrent spontaneous abortions have an extremely active immune system and an increased number of NK cytotoxic cells in both blood and decidua (Fig. 1C). pNK cells reflect dNK cell changes during miscarriage and may be a useful noninvasive predicting tool in the reproductive failure setting.
HOW CD56bright NK CELLS BECOME POLARIZED TO dNK-LIKE PHENOTYPE IN CANCER

Preclinical and clinical studies have shown that a variety of tumors may be susceptible to immune attack by NK cells (Fig. 1D). NK cells have a broad role in protecting against tumor growth and metastasis in many types of cancer, and they have distinct advantages over T cells. They are therefore candidates for therapeutic manipulation. However, tumors have strategies that allow them to evade NK cell–mediated cytotoxicity. These include disruption of receptor–ligand interactions between NK cells and tumor cells and polarization of NK cells to “nurturing” phenotype (Fig. 1B).

Several reports suggest that CD56$^{bright}$CD16$^{-}$low NK cells infiltrating tumors show a decidual-like phenotype and express CD9. They could be “good-willed” NK cells that are recruited or induced by cancer cells to resume the program necessary for fetal growth. In cancer, they deploy a program that is typically present during embryo development. Accumulation of poorly cytotoxic and proangiogenic NK cells in the tumor might occur through one or more of the following potential mechanisms: (i) tumor microenvironment (TME) reprogramming of the NK cells, (ii) migration of poorly cytotoxic peripheral CD56$^{bright}$CD16$^{-}$CD16$^{low}$ NK cells in the TME and decidualization of the NK cells, (iii) better proliferation and survival of poorly cytotoxic proangiogenic NK cells in the TME, and (iv) repression of cytotoxic NK cells in the patient with cancer.

TGFβ, which is present in the decidua and promotes the dNK phenotype, is also a common component of the TME. A number of papers have shown that treatment of cytolytic CD56$^{dim}$CD16$^{+}$ pNK cells with TGFβ polarizes them toward an immune-suppressed, noncytotoxic, proangiogenic CD56$^{bright}$CD16$^{-}$ phenotype (5, 6, 10). TGFβ converted NK cells into a different ILC1 subpopulation that was unable to control local tumor growth and metastasis (4, 11), whereas deletion of SMAD4 in the NK cells made them acquire an ILC1-like gene signature which was not able to control tumor metastasis. SMAD4 restrained noncanonical TGFβ signaling mediated by the cytokine receptor TGFβR1 on NK cells (4).

Besides TGFβ (reviewed in refs. 4, 6), hyposia and several agents have been found to have the ability to convert peripheral blood cytotoxic CD56$^{dim}$CD16$^{+}$ NK cells into CD56$^{bright}$CD16$^{-}$low NK cells that are characterized by decreased levels of granzyme B and low cytotoxicity, and express high levels of VEGF and cytokines (4–6). Among them, glycodelin-A, described above as important in dNK regulation, is present in various malignancies, such as endometrial, ovarian, breast, lung, and colon cancers (9), and is also able to polarize NK cells to a dNK-like phenotype (9). Galectin-1, expressed by dNK cells, is also overexpressed in melanoma, ovarian cancer, breast cancer, glioma, and myeloma cells (9). Galectin 1 cells have high levels of galectin-1, which suppress NK immune surveillance. Galectin cells which are deficient for galectin-1 showed reduced tumor growth in vivo, increased intratumor NK-cell infiltration, and elevated expression of granzyme B in NK cells. Targeted disruption of galectin-1–N-glycan interactions or galectin-1–specific neutralizing mAb eliminated hypoxia-driven angiogenesis and suppressed tumorigenesis in vivo.

Although the presence of enriched CD56$^{bright}$CD16$^{-}$low cells in tumor tissue is widely investigated, less attention has been paid to the expression and role of decidual markers CD9 and CD49a in tumor-associated NK cells (5, 6). There is increasing evidence that CD56$^{bright}$CD16$^{-}$low NK cells that reside in normal tissue differ from those found infiltrating tumors, some of which are more decidual-like. Historically, CD9 was considered a specific marker for dNK alone, but numerous reports find it is also expressed in tumor-infiltrating NK cells (TI-NK; refs. 5, 6), confirming the hypothesis that mechanisms similar to those involved in embryo implantation in the decidua are occurring in cancer.

