Defining Efficacious HIV-Specific CTL Responses Using Saporin-Conjugated Tetramers

Leitman EM[1], Sims S[2], Payne RP[1], Chen F[3], Riddell L[4], Buus S[5], Deeks S[6], Walker BD[7], Matthews PC[8], Goulder JR[1]

[1] Department of Paediatrics, University of Oxford, Oxford, UK; [2]Department of BSE, ETH Zurich, Basel, Switzerland; [3]Royal Berkshire Hospital, Reading, UK; [4]Northamptonshire Healthcare NHS Trust, Northampton, UK; [5]Laboratory of Experimental Immunology, University of Copenhagen, Copenhagen, Denmark; [6]Department of Medicine, UCSF, CA, USA; [7]Ragon Institute of MGH, MIT and Harvard, Charlestown, MA, USA; [8]Nuffield Department of Medicine, University of Oxford, Oxford, UK

UNIVERSITY OF OXFORD

Background

In vitro killing of HIV-infected cells by cytotoxic T lymphocytes (CTL) is a surrogate marker of CTL antiviral efficacy[1]. Tetrameric peptide-MHC complexes (tetramers) enable identification of antigen-specific CD8+ T cells in a mixed population (the terms 'CTL' and 'CD8+ T cells' are used interchangeably)[2]. Due to their rapid internalization by cognate T cells, tetramers are an effective delivery vehicle of any coupled moiety to target specific CTL. Novel tetramers conjugated to saporin (SAP), a potent toxin causing cell death through ribosome-inactivation, have been used *in vivo* to deplete murine diabetogenic T cells, but no human reports exploiting this technology exist to date[3]. Here, we used toxic tetramers to rapidly eliminate or 'zap' human CTL of different specificities in order to compare their anti-HIV potency.

Methods

- Human PBMC were treated with HLA-matched SAP-coupled tetramers (tet-SAP) or controls.
- Specific targeting, internalization[3] and depletion assessed by FACS.
- Antiviral efficacy evaluated in inhibition assays[4], using tet-SAP- or control-treated CTL (effectors) and HLA-matched NL4-3-infected targets; NL4-3 was labeled with GFP to use as a marker of infection.

Results

1. Antigen-specific CTL recognize and rapidly internalize cognate tet-PE and tet-SAP

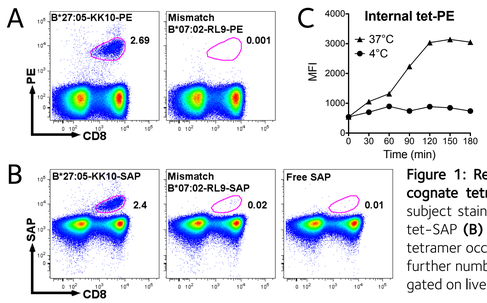


Figure 1: Recognition and kinetics of internalization of cognate tetramers by CD8+ T cells. PBMC from HIV+ subject stained with fluorescent tetramer (tet-PE) **(A)** or tet-SAP **(B)** of the same specificity. Full internalization of tetramer occurs by 2h of incubation at 37°C **(C)**. Here and further numbers in FACS plots show %tet+ cells (of CD8+); gated on live CD3+ cells.

2. Tet-SAP selectively eliminates human tet-specific CTL

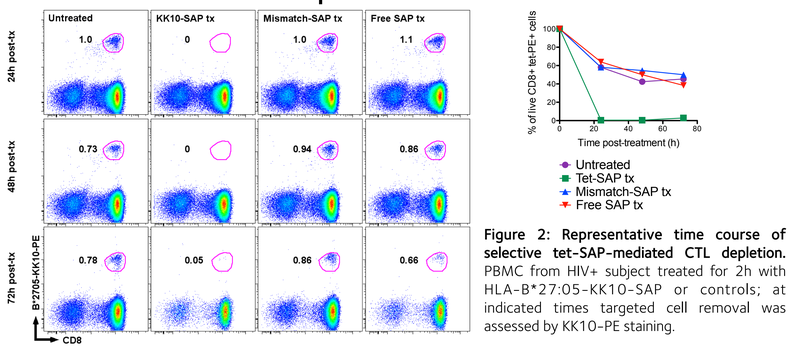


Figure 2: Representative time course of selective tet-SAP-mediated CTL depletion. PBMC from HIV+ subject treated for 2h with HLA-B*27:05-KK10-SAP or controls; at indicated times targeted cell removal was assessed by KK10-PE staining.

