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Review article

The role of cytokines in the regulation of NK cells in the tumor environment

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ABSTRACT

Natural killer (NK) cells are innate lymphoid cells that are important effectors in the first line of defense toward transformed cells. This is mediated both by direct cytotoxic mechanisms and by production of immunoregulatory cytokines. Recent evidence has shown that NK cells also display memory, similar to the cells of the adaptive immune system. Cytokines are pivotal for the maturation, activation and survival of NK cells. Interleukins (IL)-2, IL-12, IL-15, IL-18, IL-21 and type I interferons positively regulate NK cell function, either independently or in cooperation, whereas other cytokines, such as IL-23 and IL-27, may enhance or suppress NK cell function depending on the context. In the tumor microenvironment, TGFβ, IL-10 and IL-6 suppress NK cell activity not only directly, but also indirectly, by affecting immunosuppressive cells and by antagonizing the effect of stimulatory cytokines, thereby dampening the antitumor response of NK cells and promoting subsequent tumor evasion and progression. Increased understanding of the NK cell response to cytokines has provided a better understanding of their impaired function in tumors which may aid in the development of novel immunotherapeutic strategies to enhance NK cell responses in cancer patients.

1. Introduction

Natural killer (NK) cells have an unique ability to directly lyse transformed, virus-infected or stressed cells without prior sensitization or major histocompatibility complex (MHC) class I restriction [1,2]. They were initially defined and shown to be important effectors of the innate immune system and are included in the recently redefined family of innate lymphoid cells (ILC) as type 1 ILC [3]. Upon activation by target cells, NK cells may also produce large amounts of IFNγ. All NK cells express both activating and inhibitory receptors and the balance between the signals triggered by these receptors dictates whether NK cells will become activated and display effector functions [4].

NK cells are characterized by a $CD3^{-}CD56^{+}CD16^{+}$ and NKp46 natural cytotoxicity receptor (NCR)-positive phenotype and comprise 5–20% of peripheral blood lymphocytes in normal individuals. Morphologically, resting human NK cells have been identified as large granular lymphocytes (LGL) distinct from T and B cells [5]. According to the density of expression of the CD56 receptor, NK cells are divided into two subsets that differ in terms of phenotype, effector function and tissue localization. The $CD56^{dim}$ subset is cytotoxic and represents a majority of peripheral blood NK cells, expresses high levels of CD16, mediates antibody-dependent cell-mediated cytotoxicity (ADCC) [6] and is characterized by HLA class I specific inhibitory receptors, KIRs, that prevent killing of autologous normal cells. In contrast, a minor immunoregulatory $CD56^{bright}$ NK cell subset ($CD16^{-/low}KIR^{-/low}$, perforin low) subset expresses distinct cytokine and chemokine receptors and is poorly cytotoxic. However, these cells are major producers of cytokines in response to IL-12, IL-18 or IL-15 and can therefore regulate the adaptive immune response [7]. Until recently, NK cells have been considered as innate immune cells that lack immunogenic specificity in terms of clonal antigen receptors or memory of prior activation, which are considered as the main attributes of the adaptive immune system. However, increasing evidence indicates that NK cells do exhibit memory that results from prestimulation with different cytokines including IL-12, IL-18 and IL-15 which is manifested by enhanced functional capacities after restimulation [8].

The differential expression of various cytokine receptors during NK cell development implies that different cytokines are relevant for transition from one development stage to the next. Human NK cells develop from hematological stem cells (HSCs) in the bone marrow and

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complete their differentiation and maturation in peripheral organs, especially lymphoid tissues [9]. The proliferation and differentiation of HCSs requires fms-like tyrosine kinase 3 ligand (FL), kit ligand (KL), IL-3, and IL-7, which interact with their respective receptors. There are five grading stages in NK cell development [10]. "Stage I" or pro-NK cells characterized as CD34+ and c-Kit cytokine receptor positive acquire IL-2/15Rβ cytokine receptor and are able to give rise to IL-15 responsive, "stage II" pre-NK cells. These pre-NK cells differentiate to "stage III" immature NK cells (iNK) that represent committed NK cell lineage. iNK cells subsequently differentiate to "stage IV" NK cells, also defined as $CD56^{bright}$ NK cells. Finally, $CD56^{bright}$ NK cells differentiate into "stage V" mature cytotoxic $CD56^{dim}$ NK cells [10–12].

Complete NK cell development and maturation occurs in peripheral organs, especially lymphoid tissues. During this process, NK cells are rendered tolerant and licensed for functional activation via interaction of NK cell inhibitory receptors with self MHC class I molecules [13]. However, for additional full activation, NK cells need to undergo "priming" by different cytokines, one of the most important being IL-15 that is trans-presented by dendritic cells (DC)s [14].

NK cells are not only found in peripheral blood but also populate different organs as well as most peripheral tissues under steady-state conditions. NK cells enter the tumor site by extravasation through the tumor vasculature. The major chemokine receptor involved in NK cell migration toward the tumor is CXCR3 that binds to the tumor-derived chemokine (C-X-C motif) ligands CXCL9, 10, and 11 [15,16]. In melanoma, increased CXCL10 expression results in increased infiltration of adoptively transferred CXCR3-positive expanded NK cells, reflecting the role of CXCL10-induced chemoattraction [16]. However, tumor infiltrating NK cells often display a suppressed function and phenotype. Accumulating evidence indicates that tumor cells, various immunosuppressive cells as well as residing cells produce microenvironmental factors such as cytokines and other immunosuppressive mediators that negatively affect NK cell function (Fig. 1). Immunosuppressive cytokines, transforming growth factor beta (TGFβ), interleukin (IL)-10 and IL-6 inhibit NK cells directly as well as indirectly by affecting antigen presenting cells (APC) or regulatory T cells (Treg)s and myeloid-derived suppressor cells (MDSC)s to produce additional immunosuppressive factors [17]. Attenuated NK cell function in the tumor microenvironment may be restored by stimulatory cytokines such as IL-2, IL-15, IL-18, IL-21 and interferon (IFN)α. Although these cytokines can act independently, owing to some redundancy in their effects on NK cell activity, some of them, like IL-18 and IL-12, may also exert cooperative effects [18]. Interestingly, other cytokines like IL-23 and IL-27 may enhance or suppress NK cell function depending on the context, in particular the presence of a protective acute or aberrant chronic inflammation in the tumor microenvironment (Table 1) [19].

Understanding the primary roles and modes of action of each cytokine in regulating the different aspects of the antitumor immune-response is critical for the development of novel immunotherapeutic strategies that should include activation or expansion of NK cells.

