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Genetically engineered fixed K562 cells: potent "off-the-shelf" antigen-presenting cells for generating virus-specific T cells

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Abstract

Background aims. The human leukemia cell line K562 represents an attractive platform for creating artificial antigen-presenting cells (aAPC). It is readily expandable, does not express human leukocyte antigen (HLA) class I and II and can be stably transduced with various genes. *Methods.* In order to generate cytomegalovirus (CMV) antigen-specific T cells for adoptive immunotherapy, we transduced K562 with HLA-A*0201 in combination with co-stimulatory molecules. *Results.* In preliminary experiments, irradiated K562 expressing HLA-A*0201 and 4-1BBL pulsed with CMV pp65 and IE-1 peptide libraries failed to elicit antigen-specific $CD8^+$ T cells in HLA-A*0201^+ peripheral blood mononuclear cells (PBMC) or isolated T cells. Both wild-type K562 and aAPC strongly inhibited T cell proliferation to the bacterial superantigen staphylococcal enterotoxin B (SEB) and OKT3 and in mixed lymphocyte reaction (MLR). Transwell experiments suggested that suppression was mediated by a soluble factor; however, MLR inhibition was not reversed using transforming growth factor-β blocking antibody or prostaglandin E_2 inhibitors. Full abrogation of the suppressive activity of K562 on MLR, SEB and OKT3 stimulation was only achieved by brief fixation with 0.1% formaldehyde. Fixed, pp65 and IE-1 peptide-loaded aAPC induced robust expansion of CMV-specific T cells. *Conclusions.* Fixed gene-modified K562 can serve as effective aAPC to expand CMV-specific cytotoxic T lymphocytes for therapeutic use in patients after stem cell transplantation. Our findings have implications for broader understanding of the immune evasion mechanisms used by leukemia and other tumors.

Key Words: *artificial APC, cytomegalovirus, cytotoxic T cell, fixation*

Introduction

The treatment of viral infection and malignant diseases by adoptive transfer of *in vitro* expanded antigen-specific T lymphocytes is an emerging approach with promising clinical efficacy (1–3). Professional antigen-presenting cells (APC) such as dendritic cells (DC) are key elements in the generation of virus-specific or tumor antigen-specific T cells in sufficient numbers for clinical use from naïve CD8 and CD4 lymphocytes (4). DC express major histocompatibility complex (MHC) class I and II (5) together with co-stimulatory molecules. Critically, 4-1BBL (CD137L) plays an important role in expanding antigen-specific CD8 T cells (6–9). Although DC are highly effective in stimulating T cells, they need to be matured in culture for up to 7 days before they can function as APC (10–12). In addition, the generation of DC is associated with high costs, and DC themselves cannot be expanded. These constraints have motivated a number of investigators to make aAPC with comparable ability to engage and co-stimulate CD4 and CD8 lymphocytes. The mouse NIH3T3 fibroblast lines (13) and the chronic myeloid leukemia K562 line have been used for this purpose (14). In contrast to DC, such aAPC have the advantage of being infinitely renewable and expandable, "off-the-shelf" products. With the potential to be distributed worldwide, such aAPC would improve the standardization, speed and reliability of generating T cell products. Several investigators have used

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genetically engineered K562 based aAPC to generate tumor-specific T cells for adoptive immunotherapy (15,16). We set out to generate a library of K562 transduced with common MHC class I and II antigens and co-stimulatory molecules for use as aAPC. We identified an inherent antiproliferative property of both wild-type and transduced K562 lines, which could be eliminated by fixation in formaldehyde. Here we describe how such fixed K562 lines transduced with human leukocyte antigen (HLA)-A*0201 and 4-1BBL can induce robust $CD8^+$ T cell responses to cytomegalovirus (CMV) IE-1 and pp65 peptide libraries.