3. Tet-SAP-mediated CTL elimination is exclusive to desired specificity

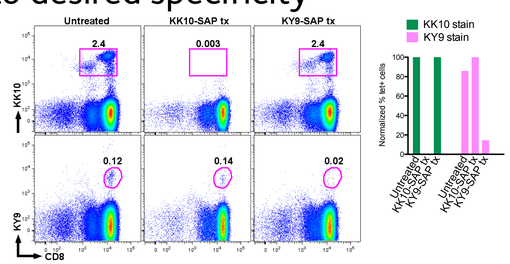


Figure 3: Tet-SAP-mediated removal of one CTL specificity is highly selective and does not affect other specificities.

4. Zapping across specificities and pathogens

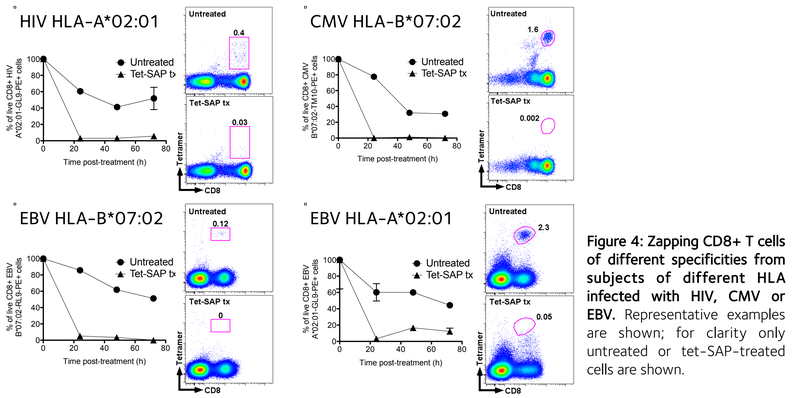


Figure 4: Zapping CD8+ T cells of different specificities from subjects of different HLA infected with HIV, CMV or EBV. Representative examples are shown; for clarity only untreated or tet-SAP-treated cells are shown.

5. Method validation: zapping vs bead depletion

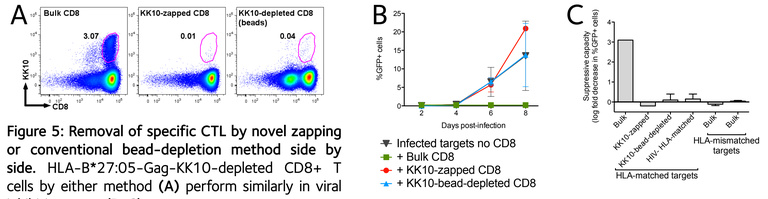


Figure 5: Removal of specific CTL by novel zapping or conventional bead-depletion method side by side. HLA-B*27:05-Gag-KK10-depleted CD8+ T cells by either method **(A)** perform similarly in viral inhibition assay **(B, C)**.

6. Functional application: HIV-suppressive capacity of zapped CTL

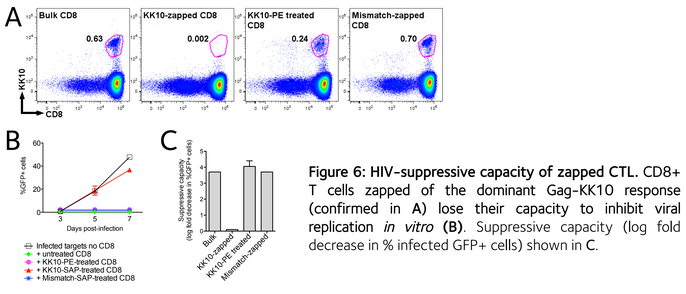


Figure 6: HIV-suppressive capacity of zapped CTL. CD8+ T cells zapped of the dominant Gag-KK10 response (confirmed in **A**) lose their capacity to inhibit viral replication *in vitro* **(B)**. Suppressive capacity (log fold decrease in % infected GFP+ cells) shown in **C**.

Conclusions

- We have optimized a novel method to eliminate human CTL of different specificities.
- This technique allows us to measure and compare the individual contribution of different CTL specificities to HIV suppression.
- This represents a rapid, cost-/labor-saving method, applicable across HLA types and pathogens.
- The approach enables us to evaluate the quality of different responses and discriminate between the most efficacious and futile CTL.

References

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Email: ellen.leitman@st-hughs.ox.ac.uk
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