Clinical Immunology 177 (2017) 3–11

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Natural killer cell adoptive immunotherapy: Coming of age

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ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form 6 February 2016
Accepted with revision 9 February 2016
Available online 14 February 2016

Keywords:
Natural killer cells
Adoptive immunotherapy
Ex vivo expansion
Hematopoietic stem cell transplant
Graft-versus-host disease
Graft versus leukemia
Acute myeloid leukemia

ABSTRACT

Cell therapy is a promising alternative to harsh chemotherapy and radiation therapy for cancer. Natural killer (NK) cells in particular have great potential for direct use in adoptive immunotherapy (AI) for cancer and to improve the graft-vs-leukemia (GVL) effect of hematopoietic stem cell transplants (HSCTs). NK cell number and function are associated with a strong GVL effect without inducing graft-versus-host disease in most settings. Clinical trials demonstrating the therapeutic role of NK cells in HSCT recipients or testing the safety and efficacy of AI with NK cells have been primarily directed at treating acute myeloid leukemia, although investigators have used NK cells for treatment of other hematological diseases, sarcomas, carcinomas, and brain tumors. Major challenges must be overcome in making NK cell-based therapy cost-effective, the most important being the need to collect or generate an adequate number of effector cells. In this review, we discuss protocols for isolation, expansion, and *in vitro* propagation of large quantities of functional NK cells that meet the criteria for clinical applications. Among the methods described are the use of bioreactors for scaling up production and expansion of NK cells in the presence of interleukins and feeder cells. We also discuss novel methodologies that optimize the generation of clinical grade NK-cell products for AI.

1. Introduction

Cell therapy is a promising alternative to chemotherapy and radiation therapy for cancer, especially in the context of an allogeneic hematopoietic stem cell transplant (HSCT) for treatment of a hematopoietic malignancy. The curative potential of a HSCT is known to depend on not only the intensity of therapy but also the GVL or graft-versus-malignancy [1] effect exerted by the immune cells present in the stem cell graft. Despite many diagnostic and therapeutic advances, HSCT remains a procedure with high morbidity and associated with mortality rates of up to 50% depending on the transplant type (related or unrelated, matched or mismatched) and conditioning regimen used [2]. The main causes of death after an HSCT are recurrence of primary disease and graft-versus-host disease (GVHD) [3].

A major focus of clinical and translational research over the past decade has been using natural killer (NK) cells to enhance GVL without worsening GVHD [4–8]. The ability to differentiate GVL and GVHD on the basis of lymphocyte type would enable transplant engineering for improved long-term survival and potentially enable adoptive immunotherapy (AI) of *in vitro* expanded activated lymphocytes with activity against malignant cells in or out of the transplant context. Researchers have explored various subgroups of lymphocytes for this purpose [9]. NK cells appear to be natural candidates for AI because they are specialized lymphocytes with activity against cancer and virally infected cells with little or no GVHD activity [8,10]. However, NK cells are found relatively infrequently in peripheral blood (PB), and *in vitro* expansion of NK cells may be accompanied by expansion of undesirable T lymphocytes, necessitating additional techniques for NK cell purification [7,11–13].

Some challenges still must be overcome to make NK cell-based therapy safe and cost-effective, the most important of which is the need for an adequate number of active cells. In this review, we describe methodologies used to obtain and expand NK cells and their possible clinical applications in AI.

2. Mode of action of NK cells as cell therapy agents

NK cells play important roles in the elimination of virally infected and malignant cells. NK cells are activated to lyse tumors that have increased expression of stress-related ligands and/or decreased expression of self major histocompatibility complex class I and produce cytokines and chemokines for their self-regulation or for regulation of other immune effectors [6]. Upon activation, NK cells induce target

Abbreviations: NK, natural killer; AI, adoptive immunotherapy; HSCT, hematopoietic stem cell transplant; GVL, graft versus leukemia; GVHD, graft-versus-host disease; PB, peripheral blood; IFN, interferon; KIR, killer immunoglobulin-like receptor; AML, acute myeloid leukemia; MM, multiple myeloma; IL, interleukin; aAPC, artificial antigen-presenting cell.

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http://dx.doi.org/10.1016/j.clim.2016.02.003
1521-6616/

cell apoptosis via contact-dependent cytotoxicity primarily mediated by perforin and granzyme B [14–17] and secretion of proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ [18–27].

NK cells can recognize and kill cells via antibody-dependent cellular cytotoxicity [28,29] mediated by CD16 engagement of the Fc end of IgG1 or IgG3. NK cells are the principal cellular mediators of cancer elimination during treatment with antibodies specific for tumor-associated antigens, such as an anti-CD20 monoclonal antibody [30,31]. NK cells are known to play a significant role in GVL without inducing GVHD at least in part because normal tissues do not express the activating ligands that are recognized by NK cells. NK cells may actually reduce GVHD by depleting recipient dendritic cells [32] and thereby eliminating antigen cross-presentation. Surprisingly, posttransplant infusions of expanded donor NK cells were associated with severe acute GVHD in five of nine patients with solid tumors who had received HSCT [33], and it is not clear why the GVHD association was seen so strongly in this trial and not in others. In allogeneic HSCT recipients, improved engraftment and decreased relapse rates are often observed when inhibitory receptors of donor NK cells (primarily killer immunoglobulin-like receptors [KIRs]) [6] are mismatched with human leukocyte antigen class I molecules in the recipient, leading to a reduced activation threshold [16,34]. This alloreactive potential of NK cells has been exploited as a tool for cell immunotherapy [8] and can be used to eradicate residual disease after allogeneic HSCTs in patients with leukemia refractory to chemotherapy [35,36].