Methods

Blood samples

Leukapheresis cells collected from healthy donors were obtained under Institutional Review Board–approved protocols for allogeneic stem cell transplantation. Peripheral blood mononuclear cells (PBMC) were isolated from leukapheresis product by Ficoll-Hypaque technique and cryopreserved via standard procedure. Cells were thawed and rested overnight at 37°C, 5% CO_2 in RPMI-1640 (Life Technologies, Carlsbad, CA, USA) supplemented with 10% human AB serum (Gemini Bio-Products, West Sacramento, CA, USA), 2 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Life Technologies) (T cell medium).

Cell lines

K562 were cultured in RPMI-1640 supplemented 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Complete Medium). 293FT cells were cultured with Dulbecco's modified Eagle's medium (Life Technologies) supplemented 10% FBS, 2 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin.

Vector construction

4-1BBL was amplified from mature DC complementary DNA and cloned into pCI to yield pCI-4-1BBL (CD137L expression vector). 4-1BBL was subsequently sub-cloned into pMSCV-IRES-GFP (17). The CD137L open reading frame (ORF) was amplified from pCI-4-1BBL and sub-cloned into pCR4-TOPO vector with TOPO TA Cloning Kit (Life Technologies) to yield pCR4-4-1BBL. The CD137L ORF was subsequently excised from the pCR4 construct using BamHI. The IRES-GFP cassette was removed from pMSCV using XhoI and SalI, and the 4-1BBL ORF was cloned into this site to yield pMSCV-4-1BBL. Codon optimized CD80, CD83, and CD64 ORFs were synthesized by GeneArt (Life Technologies) and cloned into the lentiviral vector pRRLSIN-cPPT-MSCV-WPRE (18) (kindly provided by Dr Morgan, Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA) to yield pRRL-CD80, pRRL-CD83 and pRRL-CD64. Codon-optimized HLA-DRB1*1501 and HLA-DRA were cloned into pRRL as a fusion gene separated by T2A, a spacer sequence and a furin recognition sequence (19). HLA-DM and Invariant chain (Ii) were similarly designed and cloned into pRRL.

Generation of artificial APC

The pMSCV-4-1BBL vector and helper plasmid pCI-Ampho were transfected into 293FT cells using Lipofectamin 2000 (Life Technologies) according to the manufacturer's instructions. The pRRL-CD80, pRRL-CD83, pRRL-CD64, pRRL-HLA-DR15-DRA and pRRL-HLA-DM-Ii were co-transfected with the helper plasmids pMG, pRSV-Rev and pMDL/pRRE into 293FT cells using Lipofectamin 2000. All cell-free supernatants were harvested on day 4 and day 5 post-transfection and immediately frozen at −80°C. Retroviral transduction was performed with RetroNectin (Takara Bio Inc., Otsu/Shiga, Japan) coated plates as described previously (17). After adding thawed virus supernatant to the plates, the plates were centrifuged 1700*g* for 90 min at room temperature. After centrifugation, virus supernatants were discarded, then either K562 or HLA-A*0201 transduced K562 cell line (K-A2, kindly provided by Dr Griffioen, Leiden University Medical Center, Leiden, the Netherlands), suspended in complete medium, were added to the wells and incubated at 37°C, 5% CO_2. The next day, fresh complete medium was added. After 4–5 days, cells brightly expressing HLA-A*0201, HLA-DR, CD137L, CD80 and CD83 were sorted on a Becton Dickinson FACSAria II cell sorter (BD Biosciences, San Jose, CA, USA). HLA-DM/Ii was transduced into $HLA\text{-}DR^+$ cells, and Ii-bright cells were similarly sorted (see Supplementary Figure 1).

Monoclonal antibodies

The following commercially available fluorochrome-conjugated monoclonal antibodies (mAbs) were used: (i) α-HLA-A2-fluorescein isothiocyanate (FITC), α-HLA-DR-allophycocyanin (APC), α-CD137L-phycoerythrin (PE), α-CD80-PE-Cy5, α-CD83-APC, α-CD4-V500, α-CD8-APC-H7, α-CD107a-FITC and α-tumor necrosis factor (TNF)-PE-Cy7 (BD Biosciences, San Jose, CA, USA), (ii) α-CD3-eFluor