Microchip-based fluorescence-activated cell sorting of antigen-specific CD137⁺CD8⁺ T cells in a disposable and closed cartridge system using the MACSQuant® Tyto™ Sorter

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Introduction

Adoptive T cell therapy has been shown to be a promising new strategy for treatment of a variety of malignancies. Exploitation of this potent therapeutic approach increases the need for easy and effective isolation of antigen-specific T cells in a clinical setting.

Recent technological advances like the MACSQuant® Tyto™, a benchtop microfluidic flow sorter that is fully closed, sterile, and easy to use, enable purification of target cells in compliance with GMP cell manufacturing requirements. The heart of the Tyto System is the MACSQuant TytoCard, a single-use cartridge carrying a sorting microchip, which allows for completely aseptic sorting conditions without cross-contamination between samples. Here we demonstrate the capacity of the MACSQuant Tyto to sort CD137 (4-1BB)⁺ antigen-specific cytotoxic T cells. CD137 is a member of the TNFR-family and functions as a marker for activated T cells by promoting T cell proliferation and survival. CD137 might be a promising selection marker for identifying and validating T cell responses to unknown antigens or epitopes since this method is not restricted to the knowledge of the immunogenetic peptide or HLA allele, which is necessary in the widely used peptide/MHC (pMHC) multimer or dextramer isolation of antigen-specific T cells[1]. As a model system, we used fresh peripheral blood mononuclear cells (PBMCs) and stimulated them overnight with different peptides to induce a CD137 expression. CD137-PE⁺ cytotoxic T cells were sorted in high purity and showed good viability as well as activation marker expression profiles in subsequent cell culture. These results demonstrate the applicability of the MACSQuant Tyto system for future medical-scale isolation of antigen-specific T cells.

Methods

1 Workflow overview

An overview of the workflow is shown in figure 1. Whole blood or buffy coat were obtained from healthy EBV/AdV-positive donors and PBMCs were isolated by density gradient centrifugation. Cells were suspended in RPMI 1640 medium supplemented with 5% human AB-serum at 1×10^7/mL in 24-well plates either with 1 µg/mL SEB or 1 µg/mL PepTivator® EBV Consensus and 1 µg/mL PepTivator AdV5 Hexon and stimulated overnight at 37 °C and 5% CO_2. SEB stimulation was used as a model system to generate higher frequencies of antigen-specific cells, comparable to antigen-specific expansion of T cells. Subsequently, cells were stained with CD8-VioBlue® and CD137-PE. In some experiments, additional markers were used for exclusion of unwanted cells in one channel, i.e., CD14-APC-Vio® 770, CD20-APC-Vio 770, and CD56-APC-Vio 770.

CD137⁺ CTLs were sorted on the MACSQuant Tyto and SEB-stimulated, sorted cells were cultivated for two days to validate viability and activation marker expression.

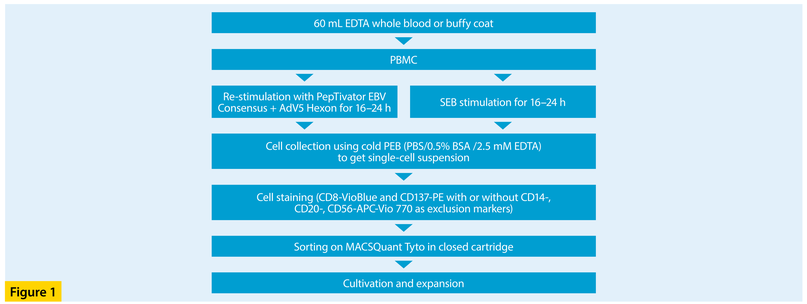

Figure 1

2 Sorting of antigen-specific CD137⁺CD8⁺ T cells

Isolation of antigen-specific T cells was performed entirely on the microfluidic flow sorter MACSQuant Tyto using a single-use cartridge. In total, 2×10^7 cells with 0.4–3×10^6 target cells were used and CD137⁺CD8⁺ CTLs were isolated with (fig. 2A,B) or without (fig. 2B) exclusion of CD14⁺, CD20⁺, and CD56⁺ cells. The sample was sorted at 4 mL/hour and a pressure of approximately 130 mbar. Depending on the sorted volume, the sort took 1 h to 2.5 h to complete.