2. Cytokines and NK cells

2.1. γ_c cytokine family

Cytokines IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 that share the γ-subunit as co-receptor, all play a central role in the constitution of NK cell subsets. Each of these cytokines binds to a specific high affinity receptor complex formed by a cytokine-specific α and common γ_c chains. Different from other members of this family, IL-2 and IL-15 can bind with high affinity to heterotrimeric receptor complexes which consist of IL-2 Rα or IL-15 Rα, respectively, and IL-2 Rβ and γ_c chains. The γ_c chain is an essential component of the receptors for these cytokines and it associates with the Janus tyrosine kinase (Jak3) which is required for signal transduction. Jak3 phosphorylates different downstream signal transducer and activator of transcription (STAT) molecules, depending on the type of the receptor complex involved [20].

2.2. IL-2 and IL-15

IL-2 and IL-15 are the most studied cytokine activators of NK cells and have a number of positive functional effects that enhance the antitumor response. IL-2, the first identified member of γ_c cytokine family, was initially defined as a T cell growth factor [21]. It is predominantly produced by activated $CD4^+$ T cells under normal biological conditions and it is characterized by its ability to induce expansion of $CD4^+$ and $CD8^+$ T cells, promote growth and differentiation of activated B cells and potentiate the cytotoxic activity of NK cells. IL-2 has also been reported to promote functional maturation of $CD56^{bright}$ NK cells from lymph nodes thereby inducing the expression of KIR, CD16, NCR and perforin [22–24]. However, more recent findings show that IL-2 also expands and activates immunosuppressive Tregs which constitutively

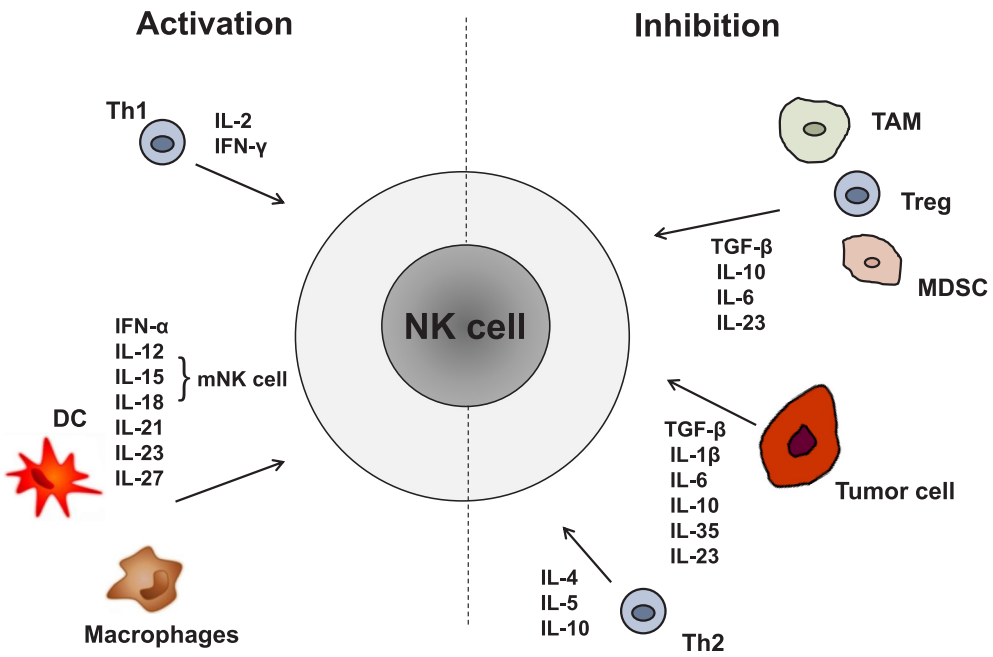

Fig. 1. Cytokine-mediated regulation of NK cell function in tumor microenvironment. Cytokines IL-2, IL-12, IL-15, IL-18, IL-21 and type I interferons, produced mainly by Th1 cells, DCs and macrophages, are positive regulators of NK cell function. This effect is antagonized by the immunosuppressive cytokines TGFβ, IL-10, IL-6 and IL-1β, as well as by IL-23, IL-35, IL-4 and IL-5, that suppress NK cell function and contribute to tumor evasion and progression. Memory NK cells result from cytokine prestimulation and manifest enhanced functional capacity after restimulation. Th1- Type 1 T helper cells; DC-Dendritic cells; TAM-Tumor associated macrophages; Treg-regulatory T cells; MDSC-Myeloid-derived suppressor cells; Th2- Type 2 T helper cells; m NK cells-memory NK cells.

Table 1
NK cell responses to cytokines.

Cytokine	Cytotoxicity	Cytokine production	Proliferation	Survival	Receptor expression
IL-2	↑↑↑	↑	↑↑	↑	↑ NKG2D, NCR, DNAM-1, KIR
IL-15	↑↑↑	↑	↑↑	↑	↑ NKG2D, NCR, DNAM-1, KIR
IL-21	↑	∅	↑	∅	↑ CD16, NCR, KIR; ↓ NKG2D
IL-12	↑↑	↑↑	↑	↑	↑ NKG2D, NKp46; ↓ CD16; = KIR
IL-23	↑/↓	↓	∅	∅	∅
IL-27	↑	↑	∅	∅	∅
IL-35	↓	↓	∅	∅	∅
IL-18	↑	↑	↑	↑	= NKG2D, CD161, KIR
IFN-α/β	↑↑	↑	↑	↑	↑ CD161, NKG2D
IFN-γ	↑	∅	∅	∅	∅
TGF-β	↓↓	↓↓	↓	↓	↓ NKG2D, NCR
IL-10	↑/↓	↑/↓	∅	∅	∅
IL-6	↑/↓	↑	∅	∅	∅

=, no change; ↑, increases; ↓, decreases; ∅, no data

express high affinity IL-2 receptors making them able to compete with NK cells for IL-2 [25].

IL-2 may display its activity through two types of receptor complexes, the high affinity trimeric IL-2 Rα, IL-2 Rβ and γ_c complex and an intermediate-affinity dimeric receptor formed by IL-2 Rβ and a γ_c chain. The intermediate-affinity IL-2 receptor is constitutively expressed on all NK cells and on minor subsets of T cells. In comparison, IL-2 Rα (CD25) is only expressed by $CD56^{bright}$ NK cells and activated T cells thereby enabling formation of the high affinity trimeric receptor that responds to low picomolar, concentrations of IL-2 [26]. The high affinity receptor can be induced on both $CD56^{bright}$ and $CD56^{dim}$ NK cells following combined cytokine activation with IL-12, IL-15 and IL-18 and, to a much lesser extent, following IL-2 or IL-15 activation. These cytokine pretreatments contribute to the newly identified NK cell memory-like response [27]. IL-2 Rβ and γ_c activate Jak1/3 that phosphorylates the STAT3/5-PI3K pathway, the MAPK pathway and ultimately NF-κB [28]. IL-2 is central for NK cell homeostasis as it induces proliferation, cytokine production and enhances the cytotoxic effector mechanisms. IL-2 also promotes survival of NK cells by inhibiting apoptosis through the induction of Bcl-2 expression [29]. However, previous reports have shown that $IL\text{-}2^{-/-}$ and $IL\text{-}2R\alpha^{-/-}$ mice have normal numbers of mature NK cells in peripheral blood, suggesting that IL-2 is not required for NK cell development [30], although IL-2 promotes NK cell activation.

