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Cytomegalovirus-specific cytokine-induced killer cells: concurrent targeting of leukemia and cytomegalovirus

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Abstract

Background aims. Human cytomegalovirus (CMV) infection and reactivation is a leading complication of allogeneic hematopoietic stem cell transplantation (HSCT). In addition to drug treatment, the adoptive transfer of virus-specific T cells to restore cellular immunity has become a standard therapy after allogeneic HSCT. We recently demonstrated potent anti-leukemic activity of interleukin (IL)-15–activated cytokine-induced killer (CIK) cells. With the use of the same expansion protocol, we asked whether concurrent CMV antigen-pulsing might generate CIK cells with anti-leukemic and anti-CMV activity. *Methods.* CIK cells expanded in the presence of interferon-γ, IL-2, IL-15 and anti-CD3 antibody were pulsed once with CMVpp65 peptide pool. CMV-specific CIK (CIK_{pp65}) and conventional CIK cells were phenotypically and functionally characterized according to their cytokine secretion pattern, degranulation capacity and T-cell receptor (TCR)-mediated and NKG2D-mediated cytotoxicity. *Results.* We demonstrated that among CIK cells generated from CMV-seropositive donors, a single stimulation with CMVpp65 protein co-expanded cytotoxic CMV-specific cells without sacrificing anti-tumor reactivity. Cells generated in this fashion lysed CMVpp65-loaded target cells and CMV-infected fibroblasts but also leukemic cells. Meanwhile, the alloreactive potential of CIK_{pp65} cells remained low. Interestingly, CMV reactivity was TCR-mediated and CMV-specific cells could be found in $CD3^+CD8^+CD56^{+/-}$ cytotoxic T-cell subpopulations. *Conclusions.* We provide an efficient method to generate CIK_{pp65} cells that may represent a useful cell therapy approach for preemptive immunotherapy in patients who have both an apparent risk of CMV and impending leukemic relapse after allogeneic stem cell transplantation.

Key Words: *CIK cells, CMV, cytotoxicity, immunotherapy, leukemia*

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established treatment for high-risk acute leukemia in patients. Survival after HSCT is significantly affected by relapse. Preemptive immunotherapy may prevent impending relapse in some cases [1]. Post-transplantation mortality is attributable in 45% of cases to recurrence and to 30% to refractory viral disease, which are the two leading causes of post-HSCT morbidity and mortality [2–5]. Among the viral infections, reactivations of Herpes viruses, such as cytomegalovirus (CMV) and Epstein-Barr virus, are the most common and occur principally because of a lack of virus-specific T cells [6]. CMV infections are asymptomatic in immunocompetent individuals, but reactivation from host or donor intracellular reservoirs or primary infection can lead to severe complications including

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uveitis, colitis and pneumonia in patients after HSCT. Because treatment with anti-viral agents (ie, ganciclovir, foscarnet) is of unspecific effectiveness, expensive and associated with substantial toxicity, adoptive immunotherapy to restore virus-specific cellular immunity is clearly an attractive alternative. A host of different approaches have proven successful for the expansion or isolation of virus-specific T lymphocytes for clinical use [7–13]. More importantly yet, several clinical trials demonstrated the efficacy of CMV-specific T cells after HSCT, whether as prophylactic or preemptive therapy, even though graft-versus-host-disease (GvHD) was observed in some of these patients [11,13–15].

We propose that a preemptive immunotherapy that combines potent anti-leukemic immunity with concomitant anti-viral immune reconstitution, yet does not cause significant GvHD, might offer an alternative approach to conventional cell therapies for patients with molecular relapse who are inherently at risk of viral reactivations. Therefore, cytokine-induced killer (CIK) cells co-enriched with CMV-specific cells might present a clinical advantage that offers the possibility of a tailored cellular product for the individual situation of the patient.

