Cytotherapy, 2014; 16: 835–844

International Society for Cellular Therapy
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Immunomagnetic selection or irradiation eliminates alloreactive cells but also reduces anti-tumor potential of cytokine-induced killer cells: implications for unmanipulated cytokine-induced killer cell infusion

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Abstract

Background aims. Cytokine-induced killer (CIK) cells may offer a novel therapeutic approach for patients with malignancies relapsing after allogeneic stem cell transplantation. Although CIK cells display negligible alloreactivity and cause minimal graft versus-host-disease (GVHD), high CIK cell doses required during relapse may pose a risk for severe GVHD, specifically in the mismatched or haploidentical transplantation setting. Manipulation of CIK cells may reduce risk for GVHD without affecting the anti-tumor potential. *Methods.* In this pre-clinical study, we provide a detailed functional comparison of conventional and irradiated, CD56-enriched or T-cell receptor α/β-depleted CIK cells. *Results. In vitro* analysis showed retained anti-leukemic and anti-tumor potential after CIK cell manipulation. Even being sequentially infused into immunodeficient mice grafted with malignant cells, cytotoxic effects were fewest after irradiation but were improved by CD56 enrichment and were best with conventional CIK cells. Hence, considering the proliferative capacity of inoculated malignancies and effector cells, a single dose of conventional CIK cells resulted in prolonged disease-free survival and elimination of rhabdomyosarcoma cells, whereas sequential infusions were needed to achieve comparable results in leukemia-bearing mice. However, this mouse model has limitations: highly effective conventional CIK cells demonstrated both limited xenogenic GVHD and low alloreactive potential *in vitro*. *Conclusions.* Our study revealed that conventional CIK cells demonstrate no significant alloreactive potential but provide the strongest anti-tumor efficacy compared with manipulated CIK cells. Conventional CIK cells may therefore be tested in high numbers and short-term intervals in patients with impending relapse even after mismatched transplantation.

Key Words: *anti-tumor potential, cytokine-induced killer cells, mouse model*

Introduction

Allogeneic stem cell transplantation (allo-SCT) is an established treatment for high-risk acute leukemia in children and may also be of benefit in patients with high-risk soft-tissue sarcoma. In both groups, the therapeutic success is significantly compromised by the high risk of relapse. In patients with impending relapse, donor lymphocyte infusion represents a curative option, which is of limited value when initiated during overt hematological relapse and which, because of the high number of T cells needed, is associated with a considerable risk of severe acute graft-versus-host disease (GVHD) (1). Especially in the mismatched or haploidentical transplantation setting, the risk for GVHD depends on the number of alloreactive cells administered and the number and potential severity of human leukocyte antigen (HLA) mismatches between donor and recipient. Because of their negligible alloreactivity and minimal risk for

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(Received 14 October 2013; accepted 8 January 2014)

http://dx.doi.org/10.1016/j.jcyt.2014.01.003

GVHD, cytokine-induced killer (CIK) cells may serve as an improved pre-emptive immunotherapeutic intervention for high-risk patients with acute leukemia or soft-tissue sarcoma after allo-SCT (2–4).

CIK cells represent an *in vitro*-generated heterogeneous population consisting of different effector cells, a small number of $CD3^{-}CD56^{+}$ natural killer (NK) cells and a large number $CD3^{+}CD56^{-}$ T cells mainly showing T-cell receptor (TCR)α/β and, to a lesser extent, TCRγ/δ phenotype, and, in consequence of the *in vitro* culture condition, an expanding number of $CD3^{+}CD56^{+}$ NK-like T cells (2,5–7). CD56- and TCRγ/δ-expressing cells are endowed with low alloreactive potential, whereas TCRα/β-expressing cells are associated with high risk for GVHD. CIK cells being applied in high numbers and short-term intervals needed for treatment of overt or impending relapse may therefore raise the risk for severe GVHD, especially in the mismatched or haploidentical transplantation setting.