Despite the dominance of immunosuppressive Treg and Th2 cells in the tumor, tumor-reactive immune cells including IL-2-producing Th1 cells, cytotoxic T lymphocytes (CTLs) and NK cells are still a part of tumor microenvironment. In contrast, it has been reported that IL-2 is largely absent from most tumors [31,32] and low quantities of endogenous IL-2 fail to induce NK cell lytic activity. In classic Hodgkin lymphoma (cHL) it has been found that the production of soluble IL-2Rα (sCD25) by Reed-Sternberg (RS) cells, which bind IL-2, prevents IL-2 interaction with T and NK cells [33]. These observations are consistent with studies in several solid malignancies showing that higher levels of sCD25 is associated with poorer prognosis and advanced disease [34].

The ability of IL-2 to stimulate NK and $CD8^+$ T cells against tumor cells was the reason for its therapeutic use aiming to increase the antitumor response. In this sense, IL-2 represents a milestone in the history of cancer immunotherapy and is still clinically used for advanced melanoma and renal cell carcinoma (RCC), although it can only induce remission in a minority of patients, when used as single agent [35,36]. The limitation in the use of IL-2 is its toxicity, including the severe vascular leak syndrome. Moreover, Tregs expanded by IL-2 produce the immunosuppressive cytokines TGFβ and IL-10, for which NK cells show high sensitivity. Therefore, therapeutic strategies have been developed that deplete Treg cells prior to administration of IL-2 thereby maximizing the antitumor effects of IL-2 [37–39].

IL-15 is a cytokine produced by activated DCs and macrophages in response to GM-CSF, interferons and agonists of Toll-like receptors. Unlike other γc cytokines that exert their effects as soluble proteins, IL-15 functions as a bound cytokine on IL-15 Rα- expressing professional APCs. IL-15 Rα presents IL-15 to target cells expressing the IL-15 Rβ and γ_c subunits in the mode of action known as transpresentation [40]. Despite sharing many biological effects with IL-2, IL-15 protein does not share homology with IL-2, and IL-15 and has unique effects on T cells. In contrast, IL-2 and IL-15 shares many properties toward NK cells. In NK cells, IL-15 signals through activation of Jak1/Jak3 and STAT1/3/5 thereby regulating NK cell activation, proliferation, survival and cytotoxicity [41,42].

IL-2 and IL-15 also regulate NK cell-mediated cytotoxicity by upregulating perforin and granzyme B, thereby activating NKG2D, NCR and KIR receptors which might facilitate target cell recognition and activation of effector functions [43–45]. Like IL-2, IL-15 supports survival of NK cells through the induction of Bcl-2. Furthermore, IL-2 and IL-15, although, being poor inducers of NK cell cytokine production by themselves, synergize with IL-12 to trigger secretion of IFNγ, Tumor necrosis factor (TNF)α and granulocyte-macrophage colony-stimulating factor (GM-CSF) [20,46].

Beside from its expected role in the differentiation and survival of NK cells, IL-15 remains a surprising evasion mechanism in different hematological malignancies [47] including cHL in which IL-15 and corresponding IL-15Rs are upregulated and RS cells utilize IL-15 for growth in an autocrine manner, thereby competing with NK cells [48].

When used as immunotherapy, IL-15 is less toxic [49] than IL-2 and does not, unlike IL-2, induce Treg cell activity [50]. Owing to its powerful immunomodulatory properties, IL-15 was originally ranked highly among the potential biological agents [50]. However, the post-treatment side-effects of IL-15 may limit its therapeutic benefit when administered beyond a certain doses. Recent studies of rIL-15 therapy of metastatic RCC and melanoma patients indicate that IL-15 can be safely administered at low doses with the best response being stable disease. Considering that IL-15 requires transpresentation by IL-15Rα, alternative forms of IL-15-based therapy have been developed and are now in clinical trials. In this sense, the combination of IL-15 with soluble IL-15Rα generates a complex termed IL-15 superagonist (IL-15 SA) that possesses greater biological activity than IL-15 alone. IL-15 SA is considered as an attractive antitumor and antiviral agent because of its ability to selectively expand NK and memory $CD8^+$ T lymphocytes. IL-15 SA caused NK cell activation as indicated by increased CD69 expression and IFN-γ, perforin, and granzyme B production. Cell depletion and adoptive transfer studies showed that the systemic toxicity of IL-15 SA was mediated by hyperproliferation of activated NK cells that produce proinflammatory cytokine IFN-γ, but not TNF-α or perforin [39,51–53].

2.3. IL-21

IL-21 is the most recently added member of the γC family secreted by DCs and Th17 cells. The IL-21 receptor is a heterodimer consisting of IL-21Rα and γC. Similar to previous cytokines, IL-21 also stimulates NK cell signaling through Jak1/3 and STAT1/3/4/5 in addition to the PI3K and MAPK pathways [54]. It has been shown that IL-21 may display both positive and negative effects on NK cells depending on their activation/maturation stage and species of origin. In humans, it is reported that IL-21 exhibits a proliferative effect on NK cells and stimulates the cytotoxic activity of NK cells by increased expression of perforin and granzyme B [55]. In humans, IL-21 also participates in the acquisition of CD16 and KIR receptors and regulates the expression of several NK cell receptors by downregulating the expression of the activating NKG2D/DAP10 receptor, while inducing several NCRs [56]. In spite of the modest effect of IL-21 alone, it has been shown that it has a favorable cooperative effect when combined with other cytokines, like IL-2, IL-15 and IL-18, on NK cell proliferation, cytotoxicity and IFN-γ production [57,58].

IL-21 is a good candidate for immunotherapy of cancer since it does not induce proliferation of Tregs. Phase I and II trials of patients with metastatic melanoma demonstrated that IL-21 was safe and well tolerated, although the clinical efficiency was limited with only a single complete remission and some partial responses [39].

2.4. IL-12 cytokine family

This family belongs to the type I cytokine superfamily and consists of IL-12, IL-23, IL-27 and IL-35 of heterodimeric cytokines formed by two subunits, an α-chain (p19, p28 or p35) and a β-chain (p40 or Ebi3) that induce NK effector function both independently and in cooperation with other cytokines. Members of this family also share the β1 and β2 receptor chains and either of these chains associates with a cytokine-specific receptor (IL-23R, IL-27R and gp130) to form a heterodimeric receptor. Signaling by all these receptors are mediated by members of the Jak1/2/Tyk2 and STAT1/3/4families dependent on the receptor [59].