Numerous reports on TI-NKs demonstrate that they have compromised cytotoxicity (5–7, 9, 10). Many of the tumors examined revealed the presence of CD56$^{bright}$CD16$^{-}$low NK cells; expression of CD9 has been reported in TI-NK of lung and colorectal cancers, and in melanoma, breast cancer, and glioblastoma (5, 6, 10, 12, 13). CXCR3, another dNK marker, is expressed in melanoma, breast cancer, and glioblastoma (5, 6, 10, 13). CXCR4 is expressed in lung and colorectal cancers (5, 7).

In certain tumors where circulating NK cells in the peripheral blood of patients with cancer (defined as “tumor associated” NK cells, or TA-NKs) have also been investigated, CD9 is present on the cell surface of a subset of circulating CD56$^{bright}$CD16$^{-}$low NK cells; dNK-like cells are present in pNK of melanoma and lung and colorectal cancers (5, 6).

NK CELLS USED AS THERAPEUTIC TOOLS COULD BECOME DECIDUAL-LIKE NURTING NK CELLS

NK-based adoptive immunotherapy has proved successful in hematologic malignancies. Immune checkpoint targeting in the context of NK inhibition has recently attracted attention for the same purpose. Besides the classic inhibitors on NK cells (KIRs, LIRs, NKG2A), there are immune checkpoints on NK cells in tumors, including PD-1, TIGIT, TIM3, LAG3, CD96, and IL18R (14). Because PD-1 is expressed not only by tumor-associated T cells but also by NK cells, blocking PD-1 may activate NK cells as well, thereby playing a crucial effector role against HLA class I–deficient tumors, which are undetectable by T cells. Recent studies have proposed that inhibition of the PD-L1/PD-1 axis can serve an important role in NK cell–induced cytotoxicity in cancer via granzyme B and perforin secretion. A combination of an anti–PD-1 or anti–PD-L1 blocker and an NK cell–specific checkpoint inhibitor, such as an anti-KIR or anti-NKG2A inhibitor, TIGIT, or TIM3, has been proposed to be of value for combined checkpoint inhibition–based immunotherapy (14).

NK-based adoptive cell therapy uses NK cells engineered with CAR-targeting tumor antigens (CAR-NK). NK cell–based therapies may also include strategies to favor migration of NK cells through tissue and tumor parenchyma. However, in the TME, and even in the peripheral blood of patients with cancer, several factors can depotentiate NK cells, turning them into cells that are immune-suppressant and proangiogenic. These dNK-like cells could be foes and not friends. It is thus not unlikely that NK cells that may be administered or recruited to fight the tumor could be hijacked to work in favor of the tumor by host signals present in the tumor.
cells, which are functional in embryo implantation but are cytotoxic, pro-proliferative, and proangiogenic CD9-positive NK cells, in glioblastoma and hepatocellular cancer (HCC), NK for tumors, and experimental studies have indeed shown remove a major obstacle to effective NK-cell immunotherapy. It is possible to hypothesize that the mechanisms that govern recruitment and enrichment of CD56^brightCD16^low NK cells in the decidua operate in a similar way in NK cells of patients with cancer. As mentioned above, these polarized NK cells are not restricted to those infiltrating the tumor, but are also found to be enriched in peripheral blood of pregnant women as well as patients with several types of cancer, where they could represent a valuable independent marker. For instance, CD56^bright NK cells with an immature phenotype CD27^− were found to be highly enriched in patients with advanced breast cancer as compared with women with benign or localized tumors. The frequency of these cells found in pNK reflected the phenotype of those infiltrating the mammary tumors. Imbalance toward a CD16^− subset, less cytotoxic and more immunoregulatory, has been speculated to mirror an immunosuppressive milieu in patients with advanced malignancies. A subset of TA-NK among these circulating NK cells has been found to express the decidual marker CD9 and, in fewer studies, also CD49a.

These polarized NK cells may therefore have switched to “decidual-like” properties, derived from a program whose normal function is to allow an embryo to grow. Decidual polarization of these cells in patients with cancer may be a case of “misusing” the properties that are characteristic of “good” fetal NK cells, but are usually deployed in the decidual setting, to turn them into possible antagonists of NK cell–based therapy.

INTERCEPTING THE “DECIDUALIZATION” OF NK CELLS

In a process resembling what takes place in the decidua, which involves activation and release of TGFβ and other factors, tumors may polarize NK cells toward CD56^bright poorly cytotoxic, pro-proliferative, and proangiogenic CD9-positive NK cells that are functional in embryo implantation but are dysfunctional in cancer.

