A workflow for detection of antigen-specific HLA-E restricted T cells in PBMC from healthy individuals

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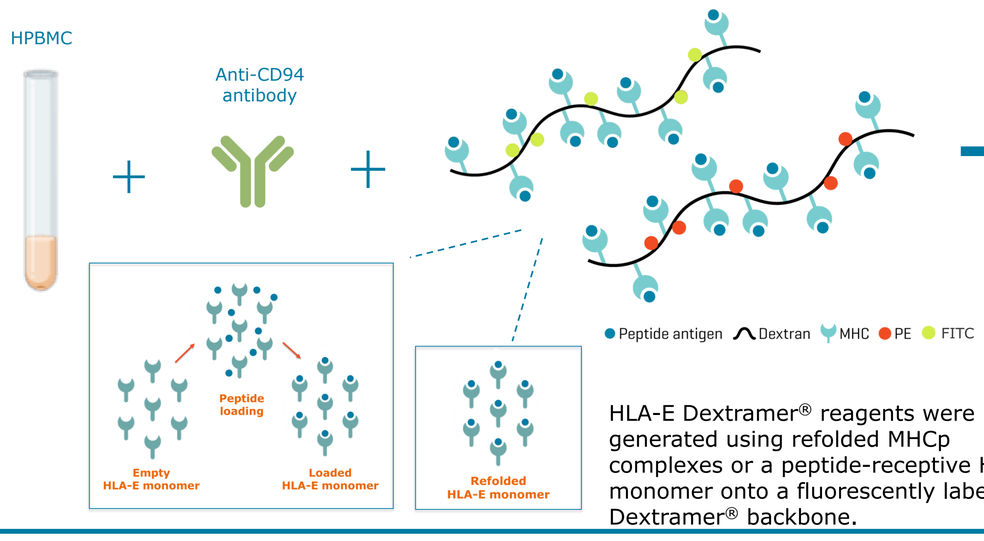
Introduction

The non-classical human HLA-E molecule exhibits monomorphic functionality and presents a promising target for immunotherapeutic strategies against cancer and infectious diseases. A reliable assay for detecting HLA-E restricted antigen-specific T cells is essential for advancing research in this field. However, due to the inherent instability of HLA-E, both its production and the development of HLA-E specific detection reagents are challenging. Moreover, studies have demonstrated that HLA-E can be recognized by both NK cells and T cells via the CD94/NKG2 receptors, highlighting the importance of blocking these non-TCR–mediated interactions and implementing a specific gating strategy to accurately characterize antigen-specific HLA-E restricted CD8+ T cells by flow cytometry-based assays. Here, we present a workflow to detect such T cells in PBMCs from healthy individuals by using HLA-E Dextramer® technology and an optimized staining protocol.

Workflow for detection of antigen-specific HLA-E restricted T cells



HLA-E Dextramer® reagents were generated using refolded MHCp complexes or a peptide-receptive HLA-E monomer onto a fluorescently labelled Dextramer® backbone.

Fig. 1. The workflow for detection of HLA-E restricted antigen-specific T cells in PBMCs by using Dextramer® Technology.
PBMCs from healthy individuals were screened using the established assay protocol in combination with flow cytometry.
An optimized workflow for specific detection of antigen-specific HLA-E restricted T cells via the staining of 1) CD94/NKG2 blocking agent; 2) HLA-E Dextramer® reagents; 3) A panel consisting of eight surface markers and in combination with flow cytometry.

