Exploring full-length T cell receptor sequencing and CITE-seq to identify and characterize pathogenic T cells in psoriasis

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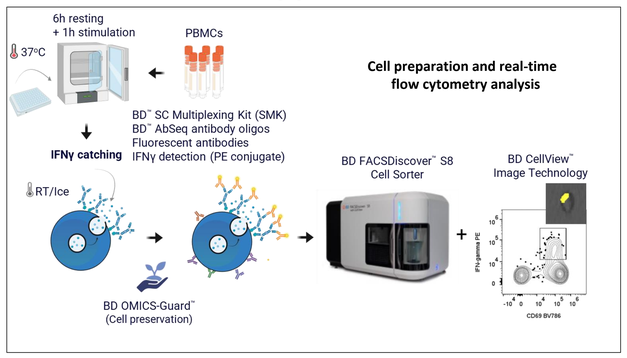
Abstract

Psoriasis is a chronic immune-mediated skin disease characterized by keratinocyte hyperproliferation and systemic inflammation. In addition to its cutaneous manifestations, psoriasis is associated with comorbidities such as cardiovascular disease and psoriatic arthritis. The disease is driven by dysregulated T helper cells, which interact with dendritic cells and keratinocytes to sustain inflammation and recruit cytotoxic CD8⁺T cells. Early in disease progression, memory T cells, expressing cutaneous lymphocyte antigen (CLA) and skin-homing chemokine receptors, migrate into the skin and circulate between skin and blood, serving as peripheral biomarkers of disease activity. CLA⁻ T cells, while less involved in lesion formation, may contribute to disseminating inflammation beyond the skin. To investigate the relationship between CLA⁺ and CLA⁻ T cells, we applied a multiomic approach combining single-cell full-length TCR sequencing, CITE-seq, and imaging flow cytometry. Imaging flow cytometry enabled real-time assessment of IFNγ secretion without compromising cell viability. TCR sequencing revealed 263 shared clonotypes between CLA⁺ and CLA⁻CD8⁺ memory T cells, suggesting common antigen recognition. CLA⁺ T cells from psoriatic donors were hyporesponsive and exhibited transcriptional exhaustion. In contrast, CLA⁻CD8⁺T cells secreted IFNγ upon stimulation and showed a transcriptional program marked by expression of type I IFN genes and capable of rescuing these cells from exhaustion. This phenotype differed from healthy controls, where CLA⁻CD8⁺T cells displayed canonical cytotoxic profiles. These findings highlight the complementary roles of CLA⁺ and CLA⁻T cells in local and systemic inflammation and demonstrate the utility of integrated multiomic technologies for understanding immune cell dynamics and accelerating biomarker discoveries in psoriasis.

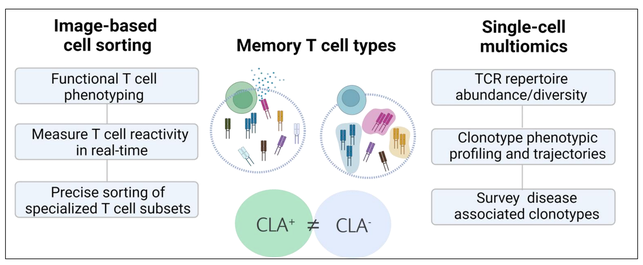
Methods

Integration of image-based cell sorting and single-cell multiomics for assessment of T-cell driven inflammation in psoriasis

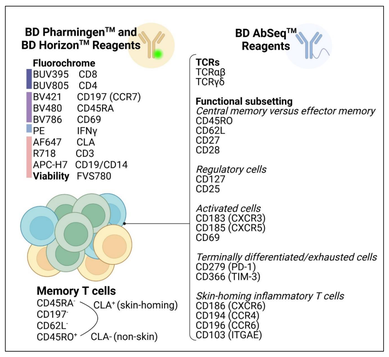
Frozen peripheral blood mononuclear cells from three healthy and three psoriatic individuals were incubated for 1 hour with CytoStim (Myltenyi Biotech) for restimulation of effector/memory T cells. After stimulation, secreted INFγ was measured using the Human IFNγ Secretion Assay (Miltenyi Biotech) and the BD FACSDiscover™ S8 Imaging Cell Sorter.



In addition, cells from the different donors and conditions were barcoded with BD® Single Cell Multiplexing Kit tags and simultaneously labeled with antibody-oligos and fluorescent antibodies. This enabled the sorting of memory subsets for downstream single-cell TCR sequencing and CITE-seq analyses.