2.5. IL-12

This cytokine is secreted by accessory cells such as monocytes, macrophages and DCs in response to microbial infection and engagement of cytokine networks or by direct cell-cell contacts with other immune cells. IL-12 enhances T and NK cell cytotoxicity, induces the production of IFNγ by T and NK cells, affects NK cell proliferation and drives the Th1 response. IL-12 was shown to be composed of p35 and p40 subunits and was originally designated as a cytotoxic lymphocyte maturation factor and a natural killer cell stimulatory factor (NKSF) based on its activities [60]. IL-12 signals via Jak2 and STAT3/STAT4 which trigger cytokine secretion and cytotoxicity by NK cells [61].

IL-12 alone enhances NK cell-mediated cytotoxicity against different target cells and facilitates target cell recognition by affecting the expression of activating NK cell surface receptors like NKp46, without affecting the expression of inhibitory KIRs such as KIR3DL1 [62]. IL-12 augments NK cell proliferation and IFNγ production in patients with IL-12 receptors β1 and β2. Patients with IL12 p40 mutations have a diminished number of NK cells that have impaired IFNγ production. More recently, IL-12 has also been associated with generation of memory-like NK cells [27].

In the tumor microenvironment, IL-12 acts mainly on lymphoid cells such as NK cells, T cells and ILCs. IL-12 secreted by DCs and macrophages is a major player in the bidirectional crosstalk of these APCs with NK or T cells. Subsequently, IFNγ produced by NK cells [63] induces NO production and upregulates co-stimulatory and MHC molecules on macrophages and DCs [64]. Therefore, by facilitating antigen presentation and cross-presentation by APCs, IL-12 further promotes the cytotoxic activity of CD8 T cells and cytokine response of CD4 T cells. However, IL-12 by itself represents a relatively weak stimulus for IFNγ production in NK cells and it has been shown that IL-12 requires cooperation with other factors to properly exert its effects on NK-derived IFNγ production [65,66].

Immunotherapy with IL-12 was introduced in the mid-90 and was shown to have unacceptable levels of adverse events. However, IL-12 is a cytokine that remains of clinical interest due to its powerful immunomodulatory effects although commercial development of rhIL-12 as a single agent is unlikely due to its side-effects. In contrast, IL-12 is considered a priority cytokine for combinations with anti-tumor monoclonal antibodies or as a vaccine adjuvant.

As the therapeutic application of IL-12 is primarily restricted to the tumor microenvironment, as opposed to the other activating cytokines such as IFN-α or IL-2, novel methods to effectively deliver this cytokine directly to the tumor site need to be improved [67,68].

2.6. IL-23 and IL-27

Besides IL-12, macrophages and DCs can produce IL-23 and IL-27 when exposed to pathogens or their products. IL-23 is composed of p19 and p40 subunit of the IL-12 family that signals through a receptor composed of IL-12 receptor β1 and IL-23R, while IL-27 is composed of the Ebi3-induced gene and the p28 subunit that signals through the receptor composed by the WSX-1 and CD130/gp130 chains [69]. IL-23 signals through Jak2/Tyk2, STAT1/3/4/5, while IL-27 displays pro- or anti-inflammatory functions through activation of STAT1 and STAT3, respectively. In addition, the pro-inflammatory effects of IL-23 also depend on induction of Tbet, a Th1 transcription factor, and of the expression of the IL-12 receptor β2 [70].

It has been reported that IL-23 activates NK cells and thereby contributes to the antitumor immune response by mediating a Th1-type response in combination with IL-12 [71]. However, other studies failed to demonstrate an effect of IL-23 on NK cells making its influence on NK cells an open question that warrants further investigation. In this sense, it has been shown that IL-23 produced by MDSCs and tumor-associated macrophages (TAM)s in the tumor microenvironment may lead to downregulation of perforin, IL-12 and IFNγ thereby suppressing T and NK cell effector functions which could promote tumor growth and development [72,73].

IL-23 has been shown to be crucial for the development of Th17 cells that are primary producers of IL-17, a cytokine with dual effects on tumor immunity. Aside from recruiting CD4, CD8, DCs and neutrophils into the tumor environment and facilitating NK cell and CTL antitumor activity, IL-17 may also promote tumor growth by inducing tumor proliferation and inhibiting apoptosis as well as by potentiating angiogenesis and recruitment of suppressive immunoregulatory cells [74]. Similarly, in the tumor microenvironment, IL-23 may regulate the function of other innate immune cell subsets, namely NKT, CTLγδ and ILC3 that express IL-23 receptors, for which reason it displays a dual effect on the antitumor immune response. In response to IL-23, ILC3 cells, initially termed NK22 [3] specialize in the secretion of IL-22 and contribute thereby to the formation of protective tumor-associated tertiary lymphoid structures [75]. In apparently contradiction, it has been reported that in colorectal cancer (CRC), ILC3-derived IL-22 can act on the epithelial cells leading to STAT3 activation and cell proliferation [76].

The stimulatory effect of IL-27 on NK cells requires cooperation with other cytokines secreted by APC, namely IL-12, IL-15 and IL-18. While IL-18 facilitates NK cell activation with IL-12, IL-27 primes NK cells for the effects of IL-18 on their antitumor response. In particular, IL-27 induces Tbet, a critical transcription factor that regulates IFNγ production in NK cells, thereby promoting IL-18-mediated IFNγ secretion [77,78].

Increased IFNγ secretion upregulates intercellular adhesion molecule (ICAM-1) on the target cells thereby facilitating the formation of

NK cell-target cell conjugates which further increase the cytotoxic activity of NK cells [79]. Aside from this, it has been reported that IL-27 may also have potential tumor-promoting effects mediated by its ability to induce suppressive tumor-resident immunoregulatory Tr1 cells and upregulate immunosuppressive molecules such as IL-10, T cell immunoglobulin and mucin domain (TIM)-3, indoleamine 2,3-dioxygenase (IDO), CD39 and programmed death ligand 1 (PD-L1) [80].

For these reasons, IL-23 and IL-27 show dual roles in tumor immunology, since they both enhance and suppress immune response. This would limit their usefulness in cancer immunotherapy which should be considered in the design of future clinical trials [59].

2.7. IL-35

While IL-12, IL-23 and IL-27 can all play a role in promoting a protective inflammatory immune responses, the newest member of IL-12 family, IL-35, is a purely immunosuppressive cytokine that, contrary to the other members of the family, is primarily expressed by Tregs. IL-35 is composed of p35 and Ebi3 subunits and upon binding to its receptor IL-35R, constituted by IL-12Rβ2 and gp130, mediates signaling via STAT1 and STAT4 [81]. The predominant mechanism of suppression associated with the activity of IL-35 is its ability to suppress T cell proliferation and effector function [82].

