High-throughput Identification of Immunogenic B- and T-cell Epitopes Meets Deep Immune Cell Phenotyping

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BACKGROUND

Identifying the major antigenic determinants mediating an immune response and their cognate immune repertoires is of utmost importance for the development of vaccines and immunotherapies. The synergy of humoral, B-cell-mediated antibody responses, and T-cell-mediated cellular responses might be the key to success in both areas.

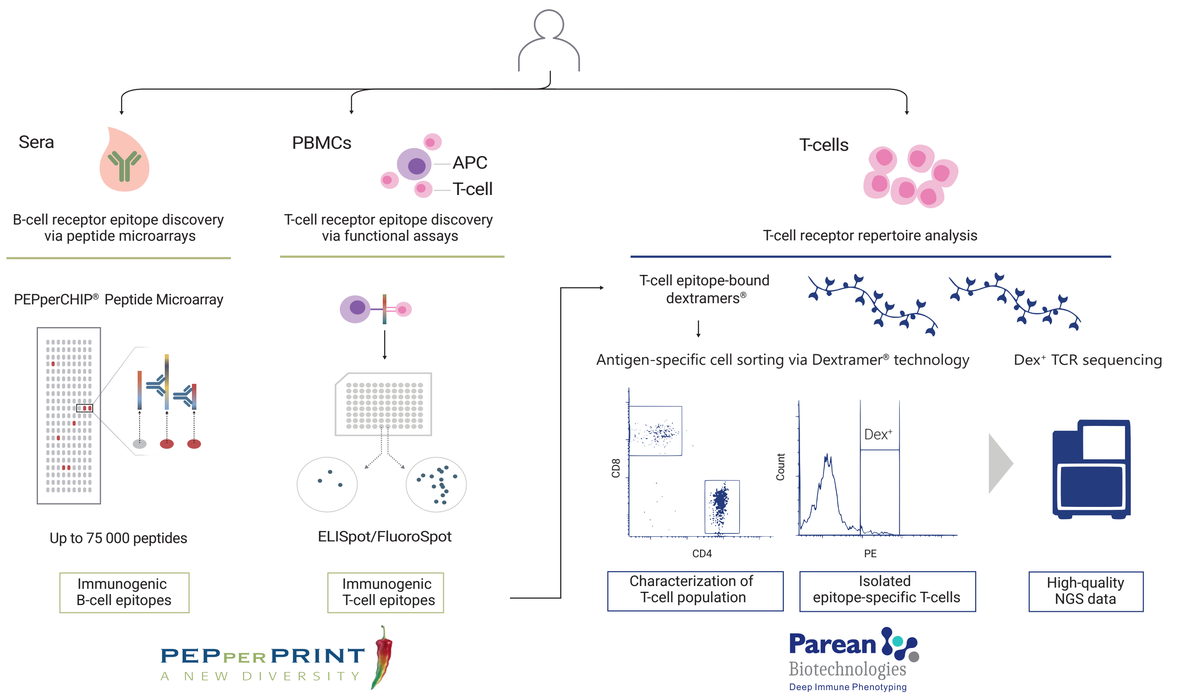

Figure 1. Combinational approach of PEPperPRINTs and Parean Biotechnologies competencies to unravel B-cell and T-cell epitopes and deep immune cell phenotyping. B-cell epitopes are identified via high-throughput screenings of sera for antibody-binding against tens of thousands of different peptides via PEPperCHIP® Peptide Microarrays. ELISpot assays in PBMC culture allow for testing peptides for T-cell antigenicity in 96 well plate format. For further analysis $CD8^+$ epitope-specific T-cells are sorted with tailored-made dextramers®. Harvested epitope-specific T-cells are then used for immune repertoire analyses via next-generation sequencing of the TCR alpha and beta chain on dedicated bioinformatic pipelines.

OBJECTIVE

In this study, we investigated the immune response towards Epstein-Barr virus (EBV)-encoded nuclear antigen-1 (EBNA1). EBNA1 is accused of playing a role in EBV-associated complications and constitutes a marker for virus-associated cancer cells, thereby offering opportunities for targeted therapeutic intervention and prevention. Here, we analyzed epitope-specific B-cell and T-cell immunity against EBNA-1 and analyzed the T-cell response down to the clonal level.

