Miltenyi Biotec

MACSQuant® Tyto™

Isolation of CD8+ antigen-specific T cells

Background

With adoptive T cell therapies (ACTs) becoming more prevalent, the need for easy and effective isolation of antigen-specific T cells in a clinical setting is growing. The MACSQuant® Tyto™ is a benchtop cell sorter that is fully closed, sterile, and easy to use. At the heart of the system is the disposable, single-use cartridge (fig. 1), which allows for completely aseptic sorting conditions with no chance of cross-contamination between samples. Here we demonstrate the MACSQuant Tyto's capacity to sort rare antigen-specific cytotoxic T cells out of fresh peripheral blood mononuclear cells (PBMCs). As a model we used PBMCs from a CMV-seropositive donor. The PBMCs contained less than 0.1% of CMV-specific T cells. CMV is a member of the herpes virus group and has a prevalence of 50–96% in human adults. [1–3] Once infected, the virus persists in the body. The infection is asymptomatic in healthy individuals, but in immunocompromised patients CMV can cause severe diseases.

Methods

Cell sample collection and preparation

Whole blood was collected from a CMV-seropositive donor using Vacutainer® CPT™ Mononuclear Cell Preparation Tubes with Sodium Heparin (BD™ Biosciences). PBMCs were isolated according to manufacturer's specifications. Cells were labeled with a PE-conjugated CMV Dextramer® (Immudex; HLA-A*0201 specifically recognizing the pp65 antigen sequence NLVPMVATV) and staining was performed according to manufacturer's protocols. Cells were subsequently labeled with a MACS® GMP CD8-APC antibody for further identification.

Isolation of antigen-specific T cells

Isolation of the antigen-specific T cells was performed entirely on the MACSQuant Tyto. The input sample contained 18.4×10^6 total PBMCs in 10 mL of MACSQuant Tyto Running Buffer (1.84×10^6 cells/mL) with 0.05% target cells. The sample was sorted at 4 mL/hour, using approximately 130 mbar of pressure. The sort took 2 h 30 min to complete.

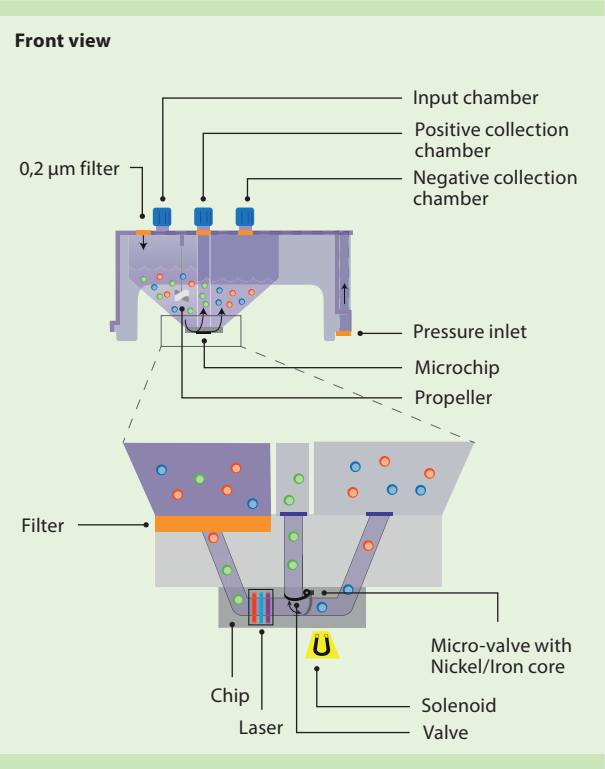

Figure 1: The sorting mechanism of the MACSQuant Tyto. Coming from the input chamber, cells enter the microchip through a microchannel where they get detected by the three lasers. Before entering the microchannel, potential cell aggregates are held back by a filter system to allow for a smooth sorting process. When a target cell (green) is identified, a magnetic pulse coming from the solenoid opens the microvalve, which then redirects the target cell into the positive collection chamber. In the default state, the valve is closed allowing non-selected cells (blue and red) to flow through into the negative collection chamber.

Analysis of target cells or analysis of cell isolation

Flow cytometry analysis was performed on the MACSQuant Analyzer 10 using the MACSQuantify® Software. Data were first gated on live single cells and then plotted to show CMV Dextramer-PE vs. CD8-APC. Cell viability was assessed by staining with the Hoechst dye.

Results

The MACSQuant Tyto enriched the target antigen-specific CD8⁺ cells from 0.05% to 93.54%, representing a 1,871-fold enrichment in a single pass through the system (fig. 2). We recovered 5,029 cells in the sorted fraction, representing a 54.67% yield of target cells from the total input fraction. The viability was greater than 98%, which demonstrates the gentleness of the sorting process.

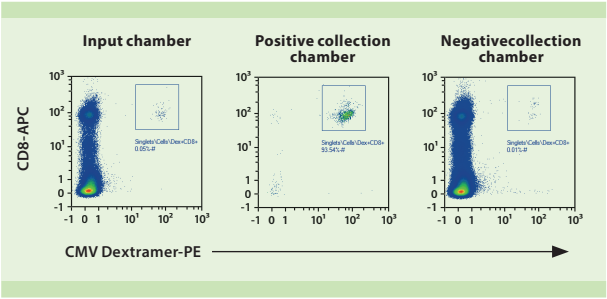

Figure 2: Enrichment of CMV-specific CD8⁺ T cells. PBMCs from a CMV-seropositive donor were labeled with CMV Dextramer-PE and CD8-APC and sorted on the MACSQuant Tyto. Dot plots show PBMCs prior to sorting (input chamber), the CMV Dextramer⁺ target cell fraction after sorting (positive collection chamber), and the cell fraction depleted of CMV Dextramer⁺ cells (negative collection chamber). Flow cytometry analysis was performed on the MACSQuant Analyzer 10.

Conclusions

These data demonstrate the MACSQuant Tyto's ability to isolate rare antigen-specific cytotoxic T cells in a closed system. We could achieve a purity above 90% even when the target cells made up less than 0.1% of the total cells. The yield amounted to more than 5,000 antigen-specific T cells. The ability to re-sort the depleted fraction preserves precious samples and provides the opportunity to increase the total target cell yield even further. Cells were sorted at a relatively low concentration of 1.84×10^6/mL because of purity considerations. If an operator needed to sort more cells in the same amount of time, the concentration could be increased, possibly at some expense to purity. Another option would be to use coincidence detection to increase purity at some expense to yield. The single-use, disposable sorting cartridge offers safety and ease of use. The operator is not exposed to patient samples in the form of aerosols and the cells can be transferred directly from the sterile cartridge into culture and expanded successfully in antibiotic-free medium (data not shown here). MACS® GMP-Grade Cartridges (in development) and Running Buffer as well as a MACS GMP-grade CD8 antibody will open new possibilities for cell product manufacturing for future medical applications.

References

1. Bate, S. L. *et al.* (2010) Clin. Infect. Dis. 50: 1439–1447.
2. Souza, M. A. *et al.* (2010) Rev. Soc. Bras. Med. Trop. 43: 359–361.
3. Staras, S. A. *et al.* (2006) Clin. Infect. Dis. 43: 1143–1151.

MACS Product	Order no.
MACSQuant Tyto Instrument*	130-103-931
MACSQuant TytoCard 24*	130-106-088
MACSQuant Tyto Running Buffer*	130-107-206
MACS GMP CD8-APC*	170-076-507

*For research use only

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