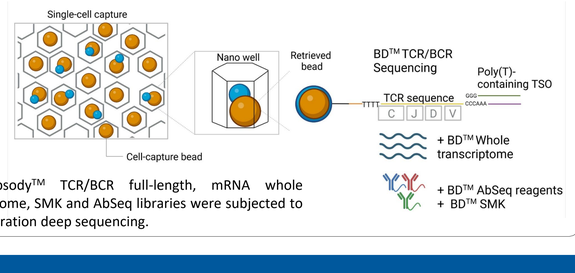
Antibody panels were designed for characterization of memory T cell subsets, including skin-derived cutaneous lymphocyte antigen (CLA)⁺ T cells. The sorted populations CD3⁺CD45RA⁻CD197⁻CLA⁻ or CD3⁺CD45RA⁻CD197⁻CLA⁺ were obtained from both unstimulated and stimulated cell cultures.



Single-cell capture for multiomics applications

BD Rhapsody™ Single-cell HT Xpress System

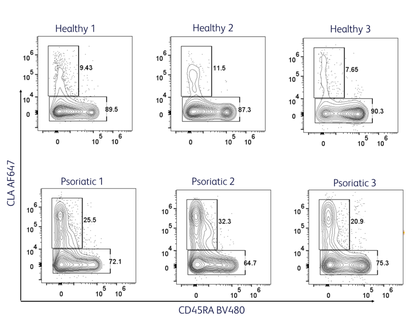
The sorted cells were captured in an 8-lane BD™ Rhapsody microwell cartridge and the single cells (in beads) were retrieved for library preparation.



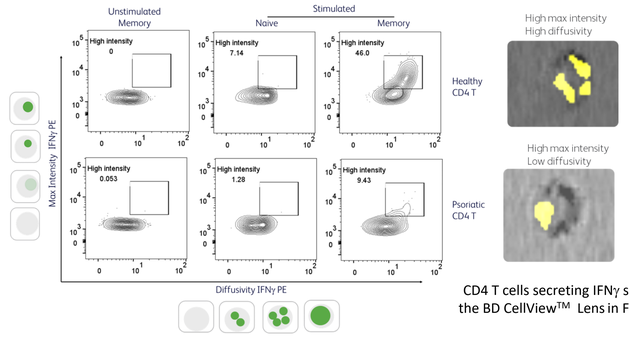
BD Rhapsody™ TCR/BCR full-length, mRNA whole transcriptome, SMK and AbSeq libraries were subjected to next generation deep sequencing.

Results – Functional fitness of circulating skin-homing T cells

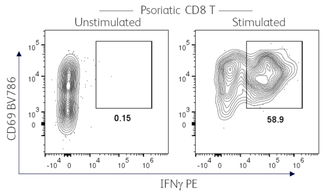
1A High frequencies of skin-homing CLA⁺ CD4T cells in psoriasis



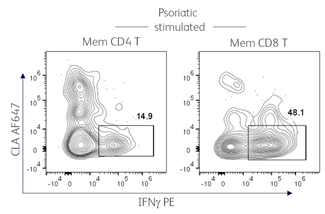
1B Defective IFNγ secretion by CD4T cells but not CD8 T cells in psoriasis



CD4 T cells secreting IFNγ seen from the BD CellView™ Lens in FlowJo v10



1C CLA⁺ skin-homing T cells are less responsive to stimulation



CD8 T cells secreting IFNγ seen from the CellView™ imaging wall

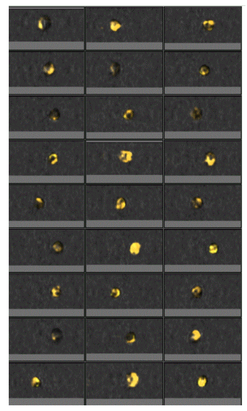
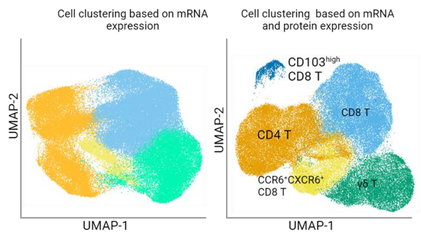


Figure 1: Functional fitness of circulating T cells measured by imaging flow cytometry. A) Assessment of the frequencies of CLA⁺ T cells among PBMCs. **B and C)** Detection of functional T cells secreting IFNγ using imaging fluorescence parameters on the BD FACSDiscover™ S8 Cell Sorter upon re-stimulation of memory T cells with CytoStim. **C)** Further assessment of IFNγ secretion reveals that CLA⁺ cells are less responsive to CytoStim stimulation and unlikely secrete IFNγ.

