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Specific targeting of CD33$^+$ leukemia cells by a natural killer cell line modified with a chimeric receptor

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Abstract

We directed the human natural killer (NK) cell line YT by gene transfer of a humanized chimeric immunoglobulin T cell receptor to CD33, a marker on myeloid leukemias. The chimeric receptor was generated using a CD33 specific single-chain Fv (scFv) fragment based on the humanized antibody HuM195, the human IgG1 Fc domains and the human CD3 ζ signal chain. YT cells transfected by electroporation with the chimeric receptor gene specifically lysed the acute myeloid leukemia (AML) cell line KG1. This gene-modified NK cell line available in unlimited source could be an attractive tool in immunotherapy.

Keywords: NK cells; CD33; Chimeric receptor; Leukemia; Targeting; Gene transfer

1. Introduction

An adoptive transfer of tumor specific cytotoxic lymphocytes could be a strategy to compensate the inability of the immune system to generate sufficient tumor directed immune cells in tumor patients. This strategy is dependent on the availability, the tumor specificity and the effector properties of the transferred immune cells. Strategies to generate tumor specific T cells by receptor gene modification have been developed. Immunoglobulin-based chimeric T cell receptors (cIgTCRs), also called "T-bodies" [1], are of particular interest. They consist of a single-chain Fv (scFv) fragment [2] fused to a signal transducing chain like the common Fc receptor γ chain or the CD3 ζ chain combining the specificity of an antibody-based recognition with the T cell function. The retroviral gene transfer of cIgTCR constructs was successfully used to generate virus or tumor specific T cells [3–5]. Natural Killer (NK) cells could be another useful source of effector cells for an adoptive immunotherapy [6]. Recent data indicated that allogeneic NK cells mediate antileukemic effects in patients after allogeneic hematopoietic transplantation and prevent graft rejection and graft versus host disease (GvHD) [7].

A further facilitation could be accomplished by the utilization of an allogeneic effector cell line instead of primary cells. Effector cell lines are available in unlimited amounts, and their production is less time and cost intensive. The cytotoxic effector cell lines TALL-104 and NK-92 have been tested for their safety in clinical trials for patients with advanced cancer [8,9]. The genetical modification of effector cell lines could extend their specificity [10–12]. In this report, we describe the targeting of the NK cell line YT by gene transfer of a chimeric receptor construct specific for CD33$^+$ myeloid leukemia cells.

2. Material and methods

2.1. Cell lines

The human natural killer cell line YT was kindly provided by J. Yodoi (Institute for Virus Research, Kyoto, Japan)

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[13]. YT cells were cultured with RPMI-1640 supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) and 50 μg/mL gentamicin (all from Gibco/BRL) with a maximum concentration of 2.5×10^5 cells per mL. The human acute myeloid leukemia (AML) cell line KG1 friendly provided by U. Karsten (Max-Delbrueck Center (MDC), Berlin, Germany) was cultured in RPMI-1640 with 10% FCS and 50 μg/mL gentamicin.

2.2. Generation of the scFv-hFc and cIgTCR constructs

The secretory leader sequence of the human IgGκ light chain was created by PCR using the oligonucleotides leader-1C (5′-CATGAAGCTT AGCAGATCTA TGGGATGGAG CTGTATCATC CTCTTCCTGG TAGC-3′) and leader-2C (5′-CAGACCATGG AGTGCACGCC TGTAGCTGTT GCTACCAGGA AGAGGATGAT AC-3′), and was digested using *Hin*dIII and *Apa*LI (restriction enzymes from New England Biolabs, Frankfurt am Main, Germany). The CD33 specific scFv fragment was constructed using the V regions of the humanized monoclonal antibody (mAb) HuM195, kindly provided by D. Scheinberg (Memorial Sloan-Kettering Cancer Center, New York, NY) [14]. The V_L domain was amplified using primer HuM195-V_L1 (5′-TCTGGTGCAC TCCATGGACA TTCAGATGAC CCAGTCT-3′) and HuM195-V_L2 (5′-CACCACTCCC GGGCTTTCCT AGACCGGAAG TGGTTCCTTT GATTTCCACC TTGGTCCCTT GA-3′) and digested with *Apa*LI and *Sma*I. The V_H domain was amplified using primer HuM195-V_H1 (5′-AAGCCCGGGA GTGGTGAAGG TAGCACTAAA GGTCAGGTTC AGCTGGTGCA GTCT-3′) and HuM195-V_H2 (5′-TGGTGGATCC TGAAGAGACA GTGACCAGAG T-3′) and digested with *Sma*I and *Bam*HI. The V_L fragment was fused to the N-terminus of the V_H fragment using the *Sma*I site in the combining linker to obtain the scFv fragment. The secretory leader and the scFv fragment were cloned into the *Hin*dIII and *Bgl*II sites of pCMX-hFc [15] to obtain pCMX-scHuM195-hFc containing the scFv-hFc fusion protein scHuM195-hFc. The fragment containing the secretory leader and the scFv scHuM195 was cloned into the *Blp*I and *Not*I sites of pCDNA3-hFcζ [15] to obtain pCDNA3-scHuM195-hFcζ containing the cIgTCR scHuM195-hFcζ. The construct has been confirmed by sequencing.

