Function of natural killer cells in immune defence against human leukaemia

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Summary

Although progress has been made in the management of acute leukaemias, most patients who fail to respond to front-line therapies with cytostatic agents and stem cell transplantation, or who relapse after an initial response die from progressive disease. Novel treatment modalities exploiting donor-derived natural killer (NK) cells generate an alloreactive graft-versus-leukaemia response and eliminate the residual malignant clones in transplanted patients. NK cells are components of the innate immunity playing an important role in the surveillance of human tumours. Recognition of malignant cells depends on a dynamic balance between antagonistic functions of an array of NK activating and inhibitory receptors. The natural cytotoxicity receptors (NCRs) are NK cell-specific and together with the NKG2D receptor are responsible for NK cell activation and tumour cell killing. The killer immunoglobulin-like receptors (KIRs) recruit phosphatases and can antagonise the activating signals and prevent the cytolytic NK cell programme. Understanding of the integration of these multiple signals at the molecular level is central for exploring the cytolytic function of NK cells. This review describes molecular mechanisms of NK receptor-ligand interactions controlling target cell recognition and addresses the potential of NK cells for the specific elimination of leukaemic clones with the goal of advancing immunotherapy of leukaemia.

Key words: NK cells; human leukaemia; NK cell receptors; signalling pathways; stem cell transplantation; immunotherapy

Introduction

Natural killer (NK) cells are potent effectors of the peripheral immune system. This lymphocyte subset, phenotypically defined as CD56+CD3−, has developed as part of protective responses against microbial pathogens and, according to recent studies, plays an important role in the surveillance and eradication of malignant cells [1, 2]. Upon recognition of tumour- or virus-transformed cells, peripheral NK cells are capable of an immediate release of the content of cytotoxic granules and production of inflammatory cytokines, such as tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). This illustrates how NK cells link the innate and adaptive arms of the immune system [3]. Unlike T lymphocytes, NK cells lack the ability to recognise antigens in the context of classical human lymphocyte antigen (HLA) molecules. Instead, NK cell function is regulated by signals delivered by an array of cell surface receptors that recognise different cellular ligands and can discriminate between target and non-target cells [4, 5]. Engagement of the activating receptors triggers the cytolytic programmes whereas inhibitory receptors antagonise the activating pathways. This equilibrium between the opposing signals defines the NK cells effector function. Recent progress in understanding this unique mechanism of receptor-dependent NK cell activity has revitalised interest in the potential immunotherapeutic value of these cells [6, 7]. Below, the molecular basis of NK cell function and current knowledge on the role of NK cells in immune responses against leukaemia are reviewed.

NK cell receptors and signalling pathways

NK cell receptors are encoded in the genome, rather than being generated by somatic recombination like the antigen-specific components of the T cell receptor. In contrast to T lymphocytes, NK cells use the diverse receptor repertoire to screen potential targets for the presence of specific cell
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Inhibitory NK cell receptor signalling

The recognition of polymorphic HLA class I is a hallmark of NK cell tolerance [9]. Human inhibitory receptors belong to two families of transmembrane glycoproteins, containing the immunoglobulin (Ig)- and lectin-like extracellular domains [10]. Among them, Killer Ig-like Receptors (KIRs) are the most important in determining the specificity towards the different alleles of classical HLA-A, -B and -C molecules. KIRs contain 2 or 3 Ig-like domains, designated 2D or 3D, and most of them contain long (L) cytoplasmic tails harbouring the immunoreceptor-tyrosine-based inhibition motifs (ITIMs). Individual KIRs have specificity for a number of different HLA alleles, e.g. KIR2DL1 for HLA-Cw2, 4, 5, 6, and KIR2DL2/3 for HLA-Cw1, 3, 7, 8, resulting in a complex receptor-ligand recognition system (table 1). Upon the engagement of clonally distributed KIRs by an appropriate HLA class I molecule, ITIMs become tyrosine phosphorylated and recruit phosphatases to counteract cellular activation signals. The predominant phosphatases associated with KIRs are SH2 domain-containing tyrosine phosphatases SHP-1 and -2, and the inositol-5-phosphatase, SHIP [11, 12]. The substrates specific for these phosphatases in NK cells have only began to be defined and they might depend upon which of the activating receptors are being modulated (figure 2).