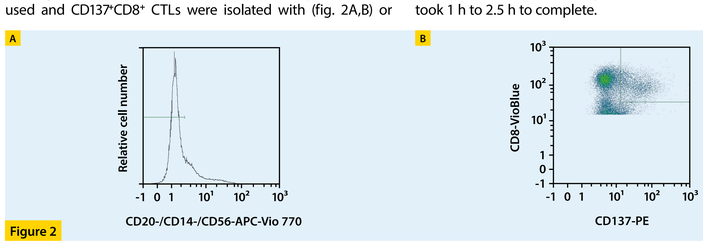

Figure 2

Results

1 Flow cytometry analysis of cell sorting

Flow cytometry analysis of the sorted fractions was performed on the MACSQuant Analyzer 10 using the MACSQuantify® Software. Data were gated on viable single cells (fig. 3A–C) and then plotted to show CD137-PE vs. CD8-VioBlue (fig. 3E). Cell viability was assessed by staining with propidium iodide (PI) prior to the measurement. For the analysis of isolated cells obtained by the depletion strategy, CD20⁺, CD14⁺, and CD56⁺ cells were evaluated separately during flow cytometry analysis (fig. 3D).

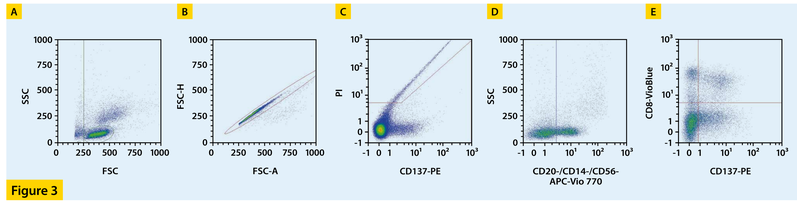

Figure 3

Samples of the input, non-target cell, and target cell fractions were analyzed according to the gating strategy depicted in figure 3. Figure 4 shows a representative flow cytometry analysis of the sorted fractions of an EBV/AdV-stimulated sample vs. an SEB-stimulated sample.

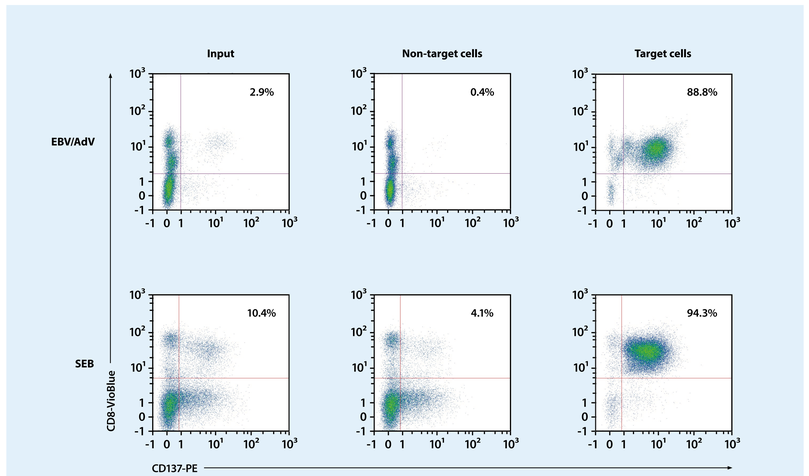

Figure 4

2 Cell sorting performance

CD137⁺CD8⁺ T cells from PBMCs (seven donors) with starting frequencies ranging from 1.8% to 12.1% were sorted on the MACSQuant Tyto. The average purity of enriched cells amounted to 90% (among viable leukocytes) and 91% (among viable lymphocytes) as shown in figure 5. The different symbols represent samples from individual donors. Samples were used for one or more experiments. The average yield of target cells in the enriched fraction and depletion of target cells in the non-target cell fraction was around 60%. For some samples however yields of up to 80% could be achieved. Consequently, sorting of 2×10^7 total cells containing 6% CD137⁺CD8⁺ T cells resulted in the recovery of about 1×10^6 target cells from a complete 10-mL sort. The addition of a dump channel for exclusion of CD19⁺, CD14⁺, and CD56⁺ cells as part of the sorting strategy, led to an increased purity of more than 95% (red asterisk). The viability of sorted cells was greater than 98% in all sorts, which demonstrates the gentleness of the sorting process.