Gating strategy

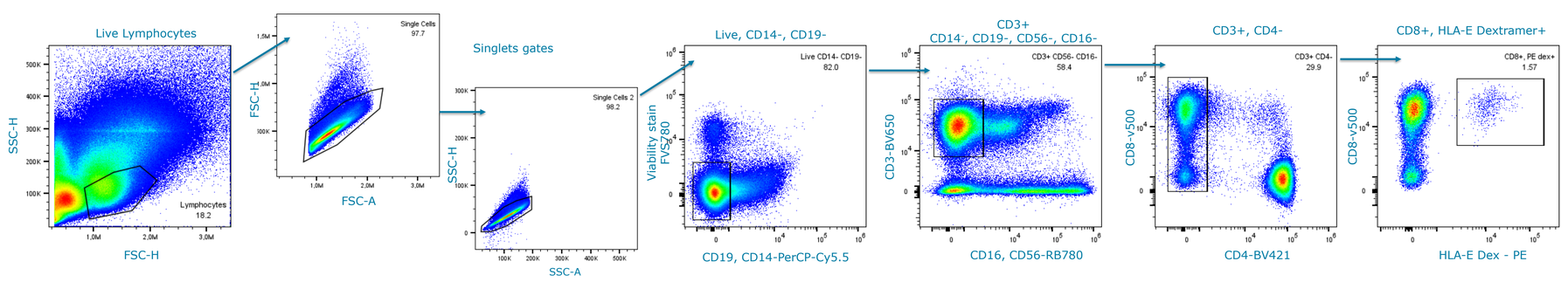
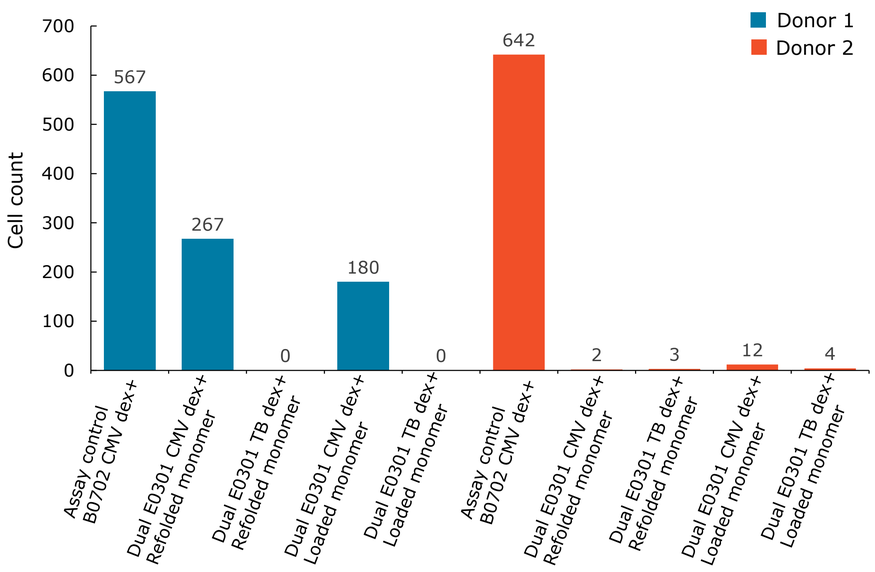


Fig. 2. Gating strategy on PBMCs for antigen-specific HLA-E restricted CD8+ T cells.
Lymphocytes were subgated to singlets, live, CD14-, CD19-, CD56-, CD16-, CD3+ to the level of CD3+ and CD8+ level. Antigen-specific, HLA-E–restricted CD8⁺ T cells were subsequently identified based on CD8 versus Dextramer®-PE⁺ or -FITC⁺ gating.

Results

	Dextramer®	Description
A	HLA-B*0702/TPRVTGGGAM/PE (CMV-pp65)	Positive assay control
B	HLA-B*0702/GPAESAAGL/APC	Negative assay control
C	HLA-E*0103/VMAPRTLIL/PE (hCMV UL40) HLA-E*0103/VMAPRTLIL/FITC (hCMV UL40)	Assembled by **refolded** HLA-E monomer
D	HLA-E*0103/RLPAKAPLL/PE (tuberculosis) HLA-E*0103/RLPAKAPLL/FITC (tuberculosis)	Assembled by **refolded** HLA-E monomer
E	HLA-E*0103/VMAPRTLIL/PE (hCMV UL40) HLA-E*0103/VMAPRTLIL/FITC (hCMV UL40)	Assembled by **loadable** HLA-E monomer
F	HLA-E*0103/RLPAKAPLL/PE (tuberculosis TB) HLA-E*0103/RLPAKAPLL/FITC (tuberculosis TB)	Assembled by **loadable** HLA-E monomer