The immunosuppressive effect of IL-35 has not been well investigated in humans. However, in mouse models of melanoma and colorectal cancer, tumor growth is primarily associated with an increase in IL-35 production in inducible Tregs (iTregs), especially in iTregs that not only produce, but respond to IL-35 [83]. Moreover, it has been reported that IL-35-induced Treg populations in the tumor microenvironment suppress CD4, CD8 and NK cell antitumor activity by upregulating the production of TGFβ and IL-10. In agreement with these observations, it has been reported that elevated plasma levels of IL-35 in patients with acute myeloid leukemia [84] and lung cancer [85] are associated with disease progression. Based on its immunosuppressive role, IL-35 may be considered as a therapeutic target that could be blocked by use of dedicated monoclonal antibodies [81].

2.8. IL-18

A member of IL-1 family, IL-18 is a proinflammatory and immunoregulatory cytokine produced by several cell types including macrophages, DCs, fibroblasts, as well as activated immune cells such as T and B lymphocytes and NK cells. It was discovered based on its ability to promote IFNγ production by T and NK cells in Th1 differentiation [86].

Produced as an inactive precursor, pro-IL-18 is converted into the mature form by casp1-mediated cleavage that binds to a heterodimeric receptor complex consisting of IL-18Rα and IL-18Rβ. The natural inhibitor of IL-18, IL-18 binding protein (BP), is constitutively produced by monocytes and macrophages and IL-18 BP secretion is part of negative-feedback loop, which proceeds from IL to 18 triggered IFNγ production to prevent exaggerated Th1 responses [87,88].

In contrast to other cytokines that transduce signals through the Jak/STAT pathway, IL-18R primarily transduces signals through the adapters MyD88 and TRAF6 leading to MAPK and NFκB activation, although minor activation of STAT3 has been reported [89]. IL-18 has shown antitumor activity by inducing Fas expression in NK cells and production of chemokines, IFNγ and TNFα that result in local recruitment and activation of DCs and effector T cells. IL-18 induces NK cell proliferation as well as the expression of the CCR7 receptor for the CCL21 chemokine which enables them to migrate to secondary lymphoid organs and suppress lymphogenic dissemination of tumor cells [90].

With respect to the effect of IL-18 on IFNγ production, it is now established that IL-12$^{-/-}$ and IL-18$^{-/-}$ double knockout mice have a profoundly impaired antitumor immune response, indicating that IL-18 alone is a poor inducer of IFNγ while efficient IFNγ production is achieved in cooperation between IL and 18 and IL-12. Accordingly, NK cells from IL to 18R$^{-/-}$ mice cannot be properly stimulated *in vivo* to secrete IFNγ by IL-12 [91]. The combined effect of IL-12 and IL-18 in the enhancement of IFNγ gene expression results from the accumulation of phosphorylated STAT4 [92], a prerequisite for IL-12-mediated IFNγ production, since it facilitates binding of AP-1 to the promoter sequence in the IFNγ gene [93]. Moreover, synergism of IL-18 with IL-12, IL-15, IFNα or IL-2 produced by DCs or T cells activates IFNγ and TNF production by NK cells thereby enabling the activation of the adaptive immune response through cooperation between NK cells and DCs [19,65].

IL-18 may also effect the migratory potential of NK cells. In the tumor microenvironment unpolarized (M0) and TAMs express a membrane form of IL-18 (mIL-18) that can be sheded by the proteolytic activity of matrix metalloproteinases (MMPs). The release of soluble IL-18 may induce expression of CCR7 on CD56^{dim} tumor-associated NK cells and promote their subsequent migration to secondary lymphoid organs (SLOs) and tertiary lymphoid structure (TLS) [90,94].

Interestingly, some studies indicate that IL-18 may also have tumor-promoting activities in terms of tumor invasiveness, angiogenesis and immunosuppression in different tumor models. In this sense, IL-18 has been shown to promote tumor immunosuppression and tumor growth in mouse models by converting Kit^{-} NK cells into Kit^{+} NK cells which overexpress PD-L1 that by DC-lysing mediate immunoablative functions [95]. Furthermore, Park et al. have shown that tumor-derived IL-18 induces PD-1 expression on immunosuppressive NK cells in triple-negative breast cancer patients [96]. Recently, it has been reported that IL-18 promotes multiple myeloma (MM) progression through the generation of MDSCs in mice models [97]. Moreover, high levels of IL-18, pro-IL-18 and IL-18BP were found in tumor tissues and in the systemic circulation of various human cancers and were associated with advanced tumor stages and/or with poor prognosis [98,99]. In this sense, it has been shown that prostate tumor cells secrete IL-18 in response to IFN-γ and that IL-18 in the tumor microenvironment may act as a autocrine/paracrine factor for the tumor growth [99]. In this sense, the apparently contradictory findings related to the biological role of IL-18 in cancer warrants the need for further investigation [88].

The relative safety of IL-18 was shown in phase I clinical trials for patients with advanced malignancies, but without any major clinical responses. The use of IL-18 in future combination therapy regiments warrants further investigation especially as it has been reported to mediate a more potent ADCC in combination with certain therapeutic monoclonal antibodies [97,100].

2.9. IFNα/β

Type I IFNs (IFNα/β) produced during viral infections by plasmocytoid DCs are strong stimuli for NK cells. IFNα/β binds to their cognate receptor which is composed of the IFNα/βAR1 and IFNα/βAR2 subunits that are pre-associated with Tyc2 and Jak1 that initiate signaling by phosphorylation of receptor components and the recruited STAT1/2/3/4/5 and 6 that by homo- or hetero-dimerization promote transcription of array of target genes [101].

The prototypical STAT signaling complex for Type I IFN is composed of STAT1, STAT2 and IRF9 that translocates to the nucleus and binds to the promotors of interferon regulated genes, (IRGs), via interferon response elements (ISRE) and gamma-activated sequence (GAS) elements. In addition, there are many non-STAT pathways such as MAPK activation that are activated by type I IFNs and that drive expression of IRGs. IFNs also induce a network of inhibitors for their own signaling, such as suppressor of cytokine signaling (SOCS) proteins that serve to avoid excessive signaling [102].

By up- or downregulating many IRGs, IFNα is responsible for antiviral, antitumor and immunoregulatory actions. Type I IFN can upregulate the activity of almost all immune cell types, including macrophages, DCs, B cells, T cells, and NK cells. In addition, type I IFNs

downregulate the proliferation and activity of immunoinhibitory cells such as Tregs and MDSCs [103,104].

