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Review

Natural killer cells in the treatment of high-risk acute leukaemia

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ABSTRACT

Several studies have shown that in patients with acute leukaemia given allogeneic haematopoietic stem cell transplantation (allo-HSCT) large part of the therapeutic effect lies on the anti-tumour effect displayed by cells of both adaptive and innate immunity. This evidence has also opened new scenarios for the treatment of patients with other haematological malignancies/solid tumours. In particular, donor-derived natural killer (NK) cells play a crucial role in the eradication of cancer cells in patients given an allograft from an HLA-haploidentical relative, especially when there is a killer inhibitory-receptor (KIR)–KIR ligand mismatched in the donor–recipient direction. Alloreactive donor-derived NK cells have been also demonstrated to kill recipient antigen-presenting cells and cytotoxic T lymphocytes, thus preventing graft-*versus*-host disease (GvHD) and graft rejection and to largely contribute to the defence against cytomegalovirus infection in the early post-transplant period. Several clinical studies have recently focused also on the influence of NK-cell activating receptors on the outcome of allo-HSCT recipients; in particular, *B/x* haplotype donors offer clinical advantages compared with *A/A* donors, even when the donor is an HLA-identical volunteer. Altogether, these data have provided the rationale for implementing phase I/II clinical trials based on adoptive infusion of either selected or *ex vivo* activated NK cells from an HLA-mismatched donor. This review summarizes the biological and clinical data on the role played by NK cells in patients with high-risk acute leukaemia, focusing also on the still unsolved issues and the future perspectives related to the approaches of adoptive NK cell therapy.

1. Introduction

Different lymphoid populations, including natural killer (NK) cells, αβTCR$^+$ and γδTCR$^+$ T lymphocytes, have been shown to mediate anti-tumour activity [1]. This effect is primarily related to the ability of lymphoid cells to kill tumour cells and/or secrete cytokines, which may act either directly on target cells, or indirectly potentiating the activity of other effector cells. Various approaches have been envisaged to increase the anti-tumour activity of αβTCR$^+$ T cells in view of their capacity to specifically recognize tumour antigens and to undergo clonal expansion *in vitro* and *in vivo* [2,3]. In this context, numerous protocols of vaccination, as well as of adoptive infusion of tumour-specific T cells, have been developed and applied, particularly to treatment of melanoma [4,5]. The reported limited clinical efficacy of these approaches may reflect the poor capacity of the infused cells to effectively traffic and home to tumour sites, this resulting in a suboptimal T cells/tumour target cell ratio [5]. Another important cause of failure has to be attributed to the marked inhibitory effect exerted by tumour cells themselves and by cells of the tumour microenvironment on T-cell proliferation and function [6]. Notably, similar inhibitory mechanisms also apply to cells of innate immunity, such as NK cells [7–10].

NK cells are major players of innate immunity, displaying a potent anti-tumour activity, which is based on their ability to recognize molecular structures highly expressed on tumour cells [11], and recognized by NK cells by means of their activating receptor [12,13]. Seminal studies have, however, shown that the signals delivered by inhibitory receptors present on the surface of NK lymphocytes are even more important than the activating signals [14,15]. The most relevant inhibitory NK receptors recognize major

Abbreviations: allo-HSCT, allogeneic haematopoietic stem cell transplantation; NK, natural killer; KIR, killer inhibitory receptor; GvHD, graft-*versus*-host disease; haplo-HSCT, haploidentical haemopoietic stem cell transplantation; HLA, human leukocyte antigen; MHC, major histocompatibility complex; TCR, T-cell receptor; GvL, graft-*versus*-leukaemia; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; NCR, natural cytotoxicity receptors; CR, complete remission; G-CSF, granulocyte colony-stimulating factor; PB, peripheral blood; CLP, common lymphoid precursors; ILC, innate lymphoid cells; LFS, leukaemia-free survival; IL, interleukin; Cy, cyclophosphamide; mPred, methylprednisolone; Flu, fludarabine; ADCC, antibody-dependent cellular cytotoxicity; CAR, chimeric antigen receptror; BiKE/TriKE, bispecific and trispecific killer engagers; CD, cluster of differentiation.