METHODS

High-density peptide microarrays are a powerful tool to monitor the humoral response via simultaneously screening tens of thousands of peptides against serum antibodies in a high-throughput manner. In this study, we used PEPperCHIP® Epstein-Barr Virus Peptide Microarrays to identify B-cell epitopes in EBNA-1. Subsequently, these epitopes were further analyzed for T-cell antigenicity via ELISpot assay. With a focus on T-cells, we unraveled the T-cell receptor (TCR) immune repertoire specific to this epitope (Figure 1). With tailored-made dextramers, we sorted epitope-specific $CD8^+$ T-cells, sequenced the immune repertoire and analyzed the TCR alpha and beta chain structures with dedicated bioinformatic pipelines. Next-generation sequencing enabled the identification of TCRs specific to this EBNA-1-derived epitope.

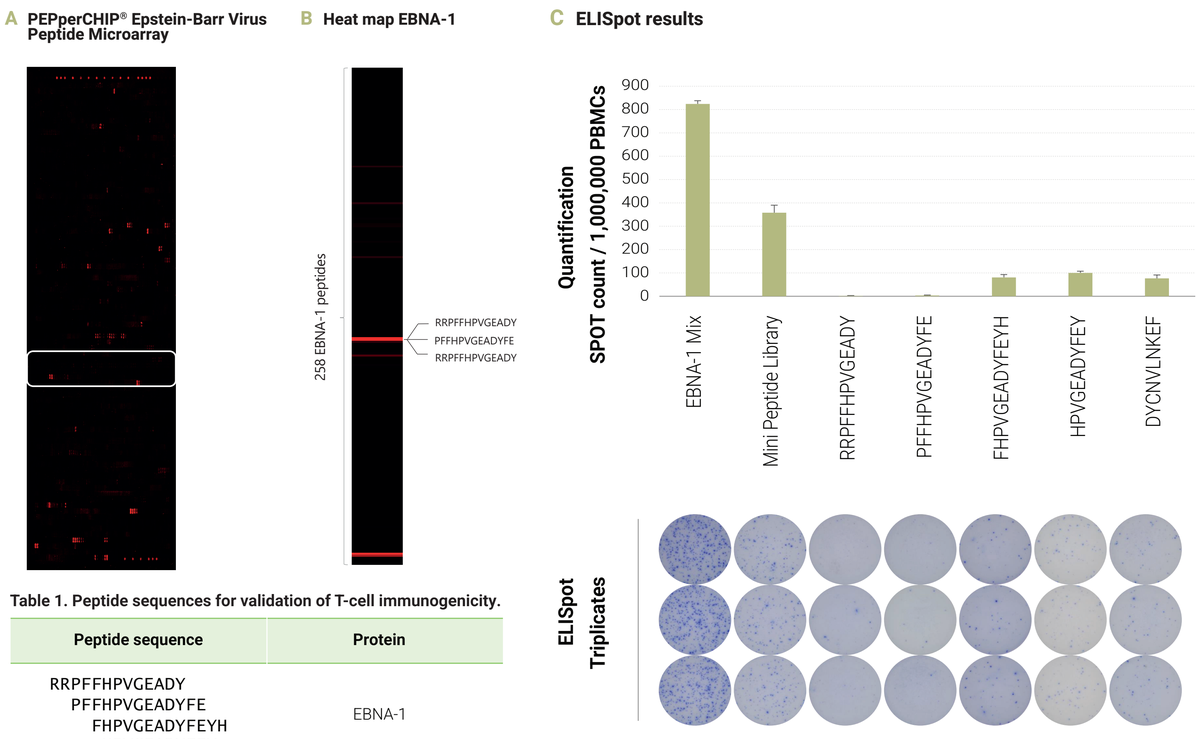

Table 1. Peptide sequences for validation of T-cell immunogenicity.

Peptide sequence	Protein
RRPFFHPVGEADY PFFHPVGEADYFE FHPVGEADYFEYH HPVGEADYFEY	EBNA-1
DYCNVLNKEF	BRLF1

Figure 2. Discovery of B- & T-cell receptor epitopes. (A) Scans of the PEPperCHIP® Epstein-Barr Virus Peptide Microarrays. The microarrays were incubated with the sera of an EBV-infected individual at a dilution of 1:150 overnight at 4°C. Fluorescence readout was performed using an INNOPSYS Imaging System. Red spots=IgG responses. The frame highlights the position of the EBNA-1 peptides. (B) Heatmap of antibody response profile against 258 EBNA-1-derived peptides. Scanned images were analyzed with the PepSlide® Analyzer software. The heatmap shows the fluorescence intensities of 258 overlapping EBNA-1 peptides sorted from the N- to the C-terminus of the protein. Color code: black = FI below 200; red = FI above 3000. (C) 500,000 PBMCs per well were stimulated in a 96-well IFN-γ ELISpot plate for 24 hours with an EBNA-1 peptide mix (EBNA-1), the mini peptide library (Table 1) or the underlying single peptides in a final concentration of 10 µg/ml. Top half: Quantification of ELISpots with the mean values and the standard deviation of triplicates of the counted spots per 1 million PBMCs. Bottom half: IFN-γ-dependent blue-colored immune complexes.