Phosphatases mediate a strong inhibition of NK-mediated cytolysis, which is dominant over signals elicited from activating receptors. Accordingly, the NK-dependent killing is impaired in both naturally-occurring murine mutants and phosphatase-knockouts. Of particular interest are SHIP-1-deficient knockout mice that have a restricted repertoire of NK receptors and a profoundly activated PI3-kinase/akt pathway in NK cells. As a consequence, SHIP-1/– NK cells fail to recognise the allogeneic targets and do not reject grafts of fully mismatched bone marrow, suggesting that SHIP plays an important role in graft rejection limiting the success of bone marrow transplantation [13]. A recent study demonstrated that SHIP-1 functions also as a negative regulator of IFN-γ production by primary human NK cell subsets [14].

Activating NK cell receptor signalling

NK cells have evolved a large series of activating receptors which trigger positive signals upon recognition of target cells [15]. At present many of these receptors are still orphans regarding the ligands which they recognise. An important family of receptors is represented by the natural cytotoxicity receptors (NCR), which include NKP30, NKP44, and NKP46 [16]. Surface density of these receptors correlates with the capacity of NK cells to kill a large variety of tumour cells [17]. The haemagglutinin protein of influenza virus has recently been suggested to interact with NKP44 and NKP46 [18, 19]. However, the cellular ligands for NCR receptors have not been identified. The

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Table 1

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<tr>
<th>Inhibitory receptors</th>
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<tr>
<td>KIR2DL1 (CD158a)</td>
<td>HLA-Cw2, 4, 5, 6</td>
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<tr>
<td>KIR2DL2/3 (CD158b)</td>
<td>HLA-Cw1, 3, 7, 8</td>
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<tr>
<td>KIR3DL1 (CD158c)</td>
<td>HLA-Bw4</td>
</tr>
<tr>
<td>KIR3DL2 (CD158k)</td>
<td>HLA-A1, -A11</td>
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<tr>
<td>KIR3DL5</td>
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<tr>
<td>KIR3DL3</td>
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<th>Activatory receptors</th>
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<tr>
<td>NKG2D</td>
<td>ULBP-1, 2, 3, 4, MIC-A, -B</td>
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<tr>
<td>NKP30</td>
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<td>NKP44</td>
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* For comprehensive receptor-ligand list, see ref. 4.
Lectin-like receptor NKG2D forms a homodimer expressed by all NK cells, a subset of CD8+ and TCR γδ+ T cells [20]. The cellular ligands for NKG2D have been recently identified [21, 22]. In humans, these ligands belong to two distinct families: the MHC class I chain-related (MIC) antigens containing a short transmembrane domain, and the UL16 binding proteins (ULBPs) linked to the membrane through the GPI anchor (table 1). Activating signals can also be elicited by KIR receptors with short (S) cytoplasmic domains lacking the ITIM sequence motif (KIR2DS and KIR3DS).

Activating signals from NCRs and NKG2D and several activating NK co-receptors are transmitted by non-covalently associated small transmembrane adaptor proteins that possess immunoreceptor tyrosine-based activation motifs (ITAMs) in their cytoplasmic domains. NK cells express the ITAM-bearing CD3ζ, FcERIγ, and DAP12 adaptor proteins [8]. The downstream events include phosphorylation of PI3-kinase, phospholipase C, Syk, Zap70 and other signalling elements commonly employed by leukocytes (figure 2) [23–25]. It remains not defined how ligand recognition by individual receptors is delegated to independent pathways and how it is assembled to produce relevant NK cell responses.

Due to unknown ligands for most of the activating receptors, including all NCRs and some KIR2/3DS receptors, the knowledge of ligand-dependent NK cell activation is largely derived from studies on NKG2D ligands. In humans, these ligands are expressed on B- and myeloid-progenitor cells (26). MIC proteins are upregulated by various forms of cellular stress, by viral infection and on tumour cells of epithelial origin [21, 27, 28]. An important characteristic of MICs is that they can be shed by some tumour cells and become detectable in the serum [29, 30]. This may lead to a permanent downregulation of NKG2D receptor, together with its functional blockade. This strategy represents a new escape tool used by tumours otherwise killed upon NKG2D recognition. The expression pattern of ULBP and MIC proteins often differs in tumours of various tissue origin, suggesting functional differences between these ligands [31, 32]. Therefore, a wide ligand reactivity of NKG2D may represent a strategy of how NK cells prevent possible escape mutants which have downregulated only one ligand.