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histocompatibility (MHC) class I molecules [11]. MHC-I$^+$ normal cells are usually protected from NK cell attack [16]. In contrast, both tumour and virus-infected cells frequently down-regulate MHC-I expression and are, therefore, susceptible to NK-mediated lysis [11]. In humans, the most important inhibitory receptors are represented by killer Ig-like receptors (KIRs) specific for allotypic determinants shared by groups of HLA-A, -B or -C alleles [17–22].

Undoubtedly, the most convincing clinical results in cancer therapies exploiting NK cell-mediated anti-tumour activity have been obtained in HLA-haploidentical haemopoietic stem cell transplantation (haplo-HSCT) performed to cure high-risk (*i.e.* otherwise fatal) acute leukaemia both in adults and in children (reviewed in [23]). In haplo-HSCT, alloreactive NK cells express inhibitory KIRs specific for HLA-class I alleles that are missing in the recipient, and/or activating KIRs, primarily KIR2DS1, recognizing given HLA ligands (HLA C2 alleles in the case of KIR2DS1) expressed by recipient cells [24,25]. This latter observation emphasizes the concept that, for tumour cell eradication, also activating receptors may play an important role, which, as recent studies have demonstrated, is not restricted only to the setting of haplo-HSCT, since patients with acute myeloid leukamia (AML) receiving the allograft from an HLA-matched unrelated donor positive for *KIR2DS1* had a lower relapse rate than those transplanted from donors negative for *KIR2DS1* [26].

In order to better exploit the anti-tumour activity of NK cells, adoptive transfer of either selected or *ex vivo* activated NK cell populations have been applied in tumour immunotherapy [27], these approaches having led to some encouraging results, although obtained in a limited number of cases.

In this review, we will focus on phenotypic and functional characteristics of NK cells that are relevant to their clinical exploitation in haplo-HSCT to treat leukaemias, paying attention also to criteria for donor selection. Novel approaches of graft manipulation that may offer further advantages in terms of optimization of NK-cell function and, thus, contribute to improving the clinical outcome, will also be analyzed. Furthermore, we will discuss in detail the results of trials based on adoptive infusion of NK cells, and the future strategies for redirecting NK cells towards tumour targets through approaches of molecular and genetic manipulation of their specificity.

2. NK cell biological aspects relevant for allo-HSCT

NK cells play an important role in host defence (because of their potent cytolytic activity against virus-infected cells and tumour cells, and production of cytokines and chemokines involved in inflammation) and in regulating both innate and adaptive immune response [28,29]. In humans, on the basis of the levels of CD56 antigen expression, it is possible to distinguish two main peripheral NK cell subsets, characterized by CD56^{bright} and CD56^{dim} expression, respectively. CD56^{bright} NK cells are predominant in peripheral lymphoid organs, while they represent only ~10% of peripheral blood (PB) NK cells. CD56^{bright} cells were identified as cytokine producing NK cells, while CD56^{dim} cells (which are largely prevalent in PB) as potent cytotoxic effectors. CD56^{dim} NK cells were thought to be unable to produce cytokines [30]. However, recent studies revealed that they can rapidly release large amounts of cytokines upon receptor-mediated cell activation [31,32]. Therefore, two fundamental functional activities of NK cells can be mediated by the same cells in a time frame compatible with innate response. Importantly, as will be discussed, CD56^{dim} cells include the alloreactive NK cells that play a central role in lysing leukaemia cells in haplo-HSCT.