In the tumor microenvironment, damage-associated molecular patterns (DAMPs) that are derived from dead tumor cells bind Toll-like receptors (TLR)s on APC that leads to production of type I IFNs [105]. IFNα alone or together with other stimulatory cytokines, induces NK cell proliferation, cytotoxicity and full effector function. In this sense, activation of STAT1 and STAT2 regulate IFNα-mediated cytotoxicity [43,44], whereas activation of STAT4 promotes IFNγ secretion. IFNα stimulates NK cell proliferation, NK cell cytotoxicity (due to upregulation of perforin and Fas or CD95 ligands and IFNγ production). Upregulation of NK cell cytotoxicity by IFNα is not only due to the known upregulation of cytolytic molecules, but also to upregulation of the main activating receptor NKG2D and CD161 [43,44,106]. In addition to the direct immunomodulatory effect on NK cells, IFNα induces DC to produce cytokines, IL-12, IL-15 and IL-18 thereby facilitating the NK cell-DC cross talk that results in NK cell priming [107]. Moreover, type I IFNs are known to negatively regulate proliferation of Tregs thereby reducing the accumulation of suppressive MDSCs in the tumor microenvironment that inhibit the cytotoxic activity of NK and T cells [104].

IFNα also has direct antitumor effect as a regulator of genes that affect tumor cell growth, proliferation, differentiation, survival and migration, such as p21, an inhibitor of cyclin-dependent kinases, Fas, Fas ligand, TNF-related apoptosis-inducing ligand (TRAIL) and 2′-5′oligoadenylate synthetases (OAS). The immunoregulatory effects of IFNs also contribute to their antitumor activity and include molecular mechanisms associated with the regulation of tumor antigens on tumor cells, as well as antigen presentation by upregulated MHC molecules [108]. Considering that treatment with IFNs is likely to induce the expression of immunosuppressive ligands and receptors, such as PDL1 and PD1 that could prevent prolonged antitumor immune responses, IFN would need to be combined with therapies that target the PDL1-PD1 axis in order to obtain a sustained therapeutic response [109].

Immunotherapy with IFNα has shown strong activity in hematological malignancies, including hairy cell leukemia and other lymphoproliferative and myeloproliferative neoplasms, and has formally been approved as adjuvant therapy for high risk melanoma patients. In clinical trials, IFNα is usually used in combinations, and shows a dose-related toxicity profile. Therefore, most side effects are managed without discontinuation of treatment. IFNα is commonly administered in combination with other cytokines, biological agents and/or different chemotherapeutics [104,110,111] and more recently, with therapeutic monoclonal antibodies, rituximab, bevacizumab, anti PD-1 or tyrosine kinase inhibitors, such as sorafenib [104].

2.10. IFNγ

This type II IFN is produced by T and NK cells activated either by mitogens or by cytokines such as IL-12 and IL-18. IFNγ binds to type II receptor subunits, IFNG-R1 which engages IFNG-R2 subunits thereby causing cross-phosphorylation between Jak1 and Jak2 followed by recruitment of STAT1/STAT3 that subsequently translocate to the nucleus where they bind GAS elements in the IRG promotors thereby activating a number of immunoregulatory genes [112].

IFN-γ supports antitumor immunity and potentiates the cytotoxic response of NK and CD8 T cells ($CD8^+$) by inducing the production of perforin, granzyme and Fas ligand (FasL) expression [113]. IFN-γ has a central role in the regulation of the adaptive immune response and tumor antigen presentation by upregulation of MHC molecules on APCs and tumor cells thereby promoting antitumor Th1 differentiation and CTL function [114]. In addition, it can also upregulate ICAM-1 which promotes NK: target cell interaction for efficient lysis [79]. IFN-γ is also critical for T cell, NK and NKT cell migration into the tumors through induction of CXCL9, CXCL10, and CXCL11 chemokines [115,116]. Accordingly, it has been shown that T cells in IFNγ-deficient mice fail to migrate to tumor site [117].

However, due to upregulation of both classical and non-classical, HLA-G and HLA-E, MHC class I molecules on tumor cells, IFNγ also induces resistance to the NK cell responses since these ligands engage inhibitory NK cell receptors [118].

Aside from the immunomodulatory effects, IFNγ has direct antitumor effects since it displays antiproliferative and proapoptotic activity against cancer cells and act as an inhibitor of tumor angiogenesis [119].

Although the primary biological activities of IFNγ in tumor-associated inflammation are beneficial, a protumorigenic role has recently been shown for the equilibrium and/or evasion stages of immunosurveillance. This is associated with the ability of IFNγ to limit the inflammatory responses and prevent tissue destruction after prolonged inflammation. In the tumor microenvironment this effect is manifested through IFNγ-mediated upregulation of Tregs and MDSCs that through IDO production suppress NK and CTL cells [120]. Moreover, IFNγ attenuates tumor infiltration and thus the protective role of neutrophils and other myeloid cells. The tumor promoting effects of IFNγ can also be mediated by induction of the inhibitory checkpoint ligand PD-L1 on tumor cells that upon binding the PD-1 receptor on CTLs and NK cells prevents a prolonged antitumor immune responses [121]. In this sense, during chronic inflammation, the tumor promoting effects of IFNγ can exceed its antitumor effects depending on the context of the tumor type, microenvironmental factors and signal intensity [122].

In spite of its numerous immunostimulatory effects, IFNγ has limited clinical utility in cancer immunotherapy as its application in a variety of malignancies has not given enough support for a routine application. However, an ongoing clinical trial is testing IFNγ in combination with replicative oncolytic adenovirus in patients with glioblastoma and glyosarcoma. Interestingly, recently described adverse, tumor-promoting effects of IFNγ might suggest that inhibition of the IFNγ pathway could be a new therapeutic target in clinical management of malignancies [104].

NK cell activity is tightly controlled to avoid damage to self tissues by immunoregulatory cytokines such as TGF-β, IL-10 and IL-6. However, in the tumor microenvironment, these cytokines are produced by infiltrating suppressive immune and tumor cells that antagonize the stimulatory effects of IL-2, IL-12, IL-15, and IL-18 thereby inhibiting NK cell activation, proliferation, differentiation and IFN-γ production [123–125] resulting in dampening of the NK cell antitumor response (Fig. 1) [126,127].

2.11. TGFβ

The TGFβ signaling pathway has pleiotropic functions that regulate cell growth, differentiation, apoptosis, motility, invasion, as well as the immune response. The TGFβ superfamily is composed of a large set of structurally and functionally related proteins, TGFβ1, -β2 and -β3, three highly homologue isoforms found in mammals, of which TGFβ1 is predominant and plays a major role in the immune system with potent immunoregulatory properties [128]. Almost all cells of the immune system can produce TGFβ that is synthesized as a biologically latent precursor in a complex with TGFβ-binding proteins. Various mechanisms, such as proteases, heat, reactive oxygen species (ROS) and an acidic environment are required to liberate the active TGFβ from the latent complex thereby allowing its binding to TGFβ receptors. TGFβ from activated Tregs can act either as a soluble or surface-bound latent form on target cells to increase Treg suppressive function [129].