2.1. Use of NK cells in the treatment of acute myeloid leukemia

Acute myeloid leukemia (AML) is a hematological malignancy consisting of a heterogeneous group of high-grade myeloid neoplasms mostly affecting the elderly (mean age, 66 years). The annual incidence of new AML cases is 4 per 100,000 in the United States [37,38]. In southern Brazil, the prevalence is reported to be 1 case per 100,000 per year [39,40].

HSCT is an effective and potentially curative treatment of AML [38,41–44] but is associated with high morbidity and mortality rates, and GVHD is its foremost serious complication [45,46]. Researchers demonstrated that patients with AML who received haploidentical transplantation of stem cells from donors with NK-cell alloreactivity had decreased risk of relapse, no GVHD, and better event-free survival rates than did those who received transplants from nonalloreactive donors [32,47].

Also, allogeneic NK cells may exert antitumor activity when delivered as AI to nontransplanted patients. In a study by Miller and colleagues, 5 of 19 AML patients with poor prognoses experienced complete remissions after AI with haploidentical NK cells, and use of KIR-mismatched donor cells resulted in an increased rate of complete remissions [48].

2.2. Other clinical applications of NK cells

Studies of the safety and efficacy of AI using NK cells have been primarily directed at the treatment of AML, although the use of NK cells for treatment of other hematological diseases has been reported. In a pilot study by Bachanova et al. [49], four of the six patients with advanced B-cell non-Hodgkin lymphoma who received haploidentical NK cell infusions had objective clinical responses.

NK cells also have been infused to treat multiple myeloma (MM) [28] following reports of evidence suggesting that NK cells have anti-MM activity [50,51] and contribute to graft-versus-myeloma reactions along with T lymphocytes [28,52–54]. In a study by Shi et al. [55], haploidentical KIR ligand-mismatched NK cells were infused into patients with relapsed or refractory MM. The results indicated that the adoptive transfer of NK cells in this setting is safe, does not diminish engraftment, and does not cause GVHD. However, the real contribution of AI with NK cells in that study is difficult to assess owing to the fact that patients also received autologous PB stem cell transplantation, so the authors pointed out suggestions to enhance the demonstration of efficacy in future studies. A more recent study demonstrated the safety of using NK cells for poor-prognosis MM, with the results further supporting the hypothesis that NK cell-based therapy can be optimized and become effective to the point of being incorporated as treatment of MM [56].

Given their increased recognition of antibody-coated and genotoxically stressed cells while maintaining tolerance to healthy cells, NK cell AI may be attractive in combination with standard chemoradiation and antibody-based therapy for disseminated solid cancers [57]. NK cells are highly cytotoxic in a wide range of solid tumor cell lines [12,58–62] and can infiltrate solid tumors [63].

In a phase 1 clinical trial, patients with advanced non-small cell lung cancer received a combination of chemotherapy and NK cells [61]. Patients with adenocarcinoma or squamous cell carcinoma were enrolled, and the combination was found safe and potentially effective. Also, Krause et al. [64] tested activated NK cells in patients with metastatic colorectal cancer or non-small cell lung cancer in a phase 1 clinical trial to evaluate their tolerability, feasibility, and safety. Overall, the results indicated that the use of the activated NK cells was safe. Regarding clinical response, these findings may be somewhat limited by the fact that the patients were in advanced disease states when they entered the study. Future clinical studies of patients with lower tumor burdens and higher cell doses may better determine the clinical value of immunotherapy with NK cells [61,64].

Geller and colleagues used allogeneic NK cells to treat recurrent ovarian and breast cancer in a phase 2 study [62]. An important finding was that sustained NK cell expansion *in vivo* may be limited by host rejection, competition with host lymphocytes, or suppression by recipient regulatory T cells or myeloid-derived suppressor cells. The authors suggested that to evaluate the clinical benefit of NK cells against solid tumors, more effective strategies to augment NK cell persistence and expansion *in vivo* are required. Also, more intense immunosuppression of the host may allow for better *in vivo* NK cell expansion [65,66].

NK cells have an important influence on the efficacy of anti-GD_2 therapy for neuroblastoma [67,68]. Furthermore, augmenting NK cell function improves direct control of metastatic disease [69] and increases the response to anti-GD_2 therapy in preclinical models [70]. NK cells are also important mediators of the graft-versus-tumor effect of HSCTs [71–73].

NK cells may also be effective against brain tumors such as medulloblastoma [74,75]. We initiated a phase 1 clinical trial in which autologous expanded NK cells are delivered locoregionally into the fourth ventricle in patients who have undergone resection of recurrent infratentorial tumors (NCT02271711).

3. Defining the protocol and obtaining the cells

The anti-tumor efficacy of NK cells depends on their maturation and activation statuses. Thus, protocols for isolation, expansion, and *in vitro* production of NK cells following Current Good Manufacturing Practices guidelines have been developed to produce large quantities of functional NK cells for clinical applications [22]. *Ex vivo* expansion of NK cells at clinical grade and scale can be performed using flasks, culture bags, gas-permeable static cell culture flasks (G-Rex), and bioreactors [7,11,13,76] depending on the initial number of cells and their intended application.

Clinical trials require large numbers of cells, with the typical number of cells for infusion ranging from 5×10^6 to 5×10^7 NK cells/kg body weight [48,49]. Infusion of as many as 1×10^8 NK cells/kg has been reported [56]. Researchers in many clinical trials have used NK cells derived via apheresis [7,77,78], a technique for harvesting of large numbers of lymphocytes from PB. Mononuclear cells are typically obtained using large-volume leukapheresis followed by depletion of $CD3^+$ T cells, possible secondary enrichment for $CD56^+$ cells, and overnight