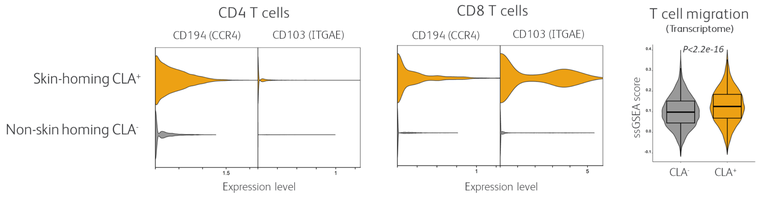
Results – Proteomic and transcriptomic characteristics of skin-homing T cells

2A Optimized detection of circulating CD103⁺ cells with CITE-seq

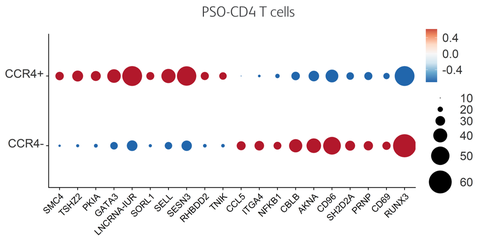


2B Skin-homing T cells are enriched for cell migration-related genes

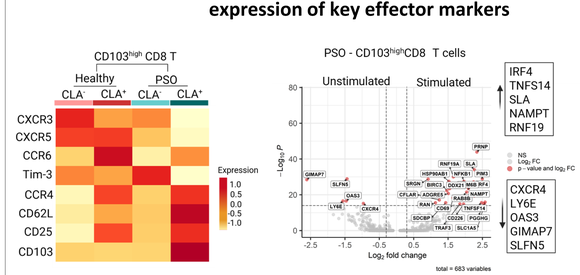
CLA⁺ cells exhibit elevated expression of CCR4, and display a gene signature associated with enhanced cell migration

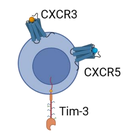


2C Skin-homing CCR4⁺ CD4 T cells from psoriatic individuals exhibit a gene expression profile consistent with a senescent/exhausted phenotype



2D CD103high CD8 T cells from psoriatic individuals exhibit impaired expression of key effector markers



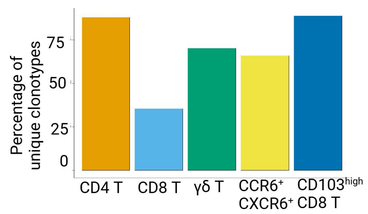


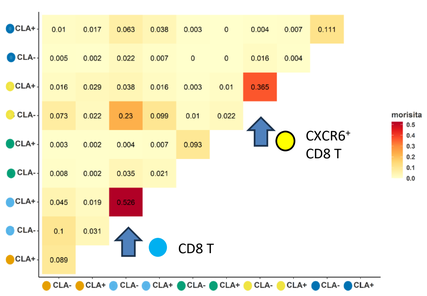
Polyfunctional responses to CytoStim are likely driven by CLA⁻ non-skin homing T cells

Figure 2: Phenotypic and functional profiling of skin-homing and non-skin homing memory T cells using CITE-seq. A) Dimensionality reduction and Seurat clustering of over 60,000 high quality cells demonstrate improved spatial cel organization when clustering is performed using integrated mRNA and protein data. **B)** Gene enrichment analysis reveals that skin-homing cells exhibit higher score for cell migration gene signatures compared to non-skin homing cells, supporting their ability to migrate between skin tissue and blood. **C)** Differential gene expression analysis of CCR4⁺ and CCR4⁻ CD4 T cells from psoriatic individuals identifies elevated expression of *SESN3, LNCRNA-IUR and TSHZ2* that may contribute to T cell exhaustion and impaired IFNγ secretion in response to CytoStim. **D)** Protein analysis highlights defective expression of CXCR3, CXCR5 and Tim-3 in CLA⁺ skin-homing T cells from psoriatic individuals. Despite lower expression of these effector targets compared to healthy individuals, the CD103^{high}CD8 T cells from the psoriatic individuals retain responsiveness to CytoStim stimulation. This response is mostly driven by the CLA⁻ cells counterpart that express effector protein markers