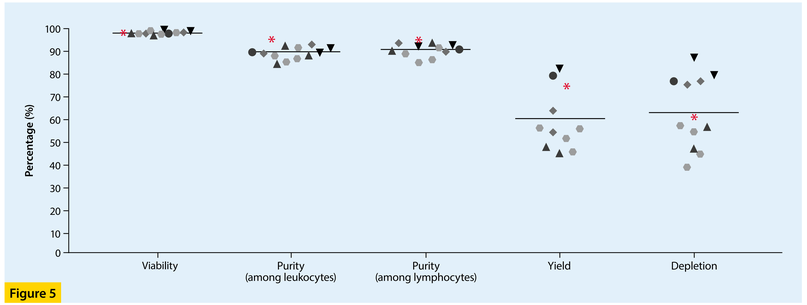

Figure 5

3 Cultivation of enriched antigen-specific T cells and activation marker expression

Unsorted and sorted SEB-stimulated cells were further cultivated in RPMI 1640 medium with 5% HSA and 20 ng/mL IL-2 for two days. Cell viability was assessed by PI staining and microscopy analysis. Sorted CD137⁺CD8⁺ cells maintained viability rates higher than 98% during two days of cultivation and displayed formation of cell clustering typical for activated T cells without visible debris and dead cells (data not shown). In addition, cultured cells were stained with CD69-FITC and CD137-PE to analyze activation marker expression. Sorted cells showed an expression pattern of activation markers similar to unsorted control samples (fig. 6A). The median fluorescence intensities (MFI) of CD69-FITC and CD137-PE of sorted and unsorted CD8⁺ cells were analyzed 30 h and 50 h after stimulation (fig. 6B). The enriched CD137⁺ CTLs showed a higher MFI, but both unsorted and sorted cells showed a decrease in CD137 expression and a beginning reduction of CD69 activation marker expression 50 h after stimulation, as expected.

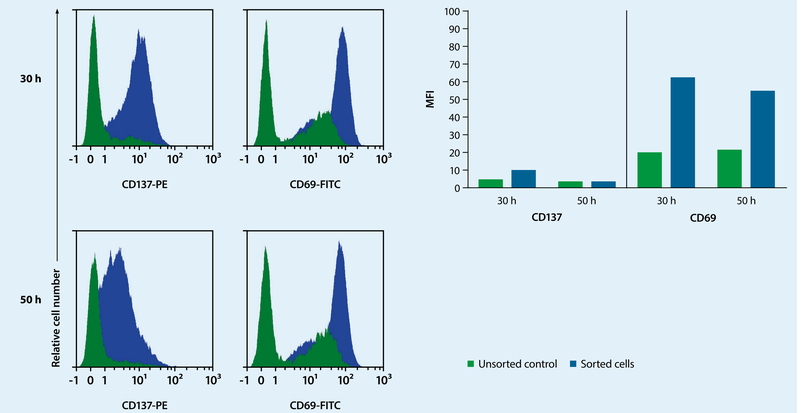

Figure 6

Conclusion

- The MACSQuant Tyto System enables isolation of antigen-specific cytotoxic T cells based on the expression of the activation marker CD137.
- Sorting strategies with additional depletion of CD14⁺ monocytes, CD19⁺ B cells, and CD56⁺ NK cells achieved purities of around 95%.
- Sorting of 2×10^7 cells containing 1.8–12% CD137⁺CD8⁺ T cells results in a target cell recovery of up to 85%.
- Upon cultivation SEB-stimulated sorted cells showed high viability rates and an activation marker expression profile similar to unsorted cells.

Reference
1. Wolfl, M. *et al.* (2007) Blood 110: 201–210.