As mentioned above, NK-cell function is finely tuned by a number of receptors with inhibitory and activating functional capability [11,23]. In an allogeneic setting, donor NK cells can kill recipient cells through the mechanism of "missing self" recognition", provided that the donor: (i) expresses a KIR-ligand which is missing in the recipient HLA genotype; and (ii) expresses the specific KIR, leading to a KIR/KIR-ligand mismatch in graft-*versus*-host (GvH) direction. According to the concept of "missing self recognition", donor NK cell alloreactivity can be predicted to occur in approximately 50% of patients given haplo-HSCT [11,23]. In comparison to alloreactive T lymphocytes, NK cells offer the advantage of inducing a graft-*versus*-leukaemia (GvL) effect without promoting graft-*versus*-host disease (GVHD) development. Indeed, healthy non-haematopoietic tissues of the recipient are protected from donor NK cell-dependent alloreactivity, since they lack activating receptors, which, by contrast, are expressed by both tumour and haematopoietic cells [11,23,33,34].

The prototypes of the NK activating receptors involved in tumour-cell recognition and lysis are collectively referred to as natural cytotoxicity receptors (NCRs). They include NKp46, NKp30 and NKp44 [35–39]. Of the NCR ligands, only the NKp30 ones have been well characterized [40–42]. Other activating receptors may play a relevant role in tumour cell killing. These include NKG2D, recognizing MICA/B and ULBP proteins on target cells [43], and DNAM-1, specific for poliovirus receptor (PVR, CD155) and Nectin-2 (CD112) [12]. Importantly, NK cells may also express activating KIRs recognizing HLA-I molecules [44]. These latter include KIR2DS1 and KIR2DS4 [44–46]. As mentioned above, the engagement of KIR2DS1 with their HLA-I-ligands (HLC2 alleles) in an allogeneic setting has been shown to strongly activate NK cells and to contribute to the anti-leukaemic effect of allogeneic HSCT [23].

Alloreactive NK cells have been demonstrated to positively affect the outcome of T-cell depleted HSCT from an HLA-haploidentical relative in both adults with AML [47,48] and children with acute lymphoblastic leukaemia (ALL) [49,50]. Indeed, in patients receiving the graft from a NK alloreactive donor, the probability of leukaemia recurrence was particularly low, while the probability of leukaemia-free survival (LFS) was found to be at least as good as that of patients transplanted in a similar disease phase from an HLA-matched sibling or unrelated volunteer. The donor NK-mediated GvL effect was particularly evident when patients with acute leukaemia were transplanted in morphological complete remission (CR) and, in children and young adults, when the donor was the mother [47,51].

In the last 2 decades, haplo-HSCT has been mainly performed through a standardized method of T-cell depletion based on the positive selection of CD34$^+$ cells from peripheral blood by magnetic beads using the CliniMACS (Miltenyi) system [47,52–54]. Propaedeutic to this clinical translation was the observation that the use of "megadoses" of granulocyte colony-stimulating factor (G-CSF)-mobilized PB-derived haematopoietic stem cells (HSC) was shown, in animal models, to be able to elude the residual anti-donor T lymphocyte reactivity of the recipient [55,56].

In view of the central role played by NK cells in terms of clearance of leukaemia blasts in patients undergoing haplo-HSCT, a precise definition of the mechanisms governing NK-cell differentiation towards mature, cytolytic, KIR$^+$ cells is particularly important [23]. It has clearly been established that NK cells originate from CD34$^+$ HSCs through discrete stages of differentiation in *vitro* and *in vivo*, identifiable on the basis of the sequential acquisition of various markers and receptors, as well as of functional characteristics. The NK-cell differentiation site is mainly bone marrow or other lymphoid tissues [30]. A first stage of differentiation is represented by the common lymphoid precursors (CLP) that may undergo differentiation also towards T and B lymphocytes [57] and other innate lymphoid cells (ILC) identified in recent years [58]. Interestingly, NK-cell differentiation from myeloid cell progenitors has also been reported [59–61]. The first wave of lymphoid cells detectable in PB 2–3 weeks after HSCT [62] is composed by CD56^{bright} cells. Although