The scFv-hFc construct scPhOx-hFc and the cIgTCR construct scPhOx-hFcζ specific for the hapten 2-phenyloxazoline-5-one (phOx) have been described [15] and were used as controls.

2.3. Expression and analysis of the scHuM195-hFc protein

293T cells were transfected with pCMX-scHuM195-hFc by calcium phosphate transfection as previously described [15]. Supernatants containing the scFv-hFc fusion protein were collected 3 days after transfection and used for FACS analysis. The AML cell line KG1 was stained with supernatant containing scHuM195-hFc protein followed by a FITC-conjugated goat anti-human IgG-Fcγ $F(ab')_2$ fragment. The scPhOx-specific scFv-hFc protein scPhOx-hFc [15] was used as control. The CD33 specific mAb WM54 (Dako, Glostrup, Denmark) followed by a FITC-conjugated goat anti-mouse IgG(H+L) $F(ab')_2$ fragment (Dianova, Hamburg, Germany) was used for comparison.

2.4. Transfection of YT cells

The plasmid pCDNA3-scHuM195-hFcζ was transfected by electroporation into the YT cell line as previously described [15]. Briefly, 10^7 YT cells resuspended in 800 μL RPMI-1640 containing 20 μg plasmid DNA were pulsed with 250 V and 975 μF using 0.4 cm electroporation cuvettes (Eurogentec, Cologne, Germany) and the GenePulser™ II with Capacitance Extender™ II (BioRad, Munich, Germany). Transfected YT cells were selected starting 48 h after electroporation by addition of 500 μg/mL G418 (Calbiochem, Bad Soden, Germany) to the culture medium. After 1-week selection, dead YT cells were removed by density gradient centrifugation using Lymphoprep™ (1077 g/mL, Nycomed, Roskilde, Denmark). Control YT cells were transfected with the cIgTCR gene construct scPhOx-hFcζ and selected with G418 and enriched using MACS as well. Mock transfected YT cells were transfected with the plasmid pCDNA3 and selected with G418.

2.5. Analysis and immunoaffinity purification of scHuM195-hFcζ expressing YT cells

The expression of the cIgTCR was detected using 1:100 diluted FITC-conjugated goat anti-human IgG-Fcγ $F(ab')_2$ fragment (Dianova). 5×10^5 cells were stained in PBS, 0.5% (w/v) BSA and 0.1% (w/v) NaN_3 (Sigma) for 15 min at 4 °C. Propidium iodide (Sigma) was added with a final concentration of 1 μg/mL to the stained cells before measurement using a FACSort™ (Becton Dickinson). Mock transfected YT cells were used as negative control. YT cells expressing the cIgTCR were enriched after 1–2 weeks selection with G418 by magnetic immunopurification (MACS™) according to the supplier's description (Miltenyi Biotec, Cologne, Germany). Briefly, 10^7 of the transfected YT cells were stained with 500 μL FITC-conjugated goat anti-human IgG-Fcγ $F(ab')_2$ fragment (Dianova) 1:100 diluted in sterile PBS supplemented with 0.5% (v/v) BSA (Sigma) and 2 mM EDTA (Serva) followed by an incubation with anti-FITC MicroBeads. MACS were performed using MS or LS columns (Miltenyi Biotec, Cologne, Germany). The enrichment was repeated after 1–2 weeks.

2.6. Cytotoxicity assay

The cytotoxicity was examined with standard ^{51}Cr-release assays as described. Briefly, the AML cell line KG1 was