Pievani *et al.* (6) recently described the dual function of $CD3^{+}CD56^{+}$ CIK cells, demonstrating both major histocompatibility complex (MHC)-restricted and MHC-unrestricted cytotoxicity as $CD8^{+}$-specific effector T and NK-like T cells, respectively. In general, similar to NK cells, these cells are involved in various immune responses including control of infections, tumor killing and tolerance induction, that is, leading to suppression of GVHD after allo-SCT (2,8–10). Furthermore, considering *in vivo* homing, these cells are able to migrate to the main sites of tumor infiltration (10–12). NK and NK-like T-effector cell subpopulations with low alloreactive but potent anti-tumor activity may be provided by CD56 enrichment of the bulk CIK cell population (5).

Accordingly, TCRα/β depletion of CIK cells may retain enriched effector cells such as NK cells and γ/δ T cells, with comparable capability (13). Similar to NK cells, γ/δ T cells are innate-like lymphocytes endowed with anti-tumor activity that is not dependent on MHC presentation of specific peptide antigens but instead relies on the recognition of ligands that bind to the activating NK cell receptor NKG2D or to the TCR (14–19). In addition, most human γ/δ T cells are capable of targeting a variety of malignant cell types (15,20,21). The high levels of CD62L in γ/δ T cells are consistent with their proposed naive/central memory phenotype and suggest that these cells can be recruited to peripheral lymphoid organs (22). Furthermore, it was demonstrated that γ/δ T cells did not cause GVHD across the MHC barrier (23).

Most commonly used CIK cell protocols last for 21 d and use interferon-γ, anti-CD3 antibody and repetitive administrations of interleukin (IL)-2 for *in vitro* activation of CIK cells (2–7). We recently used IL-15 instead of IL-2 for CIK cell activation to increase cytotoxic potential and to shorten *in vitro* expansion time of CIK cells (24). Compared with standard CIK cell protocols, IL-15-expanded CIK cells showed higher numbers of T cells with potential risk for GVHD, namely TCRα/β-positive T cells that additionally expressed high levels of activation antigen CD25.

In the present study, we analyzed whether immunomagnetic selection of CIK cell subpopulations or irradiation of CIK cells after 10 days of *in vitro* stimulation in the presence of IL-15 may segregate alloreactivity and anti-tumor potential. Manipulated CIK cells, for example, irradiated, CD56-enriched ($CD56^{+}$) and TCR$\alpha\beta$-depleted ($TCR\alpha\beta^{-}$) CIK cells, were compared with conventional, unmanipulated IL-15-activated CIK cells in terms of anti-tumor, alloreactive or xenogenic activity *in vitro* and *in vivo*, respectively. *In vivo* experiments were performed in immunodeficient NOD/SCIDγc^{-} (NSG) mice models that permit human xenografts and best mimic impending relapse after transplantation (25–27).

Methods

Acute myeloid leukemia and rhabdomyosarcoma cell lines and primary AML cells

Primary acute myeloid leukemia (AML) blasts were obtained and cultured as described previously (24). M4 subtype AML cell line, THP-1, alveolar rhabdomyosarcoma (RMS) cell line RH30 and embryonal RMS cell line TE671 were cultured according to the manufacturer's instructions (24,27,28).

Generation of CIK cells

After approval through the ethics review board of the Medical Faculty of the University of Frankfurt/Main, Germany (Geschäfts No. 298/07) and written informed consent was given by healthy donors, CIK cells were generated from peripheral blood mononuclear cells (PBMC) of healthy donors by standard Ficoll separation, as previously described (24). Cells were re-suspended at a density of 3×10^{6} cells/mL in Roswell Park Memorial Institute medium 1640, supplemented with 10% fetal calf serum, L-glutamine and antibiotics in cell culture flasks (Corning Flask with 2-µm vent cap, 225 mL, 75 cm^{2}, Corning Incorporated, Corning, NY, USA) and primed by adding 1000 U/mL interferon-γ (Imukine, Boehringer Ingelheim, RP, Germany) on day 1 and 100 ng/mL anti-CD3 antibody (MACS GMP CD3 pure, Miltenyi Biotec, NRW, Bergisch Gladbach,