CIK cells were first described and characterized by Schmidt-Wolf *et al.* [16]. They are generated *ex vivo* by culturing peripheral blood mononuclear cells (PBMC) with interferon-γ (IFN-γ), activating monoclonal anti-CD3 antibody (mAb) and interleukin (IL)-2. CIK cells are a heterogeneous population consisting of an increasing fraction of T–natural killer (T-NK) cells co-expressing CD3 and CD56 ($CD3^+CD56^+$), classical T cells ($CD3^+CD56^-$) and a minority of NK cells ($CD3^-CD56^+$). The T-NK cells derive from T-cell precursors that acquire the CD56 molecule during *ex vivo* expansion [17]. Recently, Almehmadi *et al.* [18] showed that $CD56^+$ T cells were significantly increased in CMV^+ compared with CMV^-healthy subjects.

CIK cell cytotoxicity is mainly mediated by a non–major histocompatibility complex (MHC)-restricted NK-like mechanism, namely activating NK-cell receptor NKG2D [19–21]. Recently we reported significantly enhanced, mainly NKG2D-mediated cytolytic potential against acute lymphoblastic leukemia/lymphoma cell lines as well as primary human acute myeloid and defined lymphoblastic leukemia cells with the use of IL-15–stimulated instead of IL-2–stimulated CIK cells [22]. However, NKG2D only mediates the interaction of CIK and target cells. The final execution of apoptosis is then mediated through the release of granzyme and perforin. Subfamilies of NKG2 receptors are also known for their potential involvement in CMV recognition and control of virus infection [23,24]. The degranulation capacity of cells and release of granzymes can be indicated by surface expression of CD107a (LAMP-1). In addition, the secretion of IFN-γ indicates activation but does not reflect a specific cellular cytotoxic mechanism. In addition to the non–MHC-restricted killing, Pievani *et al.* [25] described concurrent retention of T-cell receptor (TCR)-mediated cytotoxicity of $CD3^+CD56^+$ CIK cells.

In the present study, we sought to generate proof-of-principle data to answer whether it is possible to generate CIK cell products that have both antigen-specific and anti-leukemic activity. We therefore determined whether CMV-reactive cells can be co-expanded from seropositive donor mononuclear cells in CIK-inducing cocktails by antigen-pulsing with CMVpp65 and whether anti-leukemic activity and lack of alloreactivity were maintained.

Methods

Cell lines and human fibroblasts

The M4 subtype acute myeloid leukemia (AML) cell line THP-1 and chronic myelogenous leukemia cell line K562 were purchased from the European Collection of Cell Cultures (ECACC, Salisbury, United Kingdom). The B-lymphoblastoid cell line T2 (somatic cell hybrid) was kindly provided by Thomas Lehrnbecher (Department of Children and Adolescents, University Hospital, Frankfurt; Goethe University, Frankfurt/Main, Germany). All cell lines were cultured according to the manufacturer's instructions. Human foreskin fibroblasts were infected with a clinical isolate of human CMV (Strain Hi91) [26] at a multiplicity of infection of 2 at 37°C for 48 h. Subsequently, the input virus was removed and the fibroblasts were maintained in culture medium for the cytotoxic assay.

Generation of CMV-specific CIK cells

Donor PBMCs were obtained after written informed consent was given, with approval from the Ethical Review Board of the Medical Faculty of the University Hospital Frankfurt, Frankfurt/Main, Germany (Geschäfts No. 69/13). CIK cells were generated from PBMC out of 30 mL of whole blood of selected HLA-A*0201 CMV-seropositive healthy donors by standard Ficoll separation as previously described [27]. This study was restricted to HLA-A2 donors not because of HLA-restricted expansion technology but because of the availability of dextramers for enumeration of pp65-specific T cells. Briefly, PBMCs were adjusted to a density of 3×10^6 cells/mL in Roswell Park Memorial Institute 1640 medium+GlutaMax medium (Life Technologies) supplemented with 10% fetal calf serum and