Inhibition of the TGFβ axis could therefore be expected to remove a major obstacle to effective NK-cell immunotherapy for tumors, and experimental studies have indeed shown that, in glioblastoma and hepatocellular cancer (HCC), NK lytic activity can be restored by blocking the TGFβ pathway (4, 13). Galunisertib (LY2157299), which is an inhibitor of the TGFβ pathway, restores NK activity (4, 13) and could be combined with PD-1 inhibitors or CAR-NK.

NK activation of STAT3 has been described in patients with colorectal carcinoma (7) and HCC. The deletion of STAT3 in NK cells enhances tumor surveillance in in vivo models of hematologic diseases (6). Blocking STAT3 in HCC cells causes an enhancement of NK-cell cytolytic activity through NKG2D ligand upregulation, and facilitates recognition of HCC cells by NK cells. IL10 production in NK cells is dependent on STAT3. These results demonstrate the importance of STAT3 in the behavior of NK cells.

NK cells cannot survive in the absence of STAT5, but their survival can be rescued by overexpression of BCL2 (15). The rescued STAT5-deficient NK cells in transgenic mice promote tumor formation by producing enhanced levels of the angiogenic factor VEGFA (15). An inverse correlation between VEGFA and STAT5B, but not STAT5A, was detected in mouse and human NK cells (15). Gotthardt and colleagues in a Cancer Discovery article (15) found that activation of STAT5B upregulates VEGF protein and enhanced tumor-promoting NK-cell activity in mice. The authors used ruxolitinib on human healthy donor NK cells. Ruxolitinib is a Janus kinase inhibitor with selectivity for subtypes JAK1 and JAK2. Treatment of healthy NK cells with ruxolitinib enhances VEGF production. We studied polarized pNK cells isolated from peripheral blood of patients with cancer (7). In the pNK of patients with colorectal cancer, which is an altered context, STAT5 and STAT3 appear activated. Pimozide is an antipsychotic drug which has been shown to have antitumor activity (for instance against HCC and prostate cancer cells) by suppressing STAT activity. On tumor-associated poorly cytotoxic NK cells, pimozide downregulated STAT5 levels to a significant extent, and it inhibited STAT3 to a lesser extent (7). Angiogenin, which was first described in decidual NK cells, and VEGFA were upregulated in NK cells in patients with colorectal carcinoma, and treatment of pimozide reverted this phenotype in tumor NK cells. The TIMP1, TIMP2, and MMP9 axis was also found to be upregulated in tumor-associated circulating pNK cells, but this was not regulated by pimozide (7).

FUTURE DIRECTIONS

It is of pivotal importance to develop strategies for reprogramming the altered phenotype of dNK-like cells in patients with cancer, to give them back their power to kill. Recent studies have proposed that inhibition of the PD-L1/PD-1 axis can serve an important role in NK cell–induced cytotoxicity in cancer via granzyme B and perforin secretion. Blocking the inhibitory NKG2A receptor is another possibility, to augment tumor immunity by promoting NK-cell effector functions (14). There are other immune checkpoints to be pursued individually and in combination. IL15 can rescue or counteract tumor-induced downregulation of NK cytotoxicity, so an additional strategy being explored is IL15 treatment together with NK cell–based therapy.

Fine tuning and enhancing the NK-based antitumor response is strategic to NK cell–based anticancer therapies. In the case of TGFβ-mediated polarization, targeting TGFβ pathways or intercepting the intracellular pathways leading to NK cell conversion can offer new therapeutic opportunities. NKG2A, glycodelin, and galectin-1 are other important targets. Targeting STAT5, STAT3, and JAK remains controversial and should be further evaluated.

IN CONCLUSION

We should find ways to prevent “decidualization” of infused therapeutic NK cells or tissue-resident stimulated NK, not only to avoid the blunting of their weapons by the polarizing microenvironment, but also to curb their tendency to nourish cancer growth.
Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
We thank Anna Wagstaff (Cancer World, SPCC) for suggestions on the manuscript. This work has been supported by Italian Ministry of University and Research PRIN 2017 grant 2017NTK4HY and the Italian Ministry of Health Ricerca Corrente – IRCCS MultiMedica.

Published first December 4, 2020.

REFERENCES