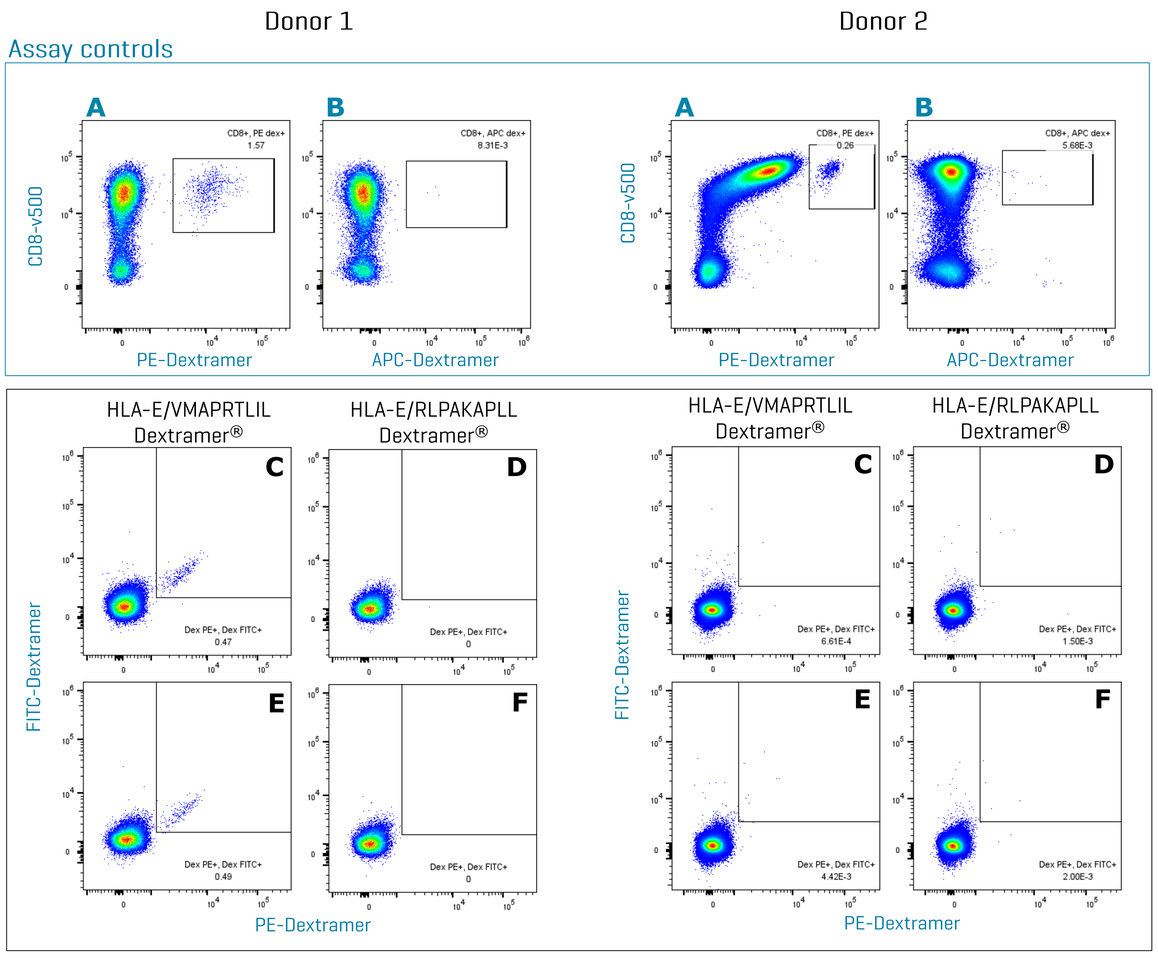


Fig. 3. CMV-specific HLA-E restricted T cells were detected in PBMC from one of the healthy individuals.

Table on the upper left shows the used Dextramer® reagents that were used to detect the HLA-E restricted T cells. A dual-staining approach was applied to ensure the detected TCR-peptide-MHC interactions were true signals. **Dot plots** on the right shows the assay was verified by the positive and negative assay control staining. Antigen-specific HLA-E-restricted T cell responses were detected in one of the donor by using this staining workflow. **Bar plot** on the lower left compares the cell count of detected antigen-specific HLA-E restricted T cells between two donors and the used Dextramer® reagents. Both plots indicate the newly developed empty HLA-E monomer can be loaded by peptides and functions as well as the conventional refolded HLA-E monomers.

Conclusions

- We were able to make stable HLA-E/peptide Dextramer® reagents using MHCp monomers made by refolding or a peptide receptive HLA-E monomer.
- By using anti-CD94 antibody we were able to prevent the binding of the HLA-E to the CD94/NKG2 receptors, allowing specific detection of TCR-mediated binding of T cells.
- Relevant cell subsets were identified by the designed antibody panel and gating strategy.
- Antigen-specific HLA-E restricted CD8+ T cells were identified by dual-staining of HLA-E Dextramer® reagents.
- we have established an optimized workflow for detection of HLA-E restricted antigen-specific T cells in PBMCs.

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