Isolation of CD8+ T cells from peripheral blood mononuclear cells (PBMCs)

Purified CD8+ T cells were isolated from peripheral blood of naïve donors by immunomagnetic sorting using CD8 MicroBeads, human (Miltenyi Biotec) and the autoMACS® Pro Separator (Miltenyi Biotec) according to the manufacturer’s protocol.

Cell transduction and evaluation of TCR expression by flow cytometry

CD8+ T cells were plated onto a 24-well plate and activated with anti-CD28 antibody (1 µg/mL), anti-CD3 antibody (100 ng/mL) and IL-2 (100 units/mL). Two days later, the indicated lentivirus and polybrene (6 g/mL) were added to each well, and the plate was spinoculated at 24,000 rpm for 60 min at 24 °C and then cultured at 37 °C overnight. Fresh media was added the following day, and the cells were cultured for an additional 3 days with a transduction efficiency ranging from 35% to 45% based on GFP expression. KK10-specific TCR expression by the transduced CD8+ T cells was determined by staining with PE-labeled HLA-B*2705/KK10 dextramer for 10 min at room temperature. Cells were also stained for human CD8 expression to confirm the purity of CD8+ T cells.
Results

Immunomagnetic sorting of CD8* T cells from PBMCs yields a 99% pure population of highly viable and functional CD8* T cells that can be efficiently transduced using lentiviral vectors (fig. 1A).

Primary CD8* T cells transduced with lentivirus expressing the TCR from the effective CTL clone or the two ineffective CTL clones displayed equivalent levels of the KK10-specific TCR clonotypes (fig. 1B) and exhibited equivalent potent inhibition (>80%) of in vitro HIV-1 infection (fig. 2).

Figure 1: Purified CD8* T cells isolated from PMBCs of an HIV naïve donor can be transduced and engineered to express HIV-specific TCRs that bind to KK10-specific dextramer. (A) Purified CD8* T cells were analyzed for viability and human CD8 expression following the 3 day activation period. (B) After 8 days of culture, the CD8* T cells were evaluated by flow cytometry for transduction by quantifying the fraction of GFP* cells. After gating on GFP* cells, their expression of the KK10-specific TCR was determined by measuring their binding to HLA-B*2705-KK10 dextramer.
Conclusion

Taken together, these data indicated that TCR clonotypes from ineffective CTLs have the intrinsic capacity to direct primary CD8$^+$ T cells to effectively kill HIV-infected cells and support the proposition that other TCR-independent factors such as epigenetic modifications may also contribute to the effective vs. ineffective functions of some CTL clones.

The autoMACS Pro Separator allows for completely automated and standardized isolation of T cells from PBMCs with reliable, user-independent results. As shown in this application, isolated cells are pure and viable, and ready to be used in downstream applications.

Figure 2: Antiviral capacity of TCR-engineered CD8$^+$ T cells. CD8$^+$ T cells were isolated from PBMC, activated and mock-transduced, transduced with lentivirus expressing EC1-TCR IC1-TCR or IC5-TCR and a GFP reporter linked by a T2A peptide or a control lentivirus expressing only the GFP reporter gene. Five days after transduction, the cells were added to GXR cells previously infected with HIV-LucR for 24 hours at a CD8$^+$ T cell/GXR cell ratio of 1:1. After 3 days, the cells were harvested and the luciferase activities in the cellular lysates were determined and reported as RLU +/- SEM. Results from a representative experiment performed in quadruplicate is shown.

To find out more about this study, please have a look at: Flerin, N. C. et al. (2017) T-cell receptor (TCR) clonotype-specific differences in inhibitory activity of HIV-1 cytotoxic T-cell clones is not mediated by TCR alone. J. Virol. 91: e02412-16.

References