TGFβ mediates its biological functions through binding to type I and type II transmembrane serine/threonine kinase receptors. Once TGFβ binds to a homodimer of TGFβ type II receptor (TβR II) on the surface of target cells, it recruits and phosphorylates TGFβ type I receptor (TβRI) that initiates the signaling cascade. Phosphorylation of TβRI leads to subsequent phosphorylation of downstream Smad-dependent, as well as Smad-independent (Erk, p38, MAPK, PI3K and Act) signaling pathways to regulate TGFβ-responsive genes. In addition to the activation of

TGFβ signaling, multiple negative regulators function at each step of the TGFβ-Smad pathway leading to signal attenuation [130,131].

The balance of the many TGFβ pleiotropic effects in different immune cells is responsible for the establishment of either immunity or tolerance thereby constituting a negative regulatory circuit of self control which maintains immune homeostasis through its impact on proliferation, differentiation and survival of multiple immune cell lineages, including NK cells. In this sense, in the context of the cancer microenvironment, TGFβ has an important role in the differentiation and induction of immunosuppressive cells that are also the major sources of TGFβ, thereby inhibiting NK and T cells function [132]. The effect of TGFβ through the Smad-dependent pathway is important to limit NK cell cytotoxicity and IFNγ production by repressing the expression of Tbet. In addition, it has been shown that TGFβ mediates downregulation of NKp30 and NKG2D NK cell receptors and inhibition of NKG2D/DAP10 signaling, as well as down-regulation of IFNα receptor and CD25 on NK cells [133].

TGF-β1 might also dampen $CD56^{dim}$ recruitment while favoring recruitment that of $CD56^{bright}$ by modifying their respective chemokine receptor repertoires [134]. In particular, TGF-β1 increases the expression of CXCR3 and CXCR4 in $CD56^{brigh}$ and CD56dim NK cells, whereas, down-regulates CX_3CR1 expression in $CD56^{dim}$ cells. This unusual chemokine receptor repertoire actually defines a peculiar $CD56^{dim}$ population [135] that shows defective migration toward tumor [134,136].

Since remodeling of the tumor microenvironment by TGFβ favors tumor growth and metastases (Table 2), including induction of anergic NK cells, it might be considered as a potential therapeutic target for enhancing NK cell-mediated antitumor immune responses. Emerging trials with TGFβ inhibitors in cancer therapy have shown encouraging results. In this sense, a TGFβ2 antisense DNA allogenic tumor cell vaccine has been explored in a phase II trial in non-small-cell lung carcinoma (NSCLC), while a human anti-TGFβ1/2/3 monoclonal antibody was tested in a Phase I trial in advanced melanoma patients and was shown to provide clinical benefit. However, although elimination of immunosuppressive cytokines seems appealing, it opens new challenges in terms of biomarkers and patient selection [129,137].

Table 2
Cytokines in tumor microenvironment and their prognostic significance.

Malignancies	Cytokine	Prognostic factor
Solid		
NSCLC	TGF-β	Negative
	IL-10	Negative
	IL-6	Negative
Breast	TGF-β	in early stages-positive, in late stages-negative
	IL-6	Negative
Melanoma	IL-6	Negative
	IL-10	Conflicting evidence
	TGF-β	Negative
Renal	TGF-β	Negative
	IL-6	Negative
Colorectal	TGF-β	Negative
	IL-6	Negative
Gastric	IL-6	Negative
	TGF-β	Negative
	IL-10	Negative
	IL-12	Positive
	IL-18	Negative
Hematological		
	IL-6	Negative
Hodgkin	IL-10	Negative
limfoma	IL-15	Negative
	TGF-β	Negative
CLL	IL-6,	Negative
	IL-10	Negative
Multiple	IL-6	Negative
myeloma	TGF-β	Not significant
AML	IL-6	Negative
	IL-10	Positive
	IL-15	Positive

NSCLC (Non-small-cell lung carcinoma), CLL (Chronic lymphocytic leukemia), AML (Acute myeloid leukemia)

2.12. IL-10

This cytokine belongs to a highly pleiotropic superfamily that includes a substantial number of cytokines (IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, IL-29 and type III IFNs) that share genetic similarity and have a conserved signaling cascade, although they mediate different activities. IL-10 is produced by monocytes, DCs, B cells, various Treg subsets, CD4+ T cells, CD8+ T cells, as well as by tolerogenic liver NK cells that have a role in anti-inflammatory responses [138]. Moreover, it has been reported that IL-10 can be secreted by the tumor cells. IL-10 exerts its biological function by forming a homodimer that binds the IL-10R1 and IL-10R2 receptor complex expressed by all responsive cell types including APC, lymphocytes, and likely also by tumor cells. Engagement of IL-10R leads to subsequent activation of JAK1 and TYK2 and STAT 1/3 [139].

IL-10 is an important cytokine that regulates homeostasis as it preferentially triggers a negative regulatory circuit that involves APCs and limits excessive NK cell activation. In this sense, IL-10 can exert indirect inhibitory effects on NK cell production of IFNγ and TNFα by dampening APC secretion of IL-12, IL-15 and IL-18 [140].

IL-10 has pleiotropic and at times seemingly contradictory effects on tumor pathogenesis and development, since it can contribute to tumor growth and promotion, as well as to tumor eradication. IL-10 is widely regarded as an immunosuppressive cytokine owing to its ability to inactivate CD4+ T cells, NK cells, and APCs and inhibit their production of the proinflammatory cytokines IL-2, IL-5, IFNγ, TNF and GM-CSF. In particular, IL-10 is mediating an immunosuppressive microenvironment that facilitates tumor escape via downregulation of IFNγ production and expression of CD80/CD86 costimulatory molecules on APCs, as well as of MHC class I molecules on tumor cells [19,126].

However, when combined with other cytokines such as IL-18, IL-10 is able to stimulate NK and CTL-mediated killing of cancer cells and to increase NK cell production of IFNγ. It has also been shown that IL-10 may enhance NK cell activity by inhibiting the secretion of ROS by TAMs, considering that ROS act as NK cell inhibitors. One explanation for the conflicting evidence regarding the role of IL-10 in cancer (Table 2) is that its function is finely controlled by a balance of cytokines and other environmental factors [141].

In spite of its diverse activities, the capacity of IL-10 to downregulate MHC class I molecules thereby making the tumor cells NK-sensitive suggest that treatment with IL-10 in combination with stimulatory cytokines, such as IL-2, may potentiate NK cell-mediated antitumor immunity [142].

