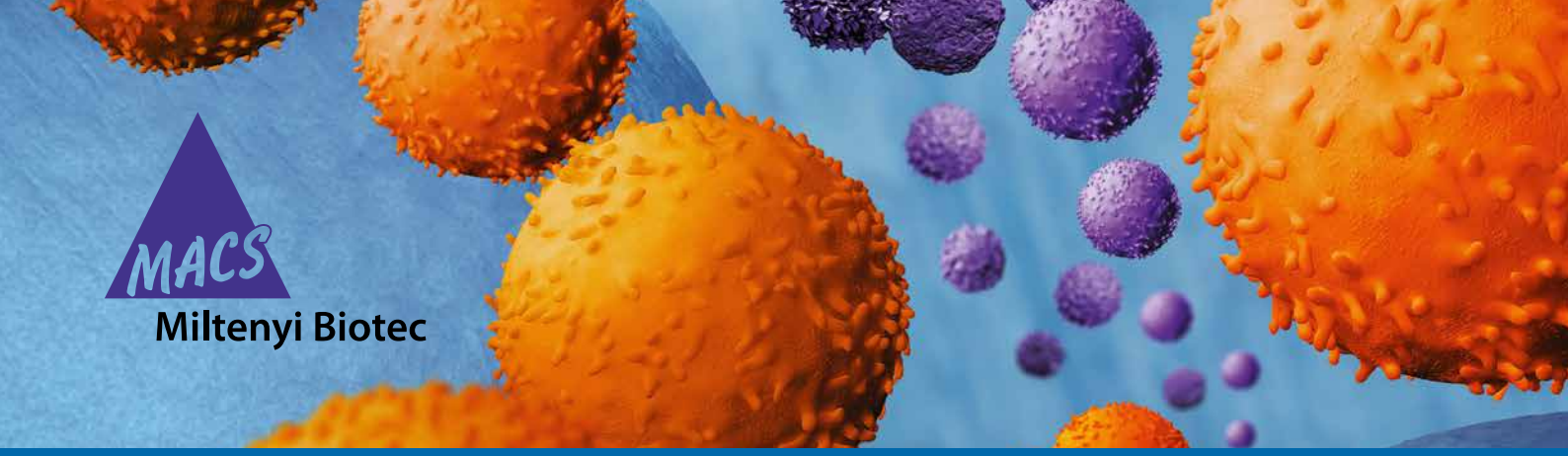

TCR clonotype sequence is only one factor defining the antiviral capacity of HIV-specific CD8+ T cells

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Background

Lentiviral vectors expressing effective human immunodeficiency virus (HIV)-specific T cell receptor (TCR) clonotypes can transform naïve CD8+ T cells into potent HIV-specific cytotoxic T cells (CTLs)[1], a possible gene therapy approach to increase HIV-specific immunity. To optimize this approach, it is crucial to identify TCR clonotypes, which confer the most potent anti-HIV activity. CTLs with superior antiviral efficacy, termed effective CTLs, are well-represented in HIV-1 controllers, or long-term non-progressors, but are rare or absent in HIV-1 progessors[2]. However, the contributory role of the HIV-1-specific CTL TCR clonotype on the potency of its antiviral activity is unclear. This compromises our ability to identify the HIV-1-specific TCR α and β chain genes that most effectively convert primary CD8+ T cells into potent HIV-1-specific CTLs.

Materials and methods

To directly evaluate the contribution of the TCR clonotype on the differences observed in effective or ineffective CTL clones, we cloned the TCR α and β chain genes from one effective and two ineffective CTL clones, specific for the same viral peptide KK10, but with different TCR clonotypes, isolated from an HLA-B*2705 elite controller into TCR-expressing lentiviral vectors. We used these lentiviral vectors to transduce Jurkat/MA cells, a T cell line engineered to measure TCR signaling using a luciferase reporter, and primary CD8+ T cells to delineate the contribution of the TCR on the functional activity of HIV-1-specific CTLs.

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.

Viral inhibition assay

HLA-B*2705-expressing GXR cells[3] were infected in 96-well plates with HIV-LucR, an infectious HIV-1 molecular clone that expresses the HIV-1JR-CSF Env and a Renilla reniformis luciferase (LucR) reporter gene. One day later, the HIV-1-infected GXR cells and either mock-transduced or lentivirus-transduced CD8+ T cells were added at an effector-to-target ratio of 1:1 and cultured for an additional 3 days. HIV-1 infection was quantified by harvesting cells, lysing them and measuring luciferase activity in the cellular lysate using a renilla luciferase reporter assay.

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).