Figure 2
NK cell receptor-dependent signalling pathways. ITAM-bearing adaptor proteins (CD3ζ, DAP12); YxxM-bearing adaptor protein DAP10; PTK, protein tyrosine kinases; PI3K, phospho-inositol-3 kinase; SHP and SHIP, phosphatases. Positive signalling (+) by the activating receptors is counteracted by the negative signalling (-) by the inhibitory receptors.

Acute leukaemias are rapidly progressing blood cell malignancies with a poor prognosis. High-dose chemotherapy and transplantation of allogeneic stem cells offer the best chance of cure, but relapses are frequent and often fatal [33]. High incidence of disease recurrence suggests that leukaemic blasts can escape recognition by the immune system. The mechanism of immune evasion from the NK surveillance may be related to an inadequate function of NK cells, caused by abnormalities either at the level of receptors or cognate ligands. Recent studies began to unravel the reactivity of NK cells in primary human leukaemias. In acute myeloid leukaemia (AML), NK cell number is profoundly reduced [34] and expression of some of the activatory NCR receptors may be downregulated resulting in low NK cell cytotoxicity against autologous leukaemic cells [35]. In chronic myeloid leukaemia (CML), NK cells are reduced in numbers and their cytokine-induced cytotoxicity is inhibited by CML blasts [36]. Unlike in many solid tumours, expression of HLA class I molecules is usually not strongly downregulated, resulting in tolerance of NK cells towards the autologous blasts. Furthermore, ligands triggering the NKG2D receptor and the putative molecules recognised by NCRs are expressed at low levels, thus contributing to poor recognition of malignant cells [26, 37]. We have recently demonstrated that inadequate ligand expression is a consequence of haematopoietic maturation arrest associated with malignant transformation [26]. This is in contrast to findings with tumours of epithelial origin, in which the stress-inducible MIC ligands are upregulated, marking the tumour tissue for destruction by NK cells [27]. In conclusion, although still fragmented, these results suggest that NK cells are important for tumour surveillance in leukaemia, providing the rationale for studies on their therapeutic value [38].
The therapeutic potential of NK cells in human leukaemia

The cure of leukaemia and other malignant blood diseases by transplantation of haematopoietic stem cells (SCT) is achieved by two mechanisms: the cytoreductive chemoradiotherapy which reduces the bulk of leukaemic blasts, and the graft-versus-leukaemia (GvL) effect exerted by donor-derived immune effector cells which eradicate residual malignant clones. Despite 3 decades of continued progress in the transplantation field, the relapse rate approaches 30% following allogeneic SCT and as much as 50% after SCT of autologous cells. In patients who are not eligible for SCT and undergo a conventional high-dose chemotherapy only, the recurrence rate of the disease is even more dramatic [39]. These clinical results underscore, on the one hand, the need for better understanding of tumour clearance mechanisms, and on the other hand, the search for new therapeutic approaches to enhance the GvL effect and improve the cure rates. Recent findings on NK receptor – ligand specificities, have reawakened an interest in the possibility of applying the NK cell-based strategies to treat leukaemia.