RESULTS

Applying high-density peptide microarrays combined with state-of-the-art ELISpot analyses discovered a highly immunogenic B- and T-cell overlapping epitope derived from the EBNA-1. TCR repertoire analysis highlighted specific highly conserved motifs, signing a restricted clonotypic stimulation (Figure 3).

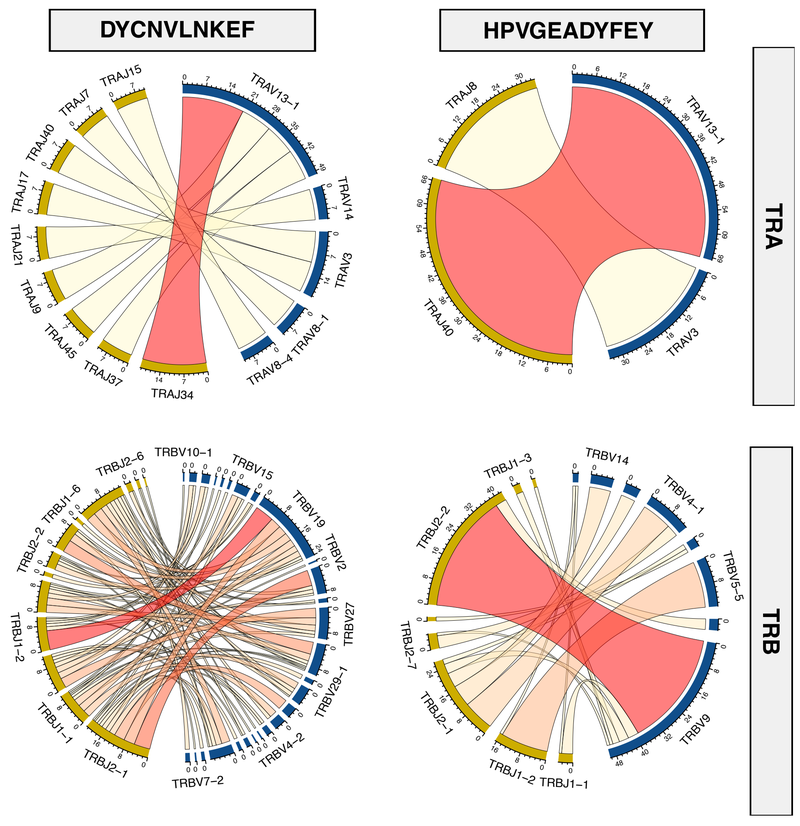

Figure 3. EBV specific V–J gene usage. Chord diagram showing frequency (%) of the given V and J usage for HPVGEADYFEY (right) and DYCNVLNKEF (left). TRA (top) and TRB (bottom) repertoires are represented by the two peptides. Only V (blue) and J (gold) genes that are detected are represented. The colors and sizes of the segments represent frequencies.

Spectratyping analysis was carried out to further examine the CDR3 regions of tetramer-positive T-cells (Figure 4). A typical Gaussian distributions of CDR3β lengths was observed for DYCNVLNKEF tetramer-positive T-cells. On the other hand, in a very clear way, the HPVGEADYFEY-specific TCR α and β spectratypes showed a highly restricted non-parametric distribution. CDR3s regions, overlapping epitope (HPVGEADYFEY), were examined for conserved amino acid residues (Figure 5).