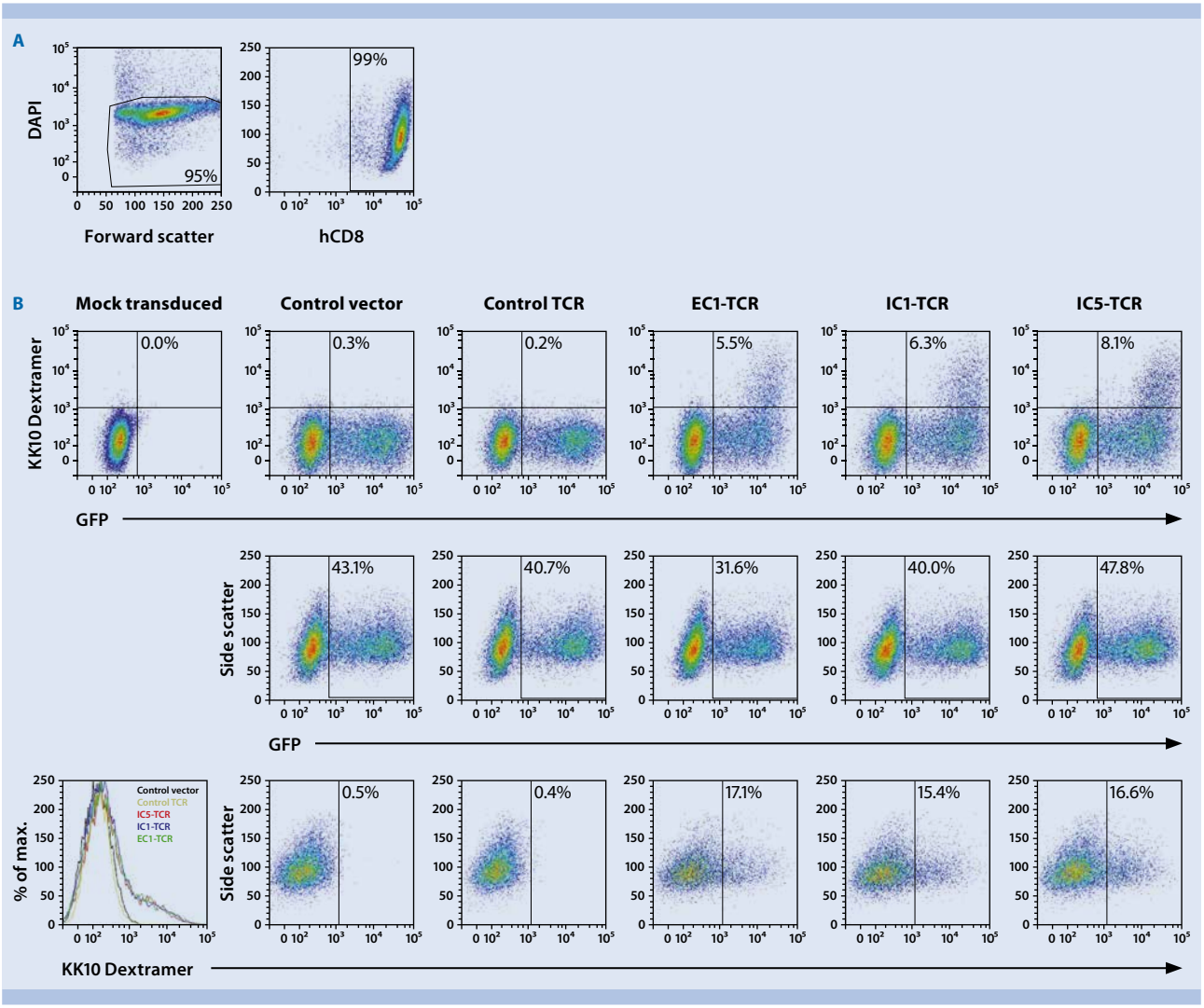

Figure 1: Purified CD8⁺ T cells isolated from PMBCs of an HIV naïve donor can be transduced and engineered to express HIV-specific TCRs that bind to KK10-specific dextramer. (A) Purified CD8⁺ T cells were analyzed for viability and human CD8 expression following the 3 day activation period. (B) After 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.

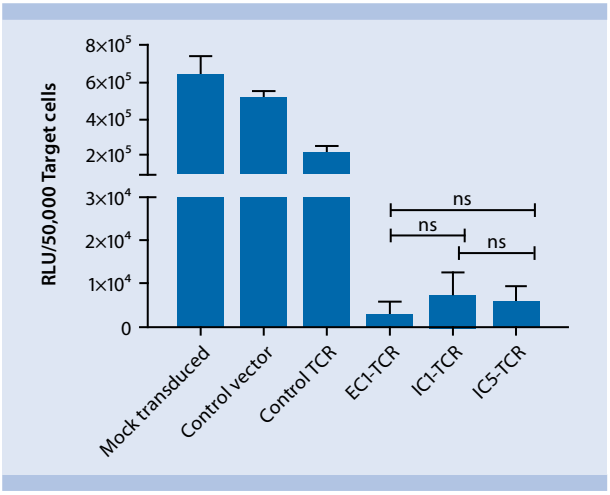

Figure 2: Antiviral capacity of TCR-engineered CD8⁺ T cells. CD8⁺ T cells were isolated from PBMC, activated and mock-transduced, transduced with lentivirus expressing EC1-TCR IC1-TCR or IC5-TCR and a GFP reporter linked by a T2A peptide or a control lentivirus expressing only the GFP reporter gene. 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 activites in the cellular lysates were determined and reported as RLU +/-SEM. Results from a representative experiment performed in quadruplicate is shown.

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.

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

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