The therapeutic potential of NK cells in human leukaemia

Clinical results, initiated by Ruggeri et al. [40], suggest that NK cells may represent a unique therapeutic tool for clearance of leukaemia, in particular in conjunction with allogeneic SCT. In AML patients grafted with stem cells from haploidentical donors, the mismatch between KIRs on NK cells of the graft and HLA class I specificities of the recipient has facilitated NK cell-mediated killing of tumour cells, thus resulting in higher remission rates. This benefit of KIR receptor-ligand alloreactivity to promote engraftment and GvL effects without causing clinically overt graft-versus-host disease (GvHD) is increasingly recognised also in other SCT settings. The survival advantage associated with KIR ligand incompatibility has been reported in numerous haematological malignancies and with grafts derived from unrelated donors [41] as well HLA-matched siblings [42, 43]. Anti-leukaemic effects of KIR/HLA class I mismatches are also suggested by the experimental finding that blocking of murine inhibitory receptors Ly-49C with specific antibody increases NK cell-mediated killing of leukaemic cells in vitro and in tumour-bearing mice [44]. In addition to the anti-leukaemic NK cytotoxicity exerted through KIR/HLA disparity, NKG2D receptor-ligand interactions were shown to play a role in the cytolytic effect of HLA-matched NK cells against CML blasts [45]. A clinical impact of leukaemia recognition by NK activating receptors awaits further evaluation.

Anti-leukaemic effect of alloreactive NK cells

Adoptive transfer of activated NK cells in an allogeneic murine transplant model has shown the participation of these cells in GvL without inducing clinically overt GvHD [48]. Pilot clinical studies of an adoptive transfer of donor-derived NK cells to consolidate engraftment in AML patients transplanted from haploidentical donors have recently been reported [49, 50]. NK cell infusions were well tolerated, none of the patients developed GvHD, and importantly, donor-type chimerism was increased in some recipients. These studies, although involving a limited number of patients, documented the feasibility of NK cell DLI, opening the way for future graft engineering to exploit the clinical benefit of anti-leukaemic NK cells.

NK cell adoptive immunotherapy in treatment of leukaemia

The field of tumour immunotherapy is dominated by T cell-based approaches while clinical trials to utilise the anti-tumour effect of NK cells had little success in the past. This may rapidly change with a significant progress in identification of NK cell receptors and some of their ligands involved in selective recognition and lysis of tumour cells. The cellular immunotherapy by infusion of immune competent cells in the clinical SCT setting is expected to amplify the GvL effect exerted by the transplanted immune system. The clinical feasibility and efficacy of adoptive transfer of donor-derived T lymphocytes has been documented in CML; consequently, donor lymphocyte infusion (DLI) after SCT has become an established therapy in haematological malignancies [46, 47]. Adaptive transfer of activated NK cells in an allogeneic murine transplant model has shown the participation of these cells in GvL without inducing clinically overt GvHD [48]. Pilot clinical studies of an adoptive transfer of donor-derived NK cells to consolidate engraftment in AML patients transplanted from haploidentical donors have recently been reported [49, 50]. NK cell infusions were well tolerated, none of the patients developed GvHD, and importantly, donor-type chimerism was increased in some recipients. These studies, although involving a limited number of patients, documented the feasibility of NK cell DLI, opening the way for future graft engineering to exploit the clinical benefit of anti-leukaemic NK cells.
Alloreactive NK cells may become an integral part of treating leukaemia with SCT from unrelated and from family donors. Based on in vitro and preclinical studies, NK cells are capable of promoting engraftment, protecting against GvHD, and exerting GvL effects [40, 51]. Therefore, including NK cells into a conditioning regimen and infusing them after SCT may prevent the progress of leukaemia, facilitate stem cell engraftment, and perhaps even serve to treat GvHD and relapse [52–54]. Many open issues include not only appropriate selection of donors and recipients, but also NK cell dose, timing of infusions and possible ex-vivo manipulations of NK cells prior to DLI. Expansion of clinical grade NK cells has been reported [55] and the use of ex-vivo cytokine-activated NK cells awaits clinical evaluation.

Also the antileukaemic potential of autologous NK cells deserves a therapeutic consideration. A number of studies documented a susceptibility of leukaemic blasts to autologous cytotoxicity by NK cells [56, 57]. According to our recent studies [33], AML-derived NK cells can be substantially expanded and activated in vitro, and are fully functional against autologous AML blasts by effectively reducing the tumour load when infused to AML-bearing NOD/SCID mice (figure 3). Adoptive transfer of autologous NK cells may represent a novel immunotherapeutic strategy in the management of leukaemia in patients not eligible for SCT, due to age or lack of a suitable donor. Clinical translation of a continuing progress in understanding the mechanisms involved in NK cell-mediated immunity will define the importance of NK cells as specialised cellular tools for enhancing the antileukaemic immune response.

Future perspectives

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