Superior Detection of Self-Reactive, Antigen-Specific T-Cells using Klickmer™ Compared to Tetramers

Dolton *et al.* Optimized Peptide–MHC Multimer Protocols for Detection and Isolation of Autoimmune T-Cells. Front. Immunol. (2018) 9: 1378.

BACKGROUND

MHC-peptide multimers have become the "gold standard" for the detection and isolation of antigen-specific T cells. Detection sensitivity is a general issue, but particularly pronounced for anticancer and autoimmune T cells. Self-reactive T-cell populations are often enriched for low-affinity T-cell receptors (TCR) due to the removal of high-affinity cells by immune tolerance mechanisms.

Here, Dolton *et al*. investigates how to optimize multimer staining using Klickmer™ or tetramer reagents for improved detection of autoreactive T-cells. Klickmer™ is a Dextramer® backbone with multiple acceptor sites for attachment of biotinylated molecules.

STUDY DESCRIPTION

Purified CD8⁺ T cells from a HLA A*0201⁺ type I diabetes patient were pre-treated with PKI dasatinib for up to 50 minutes at 37°C and stimulated with pancreatic β-cell-specific epitopes to create T-cell lines for the staining optimization. Thereafter, cells were washed with FACS buffer and mouse anti-PE unconjugated Antibody. To create autoreactive T-cell lines for the optimization study, the CD8⁺ T cells were then pulsed with peptides from insulin-β chain$_{10–18}$ (HLVEALYLV) or glutamate decarboxylase 65 (GAD65$_{114–123}$, VMNILLQYVV). The autoreactive T cell lines were then subjected to tetramer or Klickmer™ staining according to standard and the optimized protocol (**Fig. 1A**).

RESULTS

For detection of antigen-specific T cells, biotinylated peptides were conjugated onto Klickmer™ reagent at molar ratio of 4:1 and 3:1. T cells were stained with antigen-specific tetramer or Klickmer™ reagent using an optimized protocol. Optimized Klickmer™ staining extended the TCR affinity threshold amenable to multimer staining and increased the size of the detected population by over a 10-fold compared to the optimized tetramer staining. Thereby, the optimized Klickmer™ staining vastly outperformed the optimized tetramer staining (**Fig. 1B**).

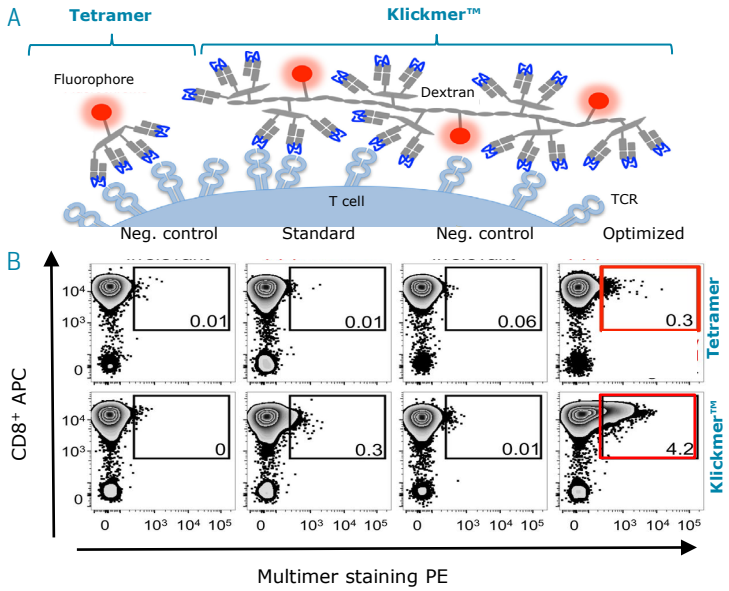

Fig. 1: Detection of preproinsulin (PPI) T-cells is further improved using Klickmer™ reagents under optimal staining conditions.

A Schematic representation of tetramers and Klickmer™ reagents as illustrated by Dolton *et al*. 2018.

B T cells were also stained with irrelevant peptides as negative control or PPI tetramers (upper panel) and Klickmer™ reagents (lower panel) using standard and optimal protocols (PKI⁺ anti-PE Ab). The percentage of cells residing in each gate is shown. The red box indicates tetramer or Klickmer™ detected cells.

CONCLUSIONS

- The application of Klickmer™ reagents extended the TCR affinity threshold amenable to MHC-peptide multimer staining, ensuring clear recovery of the self-reactive, antigen-specific T-cells from patient samples by flow cytometry
- Klickmer™ further increased the size of the detected population by over 10-fold compared to tetramer staining enabling a higher recovery of the antigen-specific autoimmune T-cells.

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