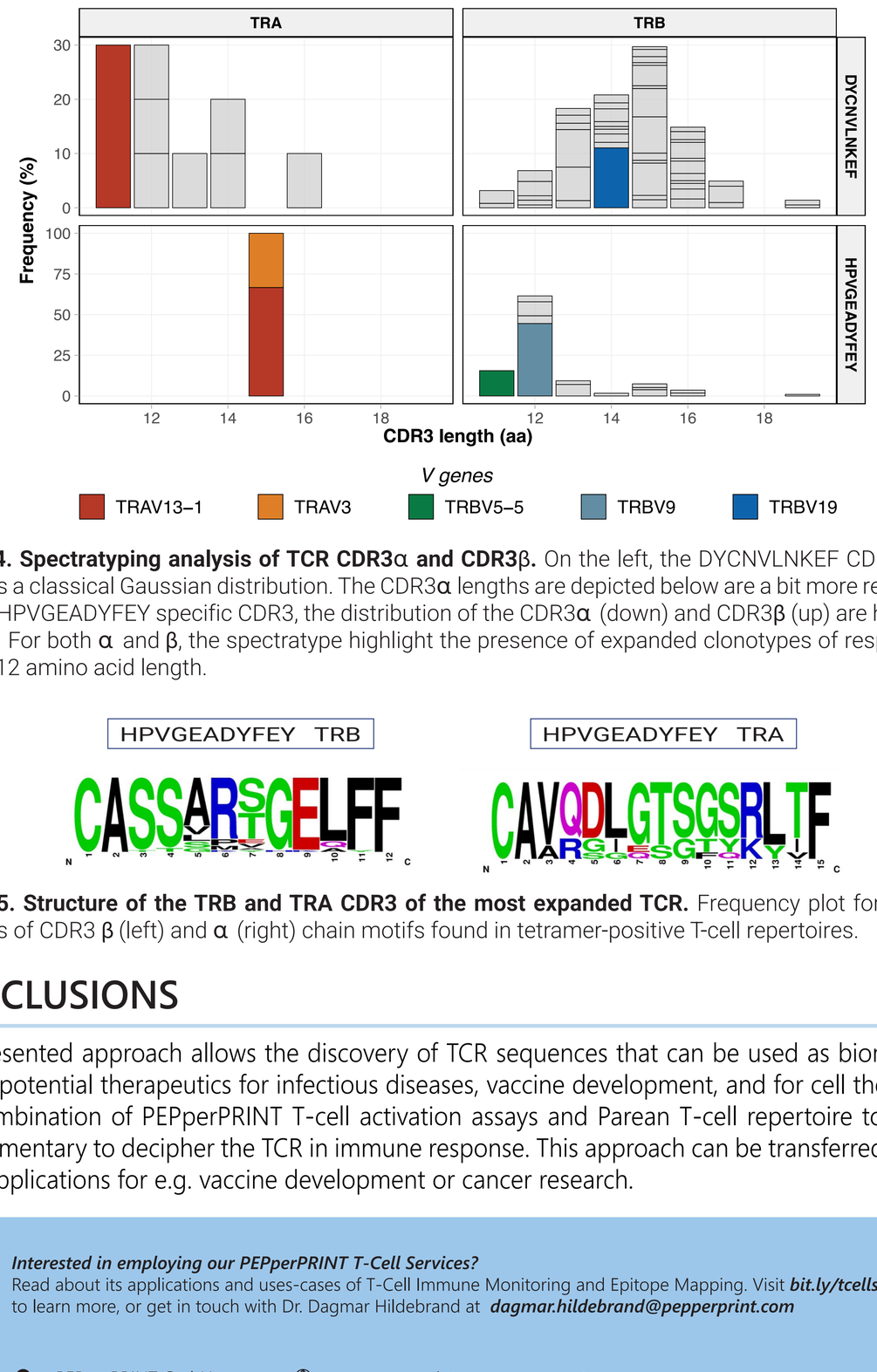

Figure 4. Spectratyping analysis of TCR CDR3α and CDR3β. On the left, the DYCNVLNKEF CDR3β (up) presents a classical Gaussian distribution. The CDR3α lengths are depicted below are a bit more restricted. For the HPVGEADYFEY specific CDR3, the distribution of the CDR3α (down) and CDR3β (up) are highly restricted. For both α and β, the spectratype highlight the presence of expanded clonotypes of respectively 15 and 12 amino acid length.

Figure 5. Structure of the TRB and TRA CDR3 of the most expanded TCR. Frequency plot for the sequences of CDR3 β (left) and α (right) chain motifs found in tetramer-positive T-cell repertoires.

CONCLUSIONS

The presented approach allows the discovery of TCR sequences that can be used as biomarkers and/or potential therapeutics for infectious diseases, vaccine development, and for cell therapies. The combination of PEPperPRINT T-cell activation assays and Parean T-cell repertoire tools are complementary to decipher the TCR in immune response. This approach can be transferred to any other applications for e.g. vaccine development or cancer research.