2.13. IL-6

IL-6 is primarily identified as B cell growth factor and a hepatocyte stimulating factor. It is produced by a variety of different cell types such as fibroblasts, endothelial cells, macrophages, T cells and myocytes. In the tumor environment, the principal source of IL-6 are the tumor cells, TAMs, CD4+ T cells and MDSCs. Signaling of IL-6 occurs through either “classical signaling” or “trans-signaling”. Classical IL-6 signaling is initiated by the binding of IL-6 to the membrane non-signaling IL-6Rα (mIL-6Rα) subunit that by binding to signal transducing subunit glycoprotein 130 (gp130), also known as IL-6Rβ, induces phosphorylation of JAK1/2 and TYK2 and subsequently of STAT1 and STAT3. While gp130 is expressed ubiquitously on most cell types, expression of the IL-6Rα subunit is restricted mainly to hepatocytes and some leukocytes, thus effectively limiting the cell types that respond to IL-6 [143]. However, trans IL-6 signaling depending on IL-6Rs rare ability to be shed from the cell surface in the form of soluble IL-6R (sIL-6R) by ADAM10 and ADAM17, resulting in the formation of a soluble IL-6 and

IL-6R complex (IL-6- sIL-6R) that can influence a wide variety of cells [144].

IL-6 is pleiotropic cytokine with pro- or anti-inflammatory properties under different (patho)physiological conditions. It is considered that the proinflammatory effect of IL-6 is mediated by the induction of Th1, Th2 and Th17 effector T cells together with inhibition of Treg differentiation. The proinflammatory effects of IL-6 rely on the ability not only to induce accumulation of TAMs and MDSCs, but also to support the development and to prevent apoptosis of these cells, as well as of tolerogenic DCs by both autocrine and paracrine signaling. IL-6 can promote the differentiation of naïve $CD4^+$ T cells towards a Th17 subset that supports proinflammatory reactions via IL-1β and IL-23 induction. Although it is involved in the induction of an acute-phase inflammatory response together with TNF-α and IL-1β, IL-6 also controls acute inflammation [145]. This is mediated by downregulating the expression of pro-inflammatory and upregulation of anti-inflammatory molecules, including the IL-1 receptor antagonist protein and TNF-soluble receptor thereby abrogating the effects of TNF-α and IL-1β [146]. During this switch, TNFα and IL-1β negatively regulate the anti-inflammatory nature of IL-6 by enhancing the IL-6-induced expression of the suppressor of cytokine signaling (SOCS3) and/or targeting IL-6-induced gene expression. Moreover, these two cytokines can prevent the induction of an anti-inflammatory environment by blocking the IL-6-mediated differentiation of recruited T cells towards the Th2 profile and by production of IL-4 [147,148].

For these reasons, IL-6 has emerged as a critical mediator of chronic inflammation and is increasingly recognized as a key cytokine for linking chronic inflammation to cancer development [149]. By supporting a chronic aberrant inflammatory tumor microenvironment, IL-6 is a key regulator of immunosuppression that is manifested in inhibition of both NK cells and CTLs. Although, it was initially reported that IL-6 augmented the activity of NK cells *in vitro*, more recent data favor a suppressive role of IL-6 in NK cell function by both direct and indirect effects [150]. In this sense, it has been shown that the IL-6 mediated downregulation of NK cell cytotoxicity *in vitro* is associated with dephosphorylation of STATs by protein tyrosine phosphatases-2 (SHP-2) which may limit STAT5 activity and perforin expression [151]. Aside from its suppressive immunomodulatory effect, IL-6 directly promotes tumor cell proliferation, survival and metastasis and release of angiogenic factors vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) [152]. It has been established that the majority of tumor promoting functions of IL-6 appear to be primarily mediated through the IL-6/NFkB/STAT3 pathway [143,147].

Taken together, the role of IL-6 in cancer is not very clear since it has both pro- and anti-inflammatory roles that are crucial for host-tumor interaction. In cancers that are associated with systemically elevated IL-6 levels, such as RCC, melanoma, lung, colorectal, and MM. IL-6 represents a poor prognostic marker (Table 2) [148,153,154]. Accordingly, blocking IL-6 using mAbs against either IL-6 or IL-6 R or inhibition of IL-6 downstream signaling pathways has shown promising results in clinical trials. Phase I and II clinical trials have established the efficacy of anti IL-6 mAbs either as a single agent or in combination with other chemotherapeutic drugs, radiation and targeted therapies [155–157].

3. Combined effects of cytokines and other stimuli on NK cell function

Cytokine-stimulated NK cells have enhanced capacity to kill tumor cells. However, usually, individual cytokines do not seem to be sufficient for full activation, proliferation, survival and effector functions of NK cells. In contrast, combination of different cytokines has been shown to promote NK cell cytotoxicity involving both perforin and Fas-mediated killing of tumor cells, as well as the production of IFNγ through which NK cells participate in a complex interaction network with Th1 lymphocytes, DC and macrophages to effectively direct the antitumor immune response [158].

Human NK cells with a history of prior activation with combinations of IL-12/15/18 have a capacity for cytokine-induced memory after restimulation with other cytokines or a tumor target cell line [159]. Such NK cells have enhanced proliferation, IFN-γ production and cytotoxicity. Memory-like NK cells upregulate CD25 (IL-2Rα) expression (resulting in expression of the heterotrimeric high affinity IL-2Rαβγ) and enhanced response to low-doses of IL-2 [160]. It have been found that memory-like NK cells show increased expression of the activating receptors NKG2D, NKp44, NKp30, NKp46 and TRAIL, but also enhanced expression of the inhibitory receptor NKG2A [27].

It has been shown that human memory-like cells when transferred to immunodeficient mice, substantially reduced the acute myeloid leukemia (AML) burden *in vivo* and improved overall survival. In the first phase of clinical trials in AML patients, adoptively transferred memory-like NK cells proliferated and demonstrated considerable anti-leukemia effect. Therefore, it may be concluded that in cancer patients, the inflammatory cytokines IL-12, IL-15, and IL-18 that are induced by chemotherapy, radiotherapy or HCT represent potential settings for memory-like NK cell induction *in vivo*. Thus, further investigations of cytokine-induced memory-like NK cells may aid in development of a promising translational immunotherapy approach for patients with hematological malignancies [161].

The combined effects of cytokines should be taken into consideration when designing cytokine-based immunotherapy for cancer patients which should not only focus on the ability of NK cells to degranulate and kill tumor targets, but also to enhance antitumor immunity through such indirect mechanisms such as cytokine production [162,163]. Considering that immunosuppressive cytokines and other factors produced by the tumor can negatively influence NK cell function and allow tumor evasion and progression, blockade of inhibitory cytokines by monoclonal antibodies and antagonists, as well as by interference with their signaling molecules appears as a feasible therapeutic approach to enhance NK cell responses in cancer patients [164].

Taken together, increased understanding of the NK cell-tumor cell interaction based on pleiotropic responses, redundancy of cytokine signaling and the dual function of many cytokines has led to a novel concept of immunotherapy that is based on application of stimulating and elimination of immunosuppressive cytokines that comprehensively enhance the NK cell antitumor response.

Conflict of interest

The authors declared that there is no conflict of interest.

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