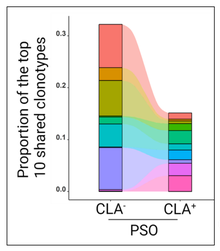
Results – Full-length TCR sequencing uncovers clones shared between skin- and non-skin homing T cells

3A Non-skin homing CD8 T cell subsets share TCR clonotypes with skin-homing CD8 T cells

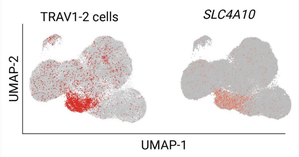




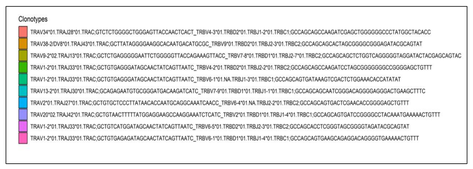
3B Skin- and non-skin-homing CD8 T cells can share TCR clonotypes while exhibiting distinct functional profiles



TCR α-chain often expressed in MAIT cells and highly used by the psoriatic individuals



Circulating clonotypes that may not reach the skin tissue but may react to psoriatic antigens



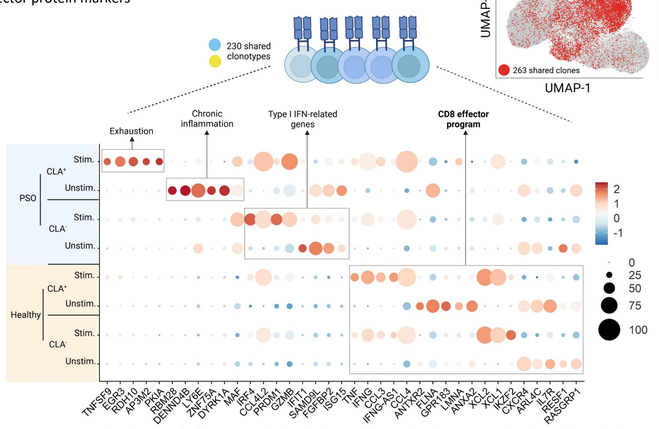


Figure 3: Identification and characterization of CD8 T cells driving inflammation using full-length TCR profiling. A) T-cell repertoire analysis reveals enrichment of unique clones among CD4 and CD103^{high}CD8 T cells. In contrast, other CD8 T cell populations exhibited less diverse repertoires. The CD8 T (blue cluster) and CXCR6⁺ CD8 T (yellow cluster) populations contained overlapping TCR clonotypes between CLA⁻ and CLA⁺ cells. The UMAP shows the location of the shared clones and the arrows in the correlation matrix indicate the highest Morisita scores, reflecting the populations with the highest TCR sequences similarities.

B) The alluvial plot depicts the top 10 clonotypes shared between skin- and non-skin homing T cells, each identified by a distinct color. The UMAP displays one of the most frequently used α-chain genes in the psoriatic individuals, which is also shared with non-skin homing cells. The shared clones from healthy individuals in addition to having similar TCR sequences, exhibit overall similar functional phenotype. In contrast, the shared clones from psoriatic individuals present distinct transcriptional programs: CLA⁺ cells are suppressed and become exhausted upon stimulation, while CLA⁻ cells retain INF I responses.

Conclusions

Using imaging flow cytometry, we assessed the functional fitness of skin-homing T cells through real-time analysis of IFNγ secretion. Additionally, we sorted memory subsets for comprehensive downstream multiomics analysis using a complete workflow solution provided by the BD™ Rhapsody Single Cell Capture System.

This workflow eliminated the need for skin tissue preparations in analyzing skin-derived T cells and further enabled the identification of TCR clonotypes shared between peripheral memory and skin-homing memory T cells.

In the psoriatic samples, CLA⁻ cells expressing overlapping clonotypes with skin-homing cells upregulated *IRF4*, *PRDM1* and other genes suggesting that these cells can respond to stimulation and sustain type I IFN-mediated inflammation during disease flares. Importantly, these cells may encounter specific antigens outside the skin, contributing to spread of the disease to other locations.

In contrast, CLA⁺ skin-homing cells exhibited signs of suppression and may undergo exhaustion upon stimulation.

Our results help to elucidate immune mechanisms involved in psoriasis, offering insights that may inform future research into targeted treatments for this and related skin inflammatory conditions.